Seasonal variations of plant nutrient levels and soil microorganisms in colliery spoil with particular reference to nitrogen.

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

in the

UNIVERSITY OF YORK

by

PETER JOHN WILLIAMS

1974

P J Williams - Biology

ABSTRACT

The seasonal variations of a number of plant nutrients, pH, spoil moisture and temperature and groups of microorganisms concerned with the recycling of nitrogen were investigated in ameliorated and untreated spoils at two colliery spoil heaps of contrasting spoil pH situatedin West Yorkshire.

In addition, the ability of unameliorated spoil to supply nitrogen for plant growth and the fate and effectiveness of nitrogen applied in the form of sewage-sludge and shoddy was assessed.

The results showed that in acid spoil (pH less than 5.0) the concentrations of a number of plant nutrients (especially aluminium and manganese) increased in the summer and autumn months to phytotoxic levels. Whilst the levels of available potassium and nitrogen were low, the vegetation was so limited by the phytotoxic concentrations of aluminium and manganese that it failed to respond to nitrogen additions.

In neutral spoil, toxicities were absent and the poor growth of the vegetation could be attributed to a deficiency of nutrients, especially nitrogen. Sewage-sludge, however, had no beneficial effect on plant yield and shoddy only had a slight effect. The ineffectiveness of these two sources of nitrogen was shown to result from leaching and denitrification losses of mineral nitrogen released from these ameliorants.

The results of the investigations are discussed in relation to the revegetation of colliery spoil heaps and a number of practical suggestions are made.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr. M.J. Chadwick, for instruction, help and friendly encouragement during the preparation of this thesis. I should also like to thank the members of the Derelict Land Research Group (V. Dennington, J.E. Cooper, M.E. Palmer, A. Tasker and T.K. Wai) for helpful discussions and my wife, Julie, for help with the writing of computer programs.

I would also like to acknowledge the help given to me by Mr. R. Hunter for reproducing some of the figures and Mrs. J. Chambers for patiently typing this work.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

CHAPTER ONE

INTRODUCTION

1. The need for reclamation of colliery spoil heaps

In Great Britain between 150,000 and 200,000 acres of land have become derelict as a result of the extractive industries and the tipping of industrial and domestic refuse. Colliery spoil heaps represent approximately 30,000 acres, or something less than 20% of the total (Chadwick 1973a).

This relatively small proportion is of great significance because spoil heaps are concentrated in localised, often densely populated areas, where they have a visual impact out of all proportion to the land area they cover. Further, they occupy land in areas where it is required for recreational and amenity purposes and the general unsightliness they produce prevents urgently needed economic development.

The reclamation of colliery spoil heaps is therefore economically and socially desirable.

2. The composition of colliery spoil heaps

Colliery spoil heaps are largely composed of the strata that originally layed adjacent to the coal seams and which were removed together with the coal during mining operations. They may also include unmarketable low grade coals, small coal particles and almost any other material removed from the earth during the opening of new seams, or discarded by the mining industry.

The mineralogical composition of colliery spoil is

shown in Table 1.1.

Table 1.1 The mineralogical composition of colliery spoil.

Inert quartz Clay minerals of mudstones and shales Coal	95%
Pyrite Siderite Ankerite Jarosite Gypsum	5%

Spoil heaps are of very variable composition and the percentage values quoted in Table 1.1 are only approximate. Minerals other than those shown can sometimes occur. The variability arises because the proportion and nature of the shale (a general term for noncarbonaceous material) and dirt (carbonaceous material containing greater than 40% ash after combustion, Fenton et al. 1962) removed during mining varies with the thickness character of the seam being worked. Some seams contain dirt bands, others do not. A single colliery may have worked a number of different seams and a single spoil heap may have received spoil from a few closely situated collieries.

The spoil may be unburnt (black) or burnt (red or white) and mixtures of both sorts commonly occur. Ignition occurs as a result of the spontaneous combustion of the carbonaceous fraction of the spoil. Burnt spoil often occurs as fused masses and Doubleday (1972a) claims that

combustion at temperatures in excess of 450°C reduces the phosphate sorbing capacity and increases the availability of potassium.

Spoils resulting from open cast or strip-mining are very similar to those resulting from deep mining and present the same sorts of reclamation problems.

3. Revegetation of colliery spoil heaps

I) The nature of the problem

Colliery spoil heaps reclaimed for agricultural, public amenity (playing fields, open space) or aesthetic reasons must be able to support a cover of vegetation. Colliery spoil does not however, represent a favourable substrate for plant growth. Natural revegetation may occur only very slowly and attempts to introduce vegetation onto bare spoil in reclamation schemes have frequently failed. The following literature review on factors affecting plant growth on spoils outlines the nature of the problem.

a) Physical factors.

Brierley (1956) working mainly in the Nottinghamshire-Derbyshire coalfield suggested that the establishment of vegetation depended upon mechanical rather than chemical factors. Conditions which facilitated the establishment of vegetation were found where large, firmly anchored rocks or any kind of stable rough material was exposed on the spoil surface (Brierley 1956).

Wood & Thirgood (1955) reported that exposure may be the cause of poor growth of trees planted on the upper slopes of tall conical heaps situated in exposed positions.

From a study of the dark coloured spoils of County

Durham, Richardson (1958) concluded that the desiccating effect of high summer temperatures on shade free slopes of spoil heaps was an important factor limiting plant growth. Similar conclusions were reported by Cornwell (1971) from a study of anthracite spoil banks in Pennsylvania.

b) Chemical factors

i) Deficiencies.

Davison & Jefferies (1966) examined freshly exposed and weathered, burnt and unburnt spoils with pH values ranging between 3.2 and 7.8 from the Northumberland and Durham coalfields. They concluded that all were severely deficient in phosphorus and nitrogen. Potassium deficiency symptoms were reported by Coates (1964) to occur in grasses and cereals grown on the Bickershaw reclamation site in Lancashire.

Beyer & Hutnik (1969) reviewed the results of investigations into poor plant growth in strip mine spoils in certain American states and commented that deficiencies of nitrogen, potassium, calcium and magnesium were found.

Knabe (1965) stated that nutrient deficiencies could account for the poor growth of vegetation on certain spoil heaps but not the complete absence of vegetation noted on some spoil heaps. One example was quoted, however, where the lack of vegetation on a neutral spoil bank in central Germany was due entirely to extreme deficiencies of nitrogen, phosphorus and potassium (Knabe 1965).

ii) Toxicities.

Spoil heaps may contain appreciable quantities of the mineral iron pyrites. On weathering, this mineral

becomes oxidised with the result that sulphuric acid is produced (see page 72). This reaction can occur to such an extent that the surface layers of a colliery spoil heap become extremely acid. Chadwick, Cornwell & Palmer (1969) reported pH values of 2.8, 3.5 and 3.8 as the means of twelve samples taken from three colliery spoil heaps in the West Riding of Yorkshire.

Knabe (1965) stated that extreme soil reaction was the most frequent cause of the failure of natural revegetation of industrial waste material. The importance of soil acidity is not usually ascribed to a direct effect of hydrogen ions but rather to the increased solubility of certain potentially phytotoxic elements, especially, aluminium and manganese.

Berg & Vogel (1968) observed both in laboratory and field studies that several legume species grown in Kentucky strip-mine spoils produced symptoms of manganese toxicity. Tree growth and survival in some Iowa spoils was reported to be inversely related to exchangeable aluminium levels (Lorio & Gatherum 1965).

Beyer & Hutnik (1969) examined spoils from the bituminous coalfield of Pennsylvania and concluded that pH and soluble aluminium were the most probable causes of spoil toxicity.

Toxicities attributable to copper, zinc and lead are not usually found in colliery spoils although they have been observed in heavy metal-ore mine spoil and smelter wastes (Bradshaw et al. 1965; Smith & Bradshaw 1972).

Knabe (1965) has suggested that the failure of

natural revegetation of certain spoil heaps could be due to the osmotic effects of high salt concentrations. This worker has also claimed that sulphur dioxide and mercaptan may be produced when spoil undergoes combustion, and these may be important factors in some situations (Knabe 1965).

c) Biotic factors

Atkinson et al. (1957) and Wood & Thirgood (1955) suggested that in Great Britain, vandalism may be the prime cause for the failure of tree planting revegetation schemes.

II) The solution of the problem?

The initial stages in revegetating a colliery spoil heap usually involves landscaping operations. These are necessary to lessen the visual impact of prominent spoil heaps being reclaimed for aesthetic reasons and to produce a suitably contoured surface where reclamation is intended to provide land for agricultural, amenity or building purposes. The reshaping of the heap is only the first and usually the least problematical, though most expensive operation in a reclamation programme.

The establishment and maintenance of a suitable cover of vegetation presents few problems if the surface of the spoil heap is covered with a sufficiently thick layer of soil to isolate the plants from the spoil, i.e. 20-25 cms. This is not usually a practical proposition because of the unavailability, and extremely high cost of transporting and spreading topsoil. This procedure can only be considered when large quantities of topsoil are available in very close proximity to a reclamation

site. Such opportunities may occur for example, when a motorway is built through an area of derelict spoil heaps. In the normal situation the vegetation must be established directly onto the spoil surface and the problems outlined in the literature may be encountered.

In early reclamation schemes the selection of ameliorants and plant species were made on an ad hoc basis. Frequently the initial results were encouraging but the rapid regression of what had initially appeared to be completely successful revegetation schemes indicated the need for a sound understanding of the factors involved in the poor growth of plants on colliery spoil heaps. A number of research groups were established and the present thesis represents an investigation undertaken in the Colliery Spoil Research and Advisory Group established at the University of York.

4. Introduction to the work presented in the present thesis

The aims of the present investigation were twofold.

a) To elucidate the extent and significance of seasonal variation of factors that might affect plant growth on colliery spoil.

b) To determine the availability and fate of naturally occurring and applied fertilizer nitrogen.

Both these topics had received very little attention in the literature; seasonal variation had not been considered and whilst it was usually thought necessary to add fertilizer nitrogen to spoil heaps during revegetation programmes (Doubleday 1972b), the fate and effectiveness of applied nitrogen was unknown.

A spoil heap called Mitchell's Main (situated near Barnsley in the West Riding of Yorkshire) was chosen for the investigation. This site was selected because it possessed characteristics typical of many spoil heaps in the West Riding of Yorkshire. Thus, Harding (1970) examined this site in winter 1967 and concluded that the spoil was moderately acid and contained very low levels of phosphorus and potassium together with high and locally toxic levels of aluminium, manganese and possibly iron, copper and zinc. Further, a field trial had been established on this site in 1967 (Chadwick 1973a), in which some ameliorants were applied to neutralize spoil acidity and others to supply nitrogen. Information had been obtained by Cooper (1973) on the initial effects of the applied ameliorants on the yield of vegetation. This field trial, therefore, represented a very suitable site at which to perform the present investigations because both acid and neutral, fertilized and unfertilized, replicated established plots of known history were available for sampling.

As a basis for the investigations, spoil samples were removed from the trial plots at very approximately monthly intervals over a period of two years. Samples were analysed for a number of plant nutrients, pH and enumerations of organisms responsible for nitrogen cycling were performed.

The plant nutrients included the elements aluminium, manganese, copper, zinc and iron, all of which were shown by Harding (1970) to occur at higher levels in Mitchell's Main spoil than in acid moorland soil and potassium for

which the reverse was true. The cations, calcium, magnesium and sodium were measured because high concentrations of these can result in spoil salinity problems (Knabe 1965). The three forms of mineral nitrogen, i.e. ammonium, nitrite and nitrate were also determined and the following groups of micro-organisms were enumerated; Fungi, Nitrosomonas, Nitrobacter, Pseudomonas and Denitrobacillus. In addition, spoil moisture determinations were performed and recordings of the spoil temperature were made.

Phosphorus determinations were not made because the standard methods of analysis for this nutrient in soils, do not give meaningful results for colliery spoils. The quantities of phosphorus extracted from colliery spoils in which plants were growing well or to which large quantities of phosphorus were added immediately prior to extraction were negligible (Chadwick, personal communication). This problem has been the subject of an investigation by Fitter (1972).

Sometime after the sampling programme at Mitchell's Main had been initiated it was decided that the investigation should be extended to include an examination of the initial effects of weathering reactions and ameliorant applications on plant growth. Such data could not be obtained at Mitchell's Main because the field trial had been established three years before the present sampling programme was begun in 1970. Further, Mitchell's Main spoil heap had been regraded in 1959, eight years before the field trial was laid down. A

suitable site for the new investigations was found at a spoil heap situated at Upton. One end of this large spoil heap was regraded (thus exposing unweathered spoil) in the summer of 1970 and a field trial was layed down immediately. The experimental design included the ameliorant treatments used at Mitchell's Main so that comparisons between the two sites could be made. With the exception of spoil temperature recordings, the determinations outlined for Mitchell's Main were repeated at the Upton site. The sampling programme at this second site was maintained for a period of twenty months.

Whilst the periodic sampling programmes at both sites provided useful information on the seasonal changes in the levels of plant nutrients, other experimental procedures had to be adopted to study the fate and recycling of nitrogen. To this end, two spoil incubation experiments and determinations of the dry weight yield and percentage nitrogen composition of the vegetation growing on the trial plots were performed.

5. Presentation of methods, results and discussions

The materials and methods used throughout the investigations are presented in Chapter Two. The results of the experimental work are detailed and discussed in the following two chapters. Chapter Three is concerned with the plant nutrients (other than mineral nitrogen), pH, spoil moisture and temperature recordings included in the repetitious sampling programmes. All the various determinations performed to elucidate the nitrogen status and recycling activity of the spoils are collected

together in Chapter Four. Overall assessment of the spoils at the two sites is made in Chapter Five and in Chapter Six, the results of the present investigations are discussed in relation to practical schemes for the revegetation of colliery spoil heaps.

CHAPTER TWO

CHAPTER TWO

MATERIALS AND METHODS

Introduction

The present chapter is divided into four sections.

Section I — Site and field trial description

Section II — Chemical and microbial determinations
In this section all the methods of analysis and enumeration used in the repeated sampling programme are described and discussed.

Section III — Incubation experiments
The experimental design of the two incubation experiments and the materials and methods used are given.

Section IV — Miscellaneous techniques
Materials and methods that were not appropriate for sections I-III are included in this section.

SECTION I SITE AND FIELD TRIAL DESCRIPTION

Mitchell's Main

The Mitchell's Main spoil heap covered approximately 40 acres of land at Wombwell (National Grid reference SE 394 043) and resulted from the mining of four coal seams, the sulphur content of which ranged from 1.6 to 2.5%.

When tipping ceased in 1956 the spoil heap was composed of two high, steep sided conical peaks. These were reduced to raised mounds connected by a saddle shaped area of spoil by regrading operations carried out in 1959. The regrading operations represented the first stage in a reclamation programme initiated by the West Riding County Council. In 1960 the spoil heap was limed (at 1 ton/acre), fertilized (400lbs/acre 7:7:7) and a grass mixture sown (30% Festuca rubra, 25% Lolium perenne, 15% Poa trivialis, 10% Agrostis tenuis, 10% Cynosurus cristatus and 10% Phleum pratense at 1cwt/acre). In 1962 a dressing of nitro-chalk was applied at 2.5 cwt/acre and the following year the whole site was planted with trees (Acer pseudoplatanus, Alnus glutinosa and A. incana, Quercus borealis, Robinia pseudoacacia and Pinus laricio).

The scheme appeared to be completely successful and won a Civic Trust Reclamation Award in 1964. By the autumn of 1964 the grass and trees had begun to die and large eroding patches had formed. The regression continued over the next few years and the south-facing slopes became almost devoid of vegetation.

In spring 1967 a field trial was established on the south-facing saddle shaped area between the two mounds. The details of this field trial are given in Table 2.1.

The main plots were arranged along the same contour with the sub-plots running down the side of the spoil heap (a gradient of approximately 1:5). The grass was sown and the trees were planted in March, 1967.

The surface layers of spoil on the trial plots were composed both of burnt and unburnt material, with the latter predominating.

When the present investigations were begun early in 1970, the original sub-divisions of the main plots were not obvious because many of the trees had died and the sown grasses had spread to form a patchy cover over the whole of the main plots. The division of main plots into sub-plots was therefore ignored throughout the present investigations.

Upton

The tipping complex at Upton (National Grid reference SE 484 143) was composed of a number of irregular spoil mounds. The spoil resulted from the mining of four seams of coal with sulphur contents ranging from 1.6 to 2.2%.

The west facing end of the tip complex was regraded in the summer of 1970 to produce a slope of maximum gradient 1:5. During the regrading operations the sub-surface layers were found to be burning and after regrading, the spoil surface was composed of approximately equal proportions of burnt and unburnt material.

A field trial was established immediately after the spoil moving operations were completed in August 1970. The details of the field trial are given in Table 2.2.

Table 2.1. Field trial design at Mitchell's Main.

	MAIN PLOTS																	
	Block I						Block II						Block III					
	3	2	0	1	5	4	4	2	5	3	0	1	5	4	0	2	1	3
Sub-plots	d	d	d	a	a	e	cb	b	0	a	0	0	0	e	e	ca	b	ca
	o	a	o	o	b	d	a	ca	b	d	cb	d	d	a	b	e	d	0
	cb	o	a	d	d	o	b	d	e	b	a	e	b	oa	a	d	cb	d
	a	b	cb	e	e	b	d	o	cb	ca	b	b	e	d	o	a	e	b
	e	ca	e	ca	cb	ca	e	a	a	o	d	a	a	o	d	o	o	a
	b	e	b	b	o	a	o	e	d	e	e	ca	cb	b	cb	b	a	e

NOTATION

Main Plots

0 Control

1 Shoddy 4 tons/acre

2 Sewage-sludge 20 tons/acre

3 Limestone 8 tons/acre in 1 application

4 Limestone 8 tons/acre in 2 applications at 6 month intervals

5 Limestone 8 tons/acre in 4 applications at 3 month intervals

Sub-plots

0 Control

a Grass at 1oz per square yard

40%	Festuca rubra (Creeping red fescue)	by weight
40%	Festuca rubra (red fescue)	
20%	Agrostis tenuis	

b Betula pendula 12"-18" 4 per sub-plot

c Alnus a A.glutinosa " " "

b A.incana 30"-36" " " "

d Robinia pseudoacacia 18"-24" " "

e Populus alba 18"-24" " " "

The sub-plots measured 3 yards along the contour and 4 yards down the slope and were contiquous with each main plot.

Main plots were separated by guard rows 2½ yards wide

Table 2.2 Field trial design at Upton

Block I

(3) (1) 7 9 (0) 8 5 4 (2) 6 10

Block II

8 5 (0) 7 9 6 10 4 (3) (2) (1)

Block III

4 (3) 5 6 8 (1) 10 7 9 (0) (2)

All plots were arranged along the same contour and each measured 2yds along the contour and 4yds down the slope.

NOTATION

(0) Control

(1) Shoddy 4 tons/acre

(2) Sewage-sludge 20 tons/acre

(3) Limestone 8 tons/acre

4 Limestone + shoddy at above rates

5 Limestone + sewage-sludge at above rates

6 Compound fertilizer 8cwts/acre ('Saingral' 17.17.17)

7 Limestone + compound fertilizer at above rates

8 1" topsoil + treatment 7

9 4" topsoil " "

10 8" topsoil " "

All plots were sown with the Mitchell's Main grass seed mixture at loz/square yard.

Only treatments enclosed in brackets were included in the present investigations.

SECTION II CHEMICAL AND MICROBIAL DETERMINATIONS

A diagramatic representation of the collection and subsequent determinations performed on each spoil sample in the repeated sampling programme is shown in Fig. 2.1. The individual determinations are described and the reasons for their adoption are discussed in the following pages.

1. Collection of spoil samples

Mitchell's Main

Spoil samples were collected from the trial plots on the following dates:-

Exp.No.1	16th March, 1970	9	11th May, 1971
2	4th May, 1970	10	7th June, 1971
3	14th July, 1970	11	22nd July, 1971
4	19th November, 1970	12	5th August, 1971
5	11th January, 1971	13	17th September, 1971
6	26th January, 1971	14	3rd November, 1971
7	25th February, 1971	15	29th November, 1971
8	16th March, 1971	16	1st February, 1972

At each sampling time, a single bulked sample was prepared for each of the three field replicates of the shoddy, sewage-sludge, limestone (treatment 3 only) and control plots. Each sample was obtained by bulking six spoil cores taken at random from within the whole of a main plot. Individual spoil cores of 7.5cm diameter and 15cm depth were taken with a fern trowel after removal of the above ground vegetation. The trowel was cleaned with 70% ethanol before each core

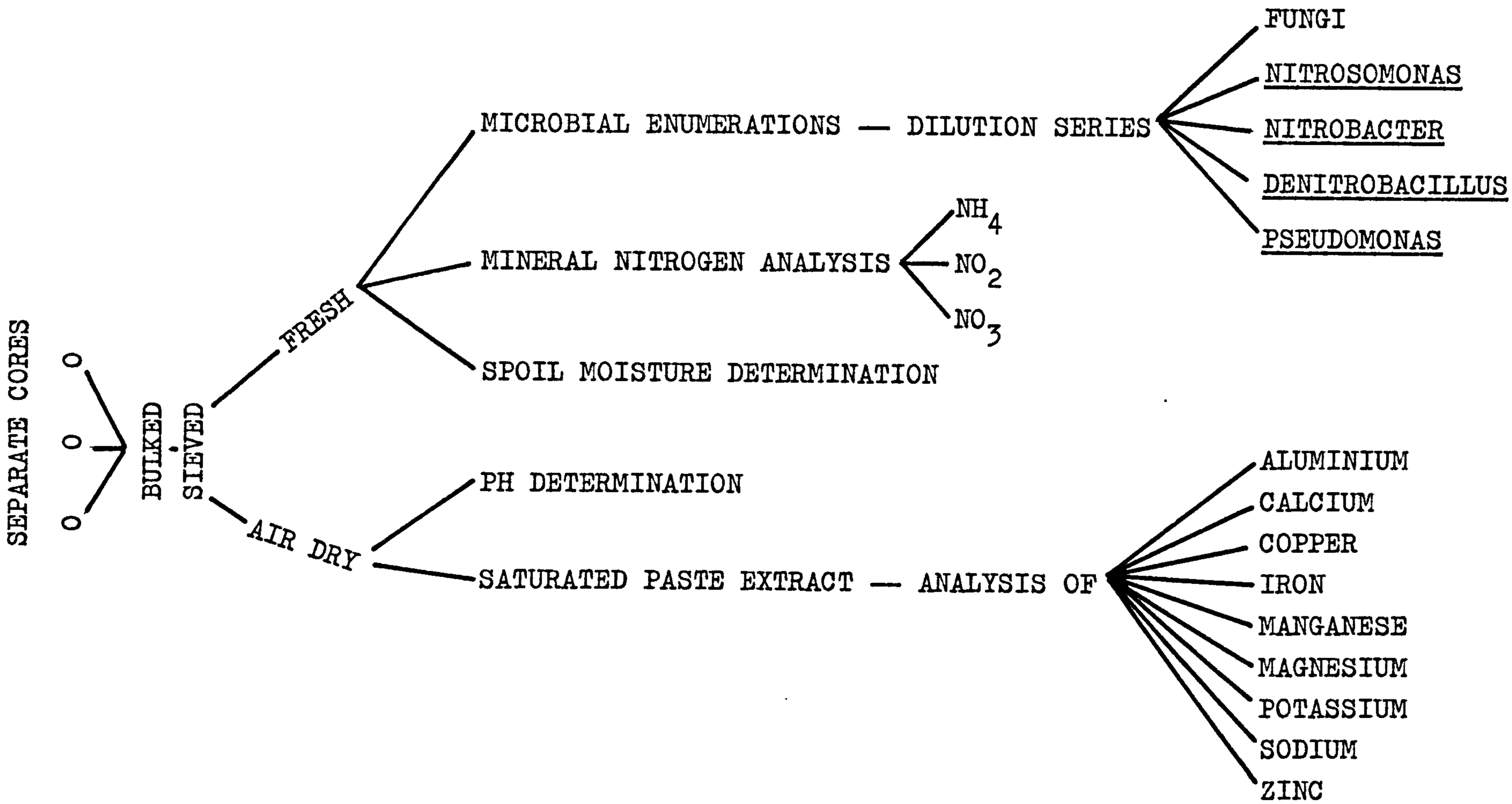


Fig. 2.1. Schematic representation of procedures.

was taken.

The cores forming a single bulked sample were placed together in a sterile, heavy duty (250 gauge) clear polythene bag that was sealed with an elastic band. (The polythene bags were sterilized by exposure to an ultra-violet light source for 24 hours). Care was taken to keep the individual cores of spoil intact during transportation to, and storage at 4°C in the laboratory. The period of storage in the laboratory was never greater than twelve hours and usually much less.

Upton

Spoil samples were collected from the trial plots on the following dates:-

Exp No. 0	12th August, 1970 (before ameliorants applied)	5	21st April, 1971
1	19th August, 1970	6	22nd June, 1971
2	16th September, 1970	7	7th August, 1971
3	9th December, 1970	8	14th October, 1971
4	10th February, 1971	9	10th January, 1972

At each sampling time a bulked sample was prepared for each of the three field replicates of the shoddy, sewage-sludge, limestone and control plots (treatments 0, 1, 2 and 3 in Table 2.2.).

Each sample was obtained by bulking three spoil cores taken at random from within each plot. The cores were collected, transported and stored in exactly the manner described for Mitchell's Main.

Discussion of the sampling regime adopted

Consideration of the time and amount of equipment

needed to carry out all the required determinations on each sample lead to the conclusion that the maximum number of samples that could be collected on any occasion was twelve. Since the number of main plots at Mitchell's Main was eighteen, all could not be sampled at once. Cooper (1973) demonstrated that at this site, all three regimes of limestone applications (main plots 3, 4 & 5 in Table 2.1) were insignificantly different with respect to pH and yield of vegetation and it was therefore decided to omit main plots 4 & 5 (those receiving the split applications of limestone) from the investigations.

Sampling at Upton was restricted to the plots common to both sites.

Because only a single sample could be taken from each plot, attempts were made to ensure that this sample was representative of the whole area of the plot. Thus, six cores of spoil were bulked at Mitchell's Main and three at Upton. In both cases the number of cores taken to form the bulked sample was considered to be the maximum that could be taken without damaging the plots.

2. Initial preparation of samples

Immediately before the determinations were to be performed, the polythene bag containing the bulked sample was removed from the cold room at $4^{o}C$ and the individual cores of spoil broken down and thoroughly mixed together by passage through a stainless steel, 1cm round holed sieve. The less than 1cm fraction was collected in a stainless steel tray. Both the sieve and collection

tray were sterilized before each sample was sieved by swabbing with 70% ethanol and flaming.

The less than 1cm material was divided into two parts, one was used in the fresh state for the preparation of a dilution series, mineral nitrogen and spoil moisture determinations, whilst the other was air dried and subsequently used for pH and plant nutrient (other than mineral nitrogen) determinations.

When soils are analysed for plant nutrients the determinations are usually performed on the less than 2mm fraction. It is assumed that this separates the "chemically inactive" larger fraction that is composed of silica grains from the "chemically active" smaller grains. Chadwick (1973b) pointed out that this was a meaningless procedure for colliery spoil because large particles of spoil are not composed of silica and may rapidly break down to form "chemically active" material. All determinations in the present investigations were therefore performed on the less than 1cm fraction of spoil.

3. Air drying of spoil samples

Because air dry soil samples are much easier to handle and store than fresh ones, many workers routinely dry their samples before analysis. Jackson (1962) states that many determinations are not significantly affected by complete air drying for storage purposes but he includes iron, phosphorus, potassium and nitrate as examples of determinations that must be made on fresh material. Other authors have reported that

considerable changes in the levels of ammonium and nitrate occur on drying and storage of samples and suggest that analysis of fresh material is preferable (Allen & Grimshaw 1962; Cooke & Cunningham 1958; Gasser 1961).

It was decided that analysis of mineral nitrogen would be performed on fresh material and the effect of air drying the spoil samples before analysis would be investigated before a decision was made for the other plant nutrients and pH. The investigation was initially carried out on a bulked spoil sample taken at Mitchell's Main in March 1970 and was repeated for an Upton sample in August, 1970. The experimental technique, and results were similar in both cases and will be described together.

At both sites a single bulked sample was obtained by pooling fifty cores of spoil taken at random from the area immediately surrounding the field trial plots. Each core was 4 cms in diameter and 15 cms deep and all were mixed together by passage through a 1 cm round holed, stainless steel sieve. The less than 1 cm fraction that was used in the determinations was divided into two parts. Duplicate saturated paste extracts (see later) were prepared immediately for one part whilst the other was air dried before the extracts were made. The saturated paste extracts prepared from both the fresh, and air dried spoil samples were analysed for a number of elements and pH determinations were performed (by the methods described later). The

results are given in Table 2.3.

The results show that air drying colliery spoil before analysis increased the concentration of most of the elements studied. With the exception of potassium, however, the magnitude of the increases was small. The pH changes induced by air drying were also small. It was, therefore, decided to use air dry material for the determination of pH and all plant nutrients except mineral nitrogen.

Although the levels of potassium were much higher in air dry than fresh spoil, this was not thought to be particularly important because the surface layers of spoil heaps often become very dry in the summer months. Further, Harding (1970) found that the levels of potassium that occurred in plants grown in spoil were well related to determinations performed on air dry material.

The following method of air drying was employed.

The spoil sample was spread to a maximum depth of 2 cm over a polythene sheet lining a shallow polypropylene tray. The tray was placed in a drying cabinet maintained at 25°C for four days after which all plant material was removed before the dry spoil was transferred to a polythene bag for storage.

4. The determination of spoil pH

Several factors affect the pH reading given by a soil:liquid suspension. Of these the dilution level and salt concentration are the most important.

Table 2.3. Comparison of various determinations made on fresh and air dry spoil samples.

	Mitchell's Main		
	Fresh (14% Moisture)	Air dry (6% moisture)	% change
Aluminium	38.0	38.0	0
Manganese	17.19	17.49	+ 1.3
Copper	0.43	0.61	+41.8
Zinc	0.70	0.80	+14.2
Iron	5.37	5.62	+ 4.6
Sodium	6.90	7.01	+ 1.5
Potassium	1.10	2.66	+141.8
Calcium	204.80	211.20	+ 3.1
Magnesium	144.96	146.23	+ 0.8
pH	3.02	2.94	- 2.6
% moisture at saturation	36	36	0

	Upton		
	Fresh (13% moisture)	Air dry (5% moisture)	% change
Aluminium	Too low to detect		
Manganese	2.07	2.39	+15.4
Copper	Too low to detect		
Zinc	0.07	0.07	0
Iron	Too low to detect		
Sodium	1029.1	1033.4	+ 0.3
Potassium	10.14	16.14	+59.1
Calcium	651.13	669.11	+ 2.7
Magnesium	157.10	161.97	+ 3.0
pH	5.41	5.86	+ 8.3
% moisture at saturation	38	37	- 2.6

All values are the mean of two replicate determinations. The values for the elements are expressed in ppm in the saturated paste extract

Soil dilution: the greater the dilution the higher the pH value recorded. This is overcome by measuring the pH at a fairly wide soil:water ratio by placing the calomel electrode in the supernatant and the glass electrode in the settled suspension (Seatz & Peterson 1964). Soluble salts: as the salt concentration increases, the pH falls. This phenomenon can be eliminated by the use of 0.01 M $CaCl_2$ in place of water as the diluent. It is assumed that the salt concentration in the sample is negligible with respect to the amount of salt added in the diluent. pH values obtained using 0.01 M $CaCl_2$ as diluent are usually between 1.0 and 0.5 pH lower than corresponding values when water is used (Seatz & Peterson 1964). The method for determining spoil pH used this background salt solution because colliery spoil may contain high levels of soluble salts (Chadwick 1973b). The method used is given below.

Duplicate 10g quantities of air dry spoil were placed into 50 ml capacity glass beakers containing 20 ml of 0.01 M $CaCl_2$ solution. The suspension was stirred intermitently for thirty minutes and allowed to stand undisturbed for a further thirty minutes.

The measurement of pH was made electrometrically using an Electronic Instruments Ltd. (EIL), model 23A direct reading pH meter.

The position of the electrodes was adjusted so that the glass electrode was partly immersed in the settled suspension and the calomel electrode in the supernatant. After switching the meter to the read position, thirty seconds equilibration time was

allowed before the reading was taken.

5. Saturated paste extract of colliery spoil

Chadwick (1973b) demonstrated that conventional methods of soil analysis based upon an extracting solution coming into rapid equilibrium with the solid phase were inappropriate for use with colliery spoil, and suggested that the determination of water soluble elements in a saturation extract provided a better guide to the suitability of colliery spoil for plant growth. Using this method of extraction, Harding (1970) found that a relationship could be established between the levels of certain nutrients in spoil and in plants grown on spoil.

Saturation paste extracts have been used in the present investigations and the following method of preparation was used.

Duplicate 250g quantities of air dry spoil were placed in 1 l glass beakers and mixed with a spatula whilst deionised water was added until saturation point was reached.

"At saturation the soil (spoil) paste glistens as it reflects light, flows slightly when the container is tipped, and slides freely and cleanly off a spatula for all soils except those of a high clay content" (Bower & Wilcox 1965).

The prepared saturated paste was covered with "Parafilm" to prevent evaporation and contamination and left for four hours at room temperature.

To obtain the extract, the saturated paste was

transferred to a Buchner funnel fitted with a Whatman no. 42 filter paper and a vacuum applied. Any cloudiness remaining in the extract was removed by a second filtration again using a Whatman no. 42 filter paper. Approximately 30 ml of extract was obtained, and this was stored in stoppered polythene bottles at 4°C until analysis was performed.

6. <u>The determination of aluminium, calcium, copper, iron, manganese, magnesium, potassium, sodium and zinc</u>.

The determination of all the above elements in the saturated paste extract were performed by atomic absorption spectrophotometry using an Evans Electroselenium Ltd. (EEL) model 240 atomic absorption spectrophotometer.

Relevant procedural details are given in Table 2.4.

Although atomic absorption spectrophotometry is a highly specific analytical method, interference problems can sometimes be encountered. Thus, when using the relatively cool air/acetylene flame, iron, aluminium and phosphorus can interfere with the determination of calcium, and aluminium and phosphorus with that of magnesium. The possibility of these interferences was overcome in the present investigations by the use of the higher temperature of the nitrous oxide/acetylene flame for the calcium and magnesium determinations.

Large quantities (of the order of 1000ppm) of both iron and phosphate in solution can interfere with the determination of potassium. This can be overcome by the addition of lanthanum chloride to the solution being analysed.

Table 2.4. Procedural details for atomic absorption spectrophotometry

Element	Flame	λ	Lamp cathode	Slitwidth	Lamp current mA	Resolution ppm (without scale expansion)	Comments
Aluminium	Nitrous Oxide/ Acetylene	396.2	EEL Aluminium	2	6.5	2	Both nitrous oxide and acetylene pressures are increased to produce a large flame with a luminous blue cone.
Calcium	"	422.7	EEL Calcium/ Magnesium	2	5.0	0.3	
Copper	Air/ Acetylene	324.8	EEL Brass	1	7.5	0.05	
Iron	"	248.3	EEL Iron	2 or 3	7.5	0.1	
Magnesium	Nitrous Oxide/ Acetylene	285.2	EEL Calcium/ Magnesium	2	5.0	0.01	
Manganese	Air/ Acetylene	280.0	EEL Manganese	2 or 3	6.0	0.02	
Potassium	"	766.5	EEL Potassium	6	7.5	0.02	It is necessary to fit a red filter in the optical beam.
Sodium	"	589.0	EEL	2	7.5	0.01	Care has to be taken to distinguish between 589.0 and the slightly less sensitive peak at 589.6
Zinc	"	213.9	EEL	2 or 3	5.0	0.05	The acetylene pressure is reduced until a clear blue flame is produced.

This was not thought to be necessary in the present investigations because the levels of iron were never very high (the highest value recorded being 16ppm) and saturated paste extracts of spoil do not usually contain more than trace quantities of phosphate (Harding 1970).

7. Spoil moisture determinations

Duplicate 100g quantities of fresh spoil were added to dry weighed beakers that were placed in an oven at 105°C for 24 hours. The beakers were transferred from the oven to a desiccator and allowed to cool before being reweighed. The percentage moisture content was then calculated.

8. Extraction and estimation of mineral nitrogen

Dennington (personal communication) investigated the suitability of a number of analytical techniques for the determination of mineral nitrogen in spoil and concluded that the most satisfactory procedure was that suggested by Bremner (1965a). This technique has been used in the present investigations and is outlined below.

A 20g quantity of fresh spoil was added to a 125ml capacity medical flat bottle containing 100ml of 2N potassium chloride solution. The suspension was shaken for 1 hour in a "Griffin" horizontal shaker (distance 4cm, frequency $200m^{-1}$) before filtration through a Whatman no. 1 filter paper. The extract was collected in a stoppered polythene bottle and stored at 4°C until the analysis was performed. The storage period was never greater than forty-eight hours.

The quantities of ammonium, nitrite and nitrate in 20ml aliquots of the potassium chloride extracts were estimated by the magnesium oxide/Devarda's alloy steam distillation technique (Bremner 1965a). Duplicate extractions and estimations were performed.

9. Microbial enumerations

Estimating numerically the populations of soil micro organisms presents difficulties not commonly encountered when determining chemical or physical features of a soil. These difficulties arise because large changes in microbial populations can occur as a result of sampling, carriage to and storage of the sample in the laboratory prior to enumeration. Further, no really satisfactory method of enumeration has been developed and the estimate of the population size often depends upon the method of enumeration used. Because of the special difficulties associated with microbial enumerations, each of the stages involved in the enumerations will be described and discussed under a separate heading.

i) Sampling, storage and sieving of samples prior to enumeration

The methods used for sampling, storage and sieving have already been described (sections 1 & 2) but the underlying microbial aspects were not discussed.

The prevention of unnecessary contamination of samples was important and it was for this reason that all the equipment used to handle the spoil samples was

sterilized. Polythene bags were used to transport and store the samples because of the ability of this material to allow gaseous exchanges without loss of moisture.

Although it is probable that the average generation time of members of the soil microflora is long, and hence rapid proliferation unlikely, it is obvious that the time interval between sampling and the beginning of the enumeration should be as small as possible. Enumerations were therefore usually begun immediately on return to the laboratory, which meant that the time interval was approximately two hours. It was not always possible, however, to do this, and in cases where a delay was inevitable, the samples had to be stored. It is generally accepted that storage at $4^{o}C$ is the most suitable and this was the procedure adopted. The storage period was never greater than twelve hours.

All enumerations were performed on the less than 1 cm fraction of the spoil. Attempts were initially made to resieve the spoil through a 2mm sieve but this proved to be impossible because of the presence of a high proportion of greater than 2mm material in the samples.

ii) <u>Preparation of the dilution series</u>

Enumeration of the organisms included in the present investigations was achieved either by plate counts (where the organisms grow to form distinct colonies on solid media) or "Most Probable Number" (MPN) determinations (where statistical interpretation is made of presence and absence data).

A common dilution series was prepared for both types of enumeration and the details are now given and discussed.

10g of fresh spoil was transferred to a 200 ml. capacity medical flat bottle containing 90 ml of sterile quarter strength Ringer's solution. The bottle was securely capped with a plastic top and the suspension shaken for 15 minutes in a "Griffin" horizontal shaker (throw = 4cm, frequency = 200 movements per minute).

The tip of a 10 ml capacity, single mark, wide mouthed, blow-out pipette was inserted into the suspension and filled and emptied three times before a 10 ml quantity was removed and transferred to a 100 ml capacity medical flat bottle containing 30 ml of sterile quarter-strength Ringer's solution. The transference was made by first touching the tip of the pipette against the bottle wall and then blowing out, after a three second interval the pipette was again blown out. A plastic cap was screwed onto the bottle and the contents mixed by hand shaking for 30 seconds. The suspension produced represented a 1/40 dilution of the original spoil sample.

Further four-fold dilutions were performed in the manner described above until the required dilution level was achieved.

The dilution factor employed in an enumeration is important because it affects the precision; the smaller the factor, the greater the precision. A ten fold factor is commonly employed but Finney (1952) states

That a two or four-fold factor is preferable. Practical considerations influenced the choice of dilution factor and a four-fold dilution series was prepared from an initial tenfold dilution of the original spoil sample.

The use of a 10g quantity of soil for the preparation of the initial dilution is often recommended (Alexander 1965a; Clark 1965; Menzies 1965). Cooper (1973) investigated the effect of the quantity of spoil used to make the initial dilution of a four-fold dilution series on the accuracy of a microbial estimation by preparing a number of dilution series from 3, 10, 20 and 40g samples of spoil. All dilution series gave rise to insignificantly different population estimates.

The quarter strength Ringer's solution used as diluent was prepared from British Drug House Chemical Company (BDH) tablets and was sterilized by autoclaving at 10psi for 10 minutes. Plastic tops were used on all dilution bottles because Withell (1942) reported that some batches of Ringer's solution became toxic after autoclaving in bottles fitted with rubber lined caps.

Brierley et al. (1927) found that maximum dispersion of microorganisms was obtained after shaking for approximately 20 minutes. The slightly shorter period of 15 minutes was used for spoil because this material disintegrated very quickly on shaking.

Wide mouthed pipettes were used to prevent the

blockage of the orifice by the spoil particles in the suspensions. Cotton-wool filter plugs were placed in the neck of each pipette to prevent contamination during the preparation of the dilution series. The pipettes were sterilized in metal containers by autoclaving at 10psi for 10 minutes and were dried in an oven at 105°C for three hours.

iii) Culture media

The media used for all determinations except fungi, were those suggested by the N.A.A.S. (1963)

a) Fungi

Glucose	5.00g	Reference: Modified
Yeast Extract (Difco Bacto)	2.00g	from (antibiotic substances omitted)
$NaNO_3$	1.00g	Schmitt-Henner &
$MgSO_4.7H_2O$	0.50g	Williams (1958)
Agar (Oxoid No. 3)	20.00g	
Oxgall (Difco Bacto)	1.00g	
$CH_3.CH_2.COONa$ (Sodium propionate)	1.00g	
Deionised H_2O	to 1000 ml.	

The ingredients were placed in a large conical flask and heated at 100°C for 10 minutes, (this served to melt the agar) and then allowed to cool to 55°C. At this time the medium was thoroughly stirred before being transferred to 200 ml capacity medical flat bottles for sterilization by autoclaving at 10psi for 10 minutes.

Preparation of plates

The agar in the medical flat bottles was melted by placing the bottles in a steam bath for 20 minutes. The melted agar was kept at 50°C in a water bath until the plates were about to be poured.

1 ml of quantities of inoculum were added to 9 cm diameter plastic petri-dishes and 15 ml of the agar at 50°C added. The liquid agar and the inoculum were mixed together by moving the petri-dishes in alternate North/South and East/West directions - five times in each direction. When set, the plates were incubated upside down in stacks of not more than three at 25°C for three days. The fungal colonies that developed were counted and marked before the plates were re-incubated for a further two days under the same conditions. The plates were re-examined and any new colonies that have appeared were counted.

b) <u>Nitrosomonas</u>

NaCl	0.30g
$MgSO_4.7H_2O$	0.14g
$FeSO_4.7H_2O$	0.03g
$(NH_4)_2SO_4$	0.66g
$CaCO_3$	10.00g
KH_2PO_4	10 ml of $^M/_{10}$ solution
Deionised H_2O	1000 ml

The K_2HPO_4 solution was prepared by adding 2.723g of KH_2PO_4 to 200 ml of deionised water and boiling for 30 minutes. After cooling the solution was made up to 200 ml.

The medium was dispensed with a "Jencons Zipette" in 2.5 ml quantities into 120 x 16 mm pyrex rimless test-tubes, capped with "Oxoid" 17 mm aluminium caps and sterilized by heating in an autoclave at 15 psi for 15 minutes.

During dispensation the chalk was kept in suspension by bubbling air through the medium.

When inoculated, tubes of the medium were incubated at 27°C for 14 days in the dark. After this time five drops of modified Griess-Ilosvay's reagents (Supplied by British Drug House Chemicals Ltd.)I and II were added. Tubes in which a red colouration developed were scored as positive. To all tubes that did not produce a red colouration, 0.1g of powdered zinc was added. Tubes that produce a red colouration after this treatment were also scored as positive.

The test for the presence or absence of Nitrosomonas is based on the fact that members of this genus can convert the ammonium in the medium to nitrite. In the presence of nitrite Griess Ilosvay's reagent produces a red compound.

Nitrobacter species can convert nitrite to nitrate and since these organisms may have been present in the inoculum, all negative results had to be further examined for the presence of nitrate. This was done by adding powdered zinc which reduced any nitrate present to nitrite which was thus detected.

c) Nitrobacter

The preparation, inoculation and incubation of this

medium was the same as that described above for Nitrosomonas with the exception that 0.175g of sodium nitrite was substituted for the ammonium sulphate.

The presence of nitrate and the absence of nitrite after incubation indicated the presence of Nitrobacter in the cultures.

d) Denitrobacillus

Glycerol	10.00g
Peptone (Fisons bacteriological)	10.00g
K_2HPO_4	1.00g
$MgSO_4.7H_2O$	0.50g
KNO_3	10.00g
Deionised H_2O	1000 ml

5 ml quantities of this medium were dispensed with a "Jencons Zipette" into 120 x 16 mm pyrex, rimless test-tubes capped with 17 mm "Oxoid" aluminium caps. Each test-tube contains a 30 x 5 mm inverted Durham tube. The medium was sterilized by autoclaving at 15 psi for 15 minutes.

After inoculation and incubation at 27°C in the dark for 7 days, the tubes were examined for the presence of a gas bubble in the Durham tube. Tubes in which at least the concavity of the Durham tube was filled with gas were recorded as positive.

Denitrobacillus spp. can, in the absence of oxygen, use nitrate as a terminal electron acceptor. The nitrate becomes reduced to nitrogen gas. The depth of culture was sufficiently great to allow anaerobic

conditions to form, at least near the bottom of the tube. The nitrogen gas formed was caught in the Durham tube and revealed the presence of Denitrobacillus. Only tubes in which at least the concavity of the Durham tube was filled with a bubble were scored because sometimes small air bubbles formed as a result of autoclaving.

e) Pseudomonas

Glycerol	3.60g
KH_2PO_4	0.50g
$CaCl_2$ anhydrous	0.10g
NaCl	0.10g
$MgSO_4.7H_2O$	0.30g
$FeCl_3$	Trace
Deionised H_2O	1000 ml

This medium was dispensed, sterilized, inoculated, incubated and scored in exactly the way described above for Denitrobacillus.

iv) Inoculation and replication of cultures

All tubes and plates of media were inoculated with 1 ml aliquots of the diluted spoil suspension using a "Summitt" syringe pipette loaded with a disposable Pasteur pipette. A new pipette was used for each dilution level.

Before the first aliquot of inoculum was removed from a dilution bottle, the pipette was filled and emptied three times. Immediately prior to the removal of an aliquot, the spoil suspension was shaken by hand, for three seconds to resuspend any settled material.

The Pasteur pipettes were sterilized in the manner described previously for the pipettes used to prepare the dilution series. The tips of the pipettes used to remove inoculum from the lowest dilution level (1/10) were broken off before sterilization to prevent blockage.

The use of a syringe pipette greatly increased the speed and accuracy with which the large numbers of cultures could be inoculated.

The number of levels of a dilution series from which aliquots are taken as inoculum is important for both plate and MPN estimations. For the plate counts of fungi, the dilution levels sampled had to be such that some plates were produced on which distinct colonies occurred. Initially, six levels were examined for samples from both sites, but this was reduced to four once the likely number could be predicted from past results.

For the MPN determinations it was desirable that the dilutions examined should range from one where all, or almost all, the replicate cultures were positive (i.e. some organisms present) to one where the reverse was true, with as many intermediate stages as possible. To achieve this, six dilution levels were initially examined for all organisms, at both sites. The choice of which six dilutions to choose was at first, arbitrary, but as sampling proceeded and estimates of the numbers were obtained, the selection became more precise.

In the case of the determinations of Nitrobacter

at Mitchell's Main, the number of levels examined was reduced to four (1/10, 1/40, 1/160, 1/640) because of the very low numbers present.

As the number of replicate cultures at each dilution level increases, so does the precision of the enumeration. The numbers of replicates used in practice had to be a compromise between precision and practicability and the following scheme was adopted. For the plate counts, four replicate plates were prepared for each dilution level examined. For the MPN determinations, two replicate tubes were used until experiment 8 (16th March 1971) at Mitchell's Main, and experiment 4 (10th February 1971) at Upton, when the number was increased to three.

v) Calculation of results

Calculations had to be performed to obtain the estimates of the population sizes of the micro-organisms in the original spoil samples. Different methods were needed for the plate count of the fungi and the MPN determinations used for all the other organisms.

a) Plate counts

The number of organisms in the original sample could be obtained by multiplying the mean number of colonies present on plates prepared from a particular dilution by the appropriate dilution factor. Because the number of colonies that developed at more than one dilution level could be counted, more than one estimate could have been produced, and these may have differed greatly. Such discrepancies arise because of competitive inhibition when the number of organisms inoculated into a

plate is high and random sampling errors when low. In the present investigations, the estimate produced was based on the results of a single dilution level. The level used as the basis for the enumeration was that at which the mean number of colonies per plate (i.e. the mean of four replicates) most closely approximated to thirty. This number was chosen because it was not so high that slower growing species were swamped by faster growing ones, or so low that random sampling errors induced large count differences between the replicates. The number of colonies on which the population estimate was made generally lay between the limit of fifteen and forty-five.

b) MPN determinations

Unlike plate counts, MPN determinations only yield estimates of population sizes after statistical interpretation. The basis of the statistical procedure is the solution of the "maximum likelihood equation" given by Halvorson & Ziegler (1933). Various authors have solved this in different ways, and for certain experimental designs the MPN values can be read directly from prepared tables (Finney 1951; Fisher & Yates 1963; Taylor 1962). None of the prepared tables were particularly suitable for the design employed in the present investigations and a computer program was developed to solve the "maximum likelihood equation" by the method suggested by Finney (1951). Details of this program are given in the Appendix.

The MPN could not be calculated for occasional sets of data where all the cultures were of similar reaction.

When the reactions were all positive, the MPN for a set of comparable data that contained a single negative value at the highest dilution level was calculated, and the unknown value was stated to be "greater than" this MPN. A similar procedure was adopted for sets of data containing only negative responses. In these cases, the values are quoted as "less than" those given by comparable sets of data containing a single positive reaction at the lowest dilution level.

Where the results of the microbial determinations are shown graphically (Chapter Four), the plotted values are the mean of the three field replicates. Where the MPN for one or more of the field replicates could not be estimated, the plotted value is based on the mean of the three field replicates using the "greater than" or "less than" estimates as real values. In such cases the true mean is stated to be "greater than" or "less than" the value plotted. Fortunately the situation did not arise in which one field replicate could only be stated to be "less than" one value whilst another was "greater than" a second value.

Although the computer program was written to include calculation of the 95% confidence limits of the MPN, these values could not meaningfully be included in the graphical presentation or statistical interpretation of results and have not, therefore, been referred to.

SECTION III INCUBATION EXPERIMENTS

1. Incubation experiment I

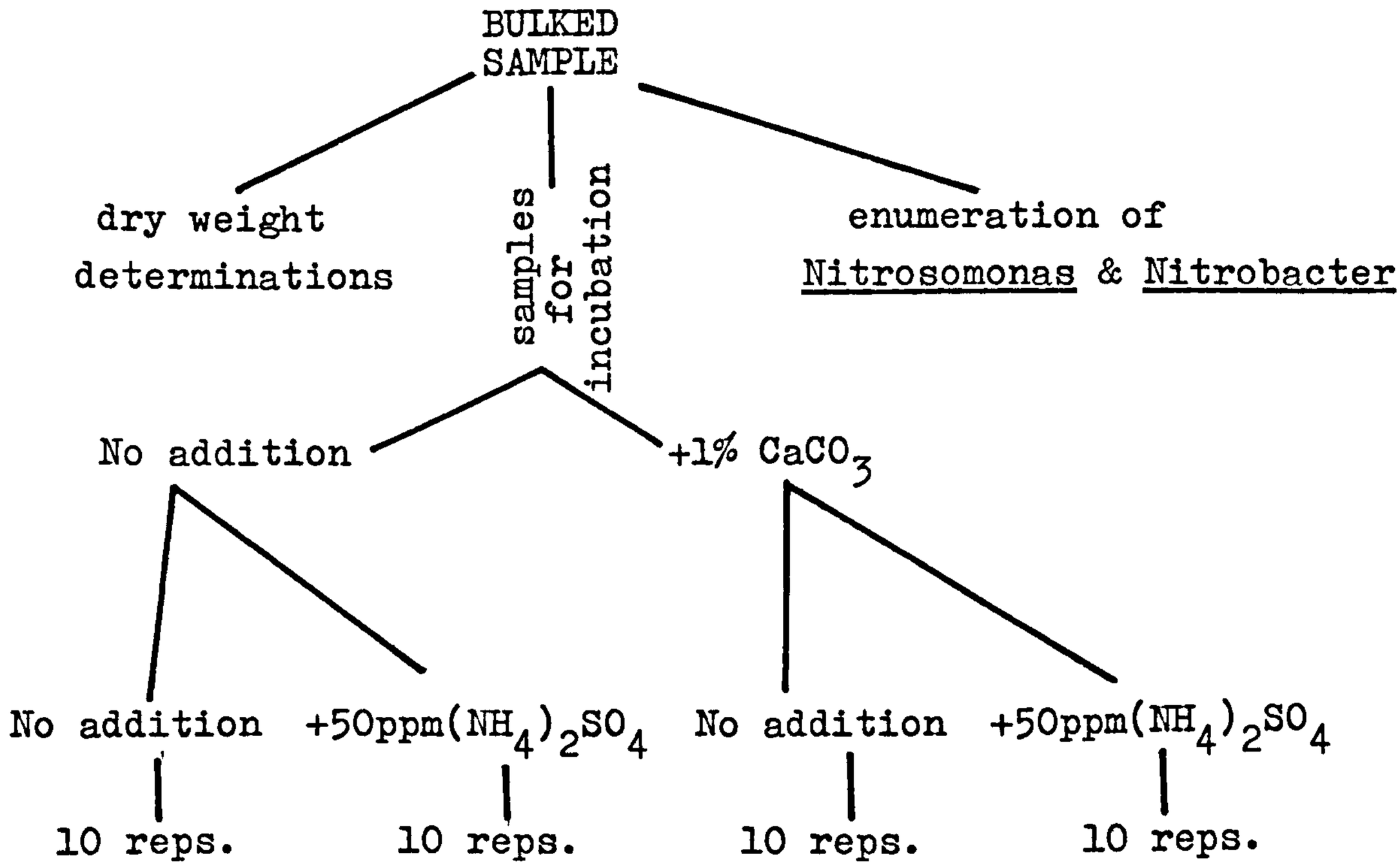
i) Sampling procedure

The trial plots at Mitchell's Main and Upton were sampled on 23rd March, 1972 and 27th March, 1972 in exactly the manner described previously. On return to the laboratory all eighteen spoil cores representing one main plot type (six cores from each of the three field replicates) were bulked and thoroughly mixed by sieving through a 1cm round holed sieve. Each treatment type was, therefore, represented by a single spoil sample.

Samples of two soil types were also collected. One was from a grassy area on an acid upland moor at Osmotherley (National Grid reference SE476 967) and the other from the edge of a Beech copse at Heslington (National Grid reference SE625 503) that one year previously had been rotovated and sown down to Rye grass. The Osmotherley sample was collected on 25th March, 1972 and the Heslington one on 27th March, 1972. A single bulked sample was prepared for each soil type by pooling and mixing ten randomly taken soil cores.

ii) Experimental design

All spoil and soil samples were treated in the manner shown diagramatically in Fig. 2.2.



2 reps. removed after 0, 10, 20 and 40 days for analysis for NH_4, NO_2 & NO_3

2 reps. removed at time 0 for pH determinations

Fig. 2.2. Experimental design of incubation experiment I

iii) Procedure

The determinations of spoil moisture and enumerations of Nitrosomonas and Nitrobacter were begun immediately on return to the laboratory. When these had been completed each sample was approximately halved. One half was weighed before being spread out over a stainless steel tray previously sterilized by swabbing with 70% ethanol and flaming. Enough 100 mesh calcium carbonate was mixed with the spoil to make the total amount present equal to 1% of the total fresh weight of the spoil. This very roughly approximated to liming the spoil at the rate

of 10 tons per acre.

Both the limed and the unlimed parts of the spoil sample were placed in sealed polythene bags and stored at $4^{o}C$ for approximately twenty-four hours until the dry weight determinations had been completed.

When this had been done, the two samples were removed from the cold room and 10g portions of each (on a dry weight basis although the spoil was still moist), were added to dry, weighed, sterile, 125ml capacity medical flat bottles. Twenty bottles were prepared from each half of the sample. Both batches of twenty were divided into two halves and one half received the equivalent of 50ppm of nitrogen (again on a dry weight basis) in the form of ammonium sulphate (0.5ml of a $4.71g.l^{-1}$ solution).

All the samples were to be incubated at 15% spoil moisture and to achieve this, the weight of the bottle plus the sample at this soil moisture regime was calculated and marked onto each bottle. The bottles were weighed after filling and where the weight was found to be less than that indicated on the bottle, deionised water was added to make up the difference. Overweight bottles lost moisture on incubation and soon achieved the correct weight.

Plastic caps were loosely fitted and all bottles were transferred and stood upright, in a dark incubator at $27^{o}C$. This time was designated time 0.

At two day intervals, the bottles were reweighed and any moisture lost through evaporation was replaced

by the addition of deionised water.

At time 0, four of the ten bottles receiving a common treatment were removed from the incubator. Duplicate determinations of pH and mineral nitrogen were performed. Further duplicate bottles were removed after ten, twenty and forty days for mineral nitrogen analysis. In all cases all three forms of mineral nitrogen were determined.

The decision to use 50ppm of nitrogen was based on the fact that this was the concentration used in the media for the enumeration of Nitrosomonas.

Ekpete & Cornfield (1966) showed that the moisture content at which soils were incubated affected both mineralization and nitrification. In the present investigations the aim was to incubate samples at a spoil moisture regime that resembled field conditions. The mean values for the spoil moisture determinations were approximately 15% (see pages 144 & 146) and this value was used. This approximated to field capacity for both spoils (see page 142).

2. Incubation experiment II

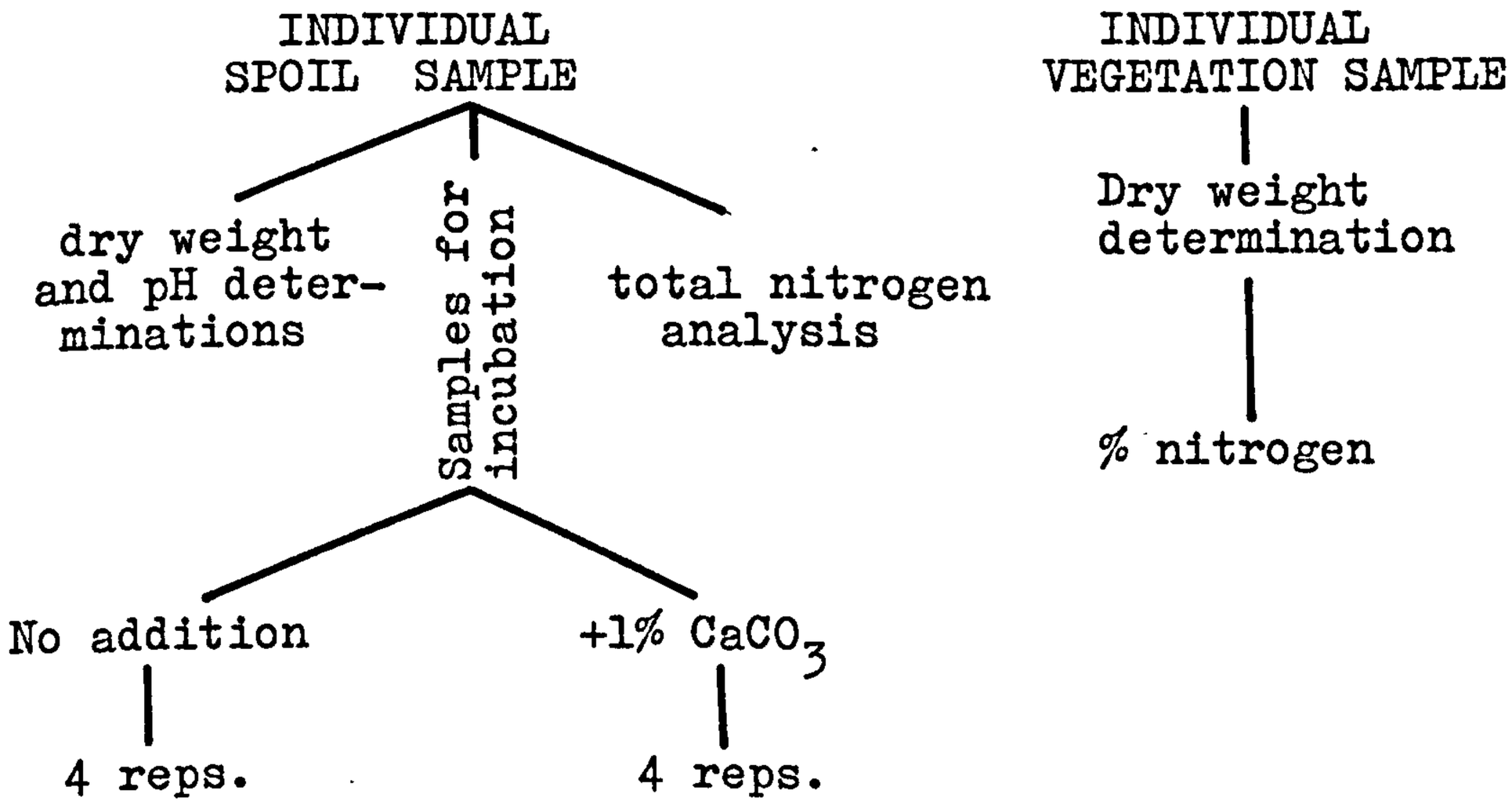
i) Sampling procedure (29th May, 1972)

On each of the shoddy, sewage-sludge, control and limestone (main plots 3 in Table 2.1) main plots of the Mitchell's Main trial, a $1/16m^2$ quadrat was selectively placed three times. One was positioned on a densely vegetated area, the second on a bare area, and the third on an intermediate area. A core of spoil of 10cms diameter and 15cms depth was taken from the centre of

the quadrat after the vegetation (where present) had been cut back to ground level and collected.

ii) Experimental design

All thirtysix spoil cores (3 cores on each of 4 treatments replicated 3 times) were treated individually throughout this experiment i.e. no bulking was performed. Each spoil core was sieved through a 1cm round holed sieve and the experimental design employed for both spoil and vegetation samples is shown in Fig. 2.3.



2 reps. for initial NH_4 and (NO_2+NO_3) determination

2 reps. for mineral nitrogen determinations after forty days incubation

Fig. 2.3. Experimental design of incubation experiment II

The preparation and conditions of incubation for the spoil samples was the same as that described for the first incubation experiment.

iii) Analysis of spoil for total nitrogen

The method used for the analysis of total nitrogen in the spoil samples utilised the method of digestion suggested by Bremner (1965b) and a steam distillation technique routinely used in the laboratories at York. The details are given below.

Air dry spoil was crushed to pass through a 100 mesh sieve in a hammer mill. A 2g quantity was accurately weighed (four decimal places) and placed in a dry, 500 ml capacity Kjeldahl flask. 20ml of deionised water was added and the flask swirled to mix the contents for one minute before being allowed to stand at room temperature for 30 minutes.

10g of potassium sulphate, 1g of copper sulphate ($5.H_2O$), 0.1g of selenium and 30ml of concentrated sulphuric acid were then added and the flask was heated gently on an electric Kjeldahl heating mantle placed in a fume cupboard, until the solution cleared. This was achieved in approximately three hours. Once cleared, the heat was increased to the point at which the solution condensed about one third of the way up the neck of the flask. The second stage of heating was continued for five hours.

After digestion, the flask was allowed to cool before approximately 150 ml of deionised water was added cautiously. The diluted solution was allowed to recool and was then filtered through a double layer of Whatman no. 1 filter paper. The filter paper was washed three times with deionised water and the volume made up to 250ml.

The digestion solution was stored in stoppered polythene bottles at $4^{o}C$ in the dark until used.

For distillation, a 5 ml aliquot of the digested solution was pipetted into the inner chamber of a Markham still. 6 ml of 50% sodium hydroxide solution was placed in the reservoir and allowed to run into the inner chamber slowly.

Steam prepared by boiling deionised water in a steam can was passed into the still for 3 minutes and the distillate was collected in 10 ml of boric acid indicator solution contained in a 50 ml capacity conical flask. Care was taken to ensure that the tip of the condenser was beneath the surface of the indicator solution so that no ammonia was lost to the atmosphere.

The boric acid indicator solution was made up inthe following way. 5.00g of boric acid crystals were crushed in 200 ml of hot deionised water. When cool this solution was added to a 2l volumetric flask containing 45 ml of a methyl red solution (0.02% methyl red in 60% ethanol) and 15 ml of a bromocresol green solution (0.1% bromocresol green in 60% ethanol). Enough tap water to turn the solution green was added before the volume was made up to 2l with deionised water. Passage of steam for 3 minutes produced about 30 ml of distillate and this was titrated against $^{N}/_{100}$ hydrochloric acid until a permanent pale pink colour occurred in the solution.

Duplicate blank digestions and distillations were performed and duplicate samples were digested and analysed.

iv) Mineral nitrogen determinations

Because the quantities of nitrite found in the first incubation experiment were so small, it was decided that the analysis of the incubated spoil samples for mineral nitrogen would be restricted to ammonium and nitrite plus nitrate together. This was achieved by use of the modification suggested by Bremner (1965a) i.e. omitting pretreatment of one aliquot with sulphamic acid.

v) Dry weight of vegetation

The whole sample was placed in a suitably sized, dry, weighed glass beaker and heated in an oven at 105°C for twenty four hours. After this time the beaker was transferred to a desiccator and allowed to cool before being reweighed.

vi) Nitrogen analysis of the vegetation samples

The whole of each sample was ground in a hammer mill to pass a 2mm sieve.

Approximately 2g (where available) of the ground sample was put in a porcelain crucible and dried at 105°C for one hour. After cooling in a desiccator, the crucible was weighed before and after its contents were transferred to a 800ml capacity Kjeldahl flask. The amount of dry material added to the Kjeldahl flask could be calculated.

3 Kjeldahl tables supplied by BDH Chemicals Ltd. (each containing 1g of sodium sulphate and 0.1g of copper sulphate (5 H_2O)) and 50ml of concentrated sulphuric acid were placed in a 300ml capacity Kjeldahl digestion flask which was then placed on an electric heating mantle situated in a fume cupboard.

The mixture was heated slowly at first, to prevent loss of sample by boiling out of the neck of the flask, and later more vigorously, until the solution attained a pale yellow colour. This signified the completion of digestion. The flask was allowed to cool before a quantity of deionised water was added. When cool the solution was filtered through a Whatman no. 42 filter paper, which was rinsed three times before the filtrate was made up to 250 ml. The filtrate was stored in stoppered polythene bottles at $4^{o}C$ until analysed.

Duplicate digestions were performed on each sample and blank digestions and determinations were performed.

Analysis of the filtrate was performed by the method described for the analysis of spoil for total nitrogen.

SECTION IV MISCELLANEOUS TECHNIQUES

1. Yield and nitrogen content of the vegetation at Mitchell's Main and Upton

Mitchell's Main

On 29th July, 1972 the above ground vegetation was removed from within three randomly positioned $1/16m^2$ quadrats on each of the three field replicates of the four main plot treatments previously sampled.

The vegetation from the three replicate quadrats was pooled before the samples were returned to the laboratory in sealed polythene bags in which they were stored at $-16^{o}C$.

Each sample was sorted into living (green) and dead (brown) components and the dry weight and percentage nitrogen content of each was determined using the methods already described.

Upton

On 9th September, 1971 the above ground vegetation was removed from within two $1/20m^{-2}$ randomly positioned quadrats on each of the three field replicates of the four treatments previously sampled at this site.

The samples were not bulked or sorted into living and dead components and only dry weight determinations were performed.

On 28th July, 1972 the sampling, sorting and analysis described above for Mitchell's Main were repeated at Upton.

2. Spoil temperature recordings at Mitchell's Main

The temperature of the spoil at Mitchell's Main was recorded semi-continuously from November, 1970 to December, 1971.

Thermocouples were placed at three depths, 1, 5 and 10 cms below the surface of a bare spoil area situated just to the west side of the trial plots. The thermocouples were positioned at each depth by inserting them horizontally into a vertical wall of a small hole dug into the spoil. After insertion of the thermocouples, the hole was backfilled. Care was taken to ensure that the thermocouples were not positioned directly over each other. Three sets each of three thermocouples (i.e. at the different depths) were buried in the spoil and all were connected to a "Grant Instruments Ltd." automatic temperature recorder which recorded the temperature at hourly intervals.

The thermostats were frequently stolen or damaged and this resulted in gaps in the recordings. The recording meter was protected against vandals by virtue of the fact that it was placed in a "padlocked" steel box sunk into the surface of the spoil heap.

3. Drying characteristics of the spoils

The drying characteristics of the spoil at the two sites was determined by the use of tensiometers (Richards 1965).

A bulked sample of each spoil type was obtained by pooling approximately forty cores of spoil (7.5cms diameter 15cm depth) taken at random from within and

around the trial site. All cores from one site were mixed by sieving through a 1cm sieve and the less than 1cm fraction (90^{+}%) was placed in a wooden box (side 30cms, length 60cms, depth 15cms).

The box was completely submerged in tap water for 10 minutes and then allowed to drain for a further 30 minutes.

The porous pots of two tensiometers were inserted into each box so that they were totally beneath the spoil surface. After removal of all bubbles in the capillary tube connecting the porous pot to the mercury manometer, the mercury level was adjusted to read zero. This time was designated time 0.

The apparatus was set up in a laboratory at an ambient temperature of 20^{o}C.

At time 0 and 12 hour intervals thereafter until the water column broke, the readings on the mercury manometers were recorded and duplicate (approximately 50g), spoil samples were taken for moisture determinations.

The mean values of the manometer and corresponding spoil moisture determinations were calculated and the drying curves constructed.

4. Washing of glassware

Used glassware was rinsed in tap water and then in chromic acid. The chromic acid was removed by three rinses in tap water followed by three rinses in deionised water.

CHAPTER THREE

CHAPTER THREE

SEASONAL VARIATION IN THE LEVELS OF PLANT NUTRIENTS (OTHER THAN NITROGEN), pH, SPOIL MOISTURE AND TEMPERATURE

INTRODUCTION

The results of the periodic determinations of pH, aluminium, manganese, copper, zinc, iron, sodium, potassium, calcium, magnesium, spoil moisture and temperature are presented and discussed in this chapter. The materials and methods employed have been given in Chapter Two and will not be referred to.

1. Presentation of results

Each 'element' included in the study is initially treated as a separate topic and presented in a distinct section. When this has been done for each of the 'elements', the effect on plant growth of the interactions of all the elements will be discussed.

The results are expressed graphically. With the exception of spoil temperature, separate axis are used for each of the spoil treatment plots (i.e. shoddy, sewage-sludge, control, limestone). Each plotted value represents the mean of the three field replicates (blocks).

The concentration of all plant nutrients are expressed in terms of parts per million (ppm) of the element in the saturated paste extract.

2. Statistical interpretation of results

All the data (except spoil temperature) have been analysed statistically using a parametric, split-

plot analysis of variance technique (Snedecor 1956). In all analyses spoil treatments (i.e. shoddy, sewage-sludge, control, limestone) formed the main plots, and sampling dates, the sub-plots. Main plot comparisons provided information on the effect of the ameliorant addition on the factor being considered, and sub-plot comparisons, the information on changes with time.

Significant main-plot or sub-plot variance ratios only indicated an overall difference between main plots or sub-plots and gave no indication of which particular groupings were different. To obtain this information other operations had to be performed. The usual technique is to calculate the least significant difference (LSD, Cochran & Cox 1957), and to compare individual sub-plot or main plot values. Differences between the compared values greater than the calculated LSD are taken to indicate that the values compared were significantly different. Snedecor (1956) suggests that a LSD should only be used for the comparison of two particular values, although it is commonly used for more. This is probably not important as long as the number of comparisons made remains small, but becomes increasingly so as the number rises. This occurs because the use of the LSD for more than one comparison is subject to Type I errors (i.e. concluding that a significant difference exists between two observations which, in fact, are similar). LSD values have been used in the present investigations to distinguish particular differences between the main plots and blocks

because the number of comparisons involved were small. LSD values were only calculated where the main plot or block variance ratio was significant ($p \geq 0.05$).

An important part of the present investigations was the assessment of seasonal variation. This information was contained in the sub-plot comparisons of the analysis of variance and in the case of Mitchell's Main was extracted by use of orthogonal comparisons (Snedecor 1956). In order to make these comparisons, the year was divided up into the four seasons on the following basis:-

Spring	March, April, May,
Summer	June, July, August,
Autumn	September, October, November,
Winter	December, January, February.

In terms of experiments, the following regime resulted:-

Spring	1, 2, 8, 9.
Summer	3, 10, 11, 12.
Autumn	4, 13, 14, 15.
Winter	5, 6, 7, 16.

Thus the sixteen sampling dates (experiments) were divided into four equal groups. Observation of the data indicated that samples taken in the spring and winter seasons were generally similar but contrasted to those taken in summer and autumn. The following orthogonal comparisons were, therefore, thought to be the most appropriate, and have been performed.

1) Spring + Winter vs Summer + Autumn

2) Summer vs Autumn

3) Spring vs Winter

The procedure of dividing up the year and examining the data for seasonal variation in this way was justifiable because the trial plots at Mitchell's Main had been established three years before the present sampling programme was begun, and had been layed down on a spoil surface that had weathered over a period of eight years. It was, therefore, unlikely that seasonal variation would have been obscured by changes induced by initial weathering reactions or ameliorant applications.

The field trial at Upton had been established on a freshly recontoured spoil heap and sampling began almost immediately. At this site seasonal variation would be confounded by initial weathering, and amelior- and applications and hence the orthogonal comparisons used to investigate significant sub-plot differences at Mitchell's Main were inappropriate. It was, therefore, decided to examine significant sub-plot differences by ranking the sub-plot mean values and calculating the least significant range (LSR, or D, Snedecor 1956). The individual sub-plot values could not be distinguished by use of a LSD because the number of comparisons was large (i.e. 9 sub-plots represents 36 comparisons) and hence the probability of committing a Type I error great. The use of the LSR in place of the LSD overcomes the difficulty and has, therefore, been calculated

whenever a significant sub-plot variance ratio was found.

In many of the analyses, untransformed data have been used. Occasionally, however, a transformation was necessary to normalise the data. Two transformations have been used, the square root, where the untransformed data approximated more to a Poisson than a Normal distribution, and a $\log_{10}$ transformation where the numerical range of the data extended over several orders of magnitude.

The presence of a number of zero values in the data invalidates the analysis of variance. Where data containing such values was used in the untransformed or $\log_{10}$ transformed state, zero values were increased to the smallest value that was an order of magnitude less than the degree of accuracy to which the determinations were made (e.g. if the determinations were measured accurately to two decimal places, zero values became 0.001). Since most of the data requiring a square root transformation was composed of very small and often zero values, the integer 1 was added to all observations before the square root was taken.

The results of the statistical analyses are shown in tabular form. The upper part of each table gives the variance table, and the lower part the means tables. LSD and LSR values are given where appropriate and in the cases of the Upton sub-plot mean tables, the results of applying the LSR values are indicated. Thus, values that are not significantly different ($p \geq 0.05$) are

indicated by a connecting line.

Although the sub-plot values in the Upton data were not grouped seasonally for orthogonal comparisons, the season in which they were taken (using the regime described for Mitchell's Main) is indicated.

Where transformed data was used in an analysis, untransformed mean values are presented in brackets, after the transformed means.

RESULTS

1. SPOIL PH

INTRODUCTION

To a large extent the nature and activity of the soil microflora and the availability of many plant nutrients is dependant upon the pH of the soil or spoil. This factor is therefore of fundamental importance and will be discussed first.

RESULTS AND INTERPRETATION

The results of the pH determinations are shown graphically in Figs. 3.1 & 3.2 and the statistical analyses are shown in Tables 3.1 & 3.2.

Mitchell's Main

The results show that the spoil at this site was extremely acid. The pH of the limestone plots was significantly higher than the others, that were not significantly different one from another. This demonstrates that the limestone applied three years before sampling was begun was not exhausted.

The pH was significantly lower in the autumn months than at other times of the year.

Upton

No significant treatment effects were found to occur. This would be expected because the results clearly show that the spoil at this site was of neutral reaction and neither shoddy nor sewage-sludge have a marked effect on spoil pH.

Significant differences are observed between the sub-plots. These do not, however, appear to be

related either to season or to progressive weathering. There was no indication that the spoil at Upton was becoming acid.

Mitchell's Main and Upton comparison

The results show that the two sites were very different in respect of spoil pH. Mitchell's Main spoil was highly acidic whilst that at Upton was of near neutral reaction. This fundamental difference will be reflected in many of the factors to be described and discussed.

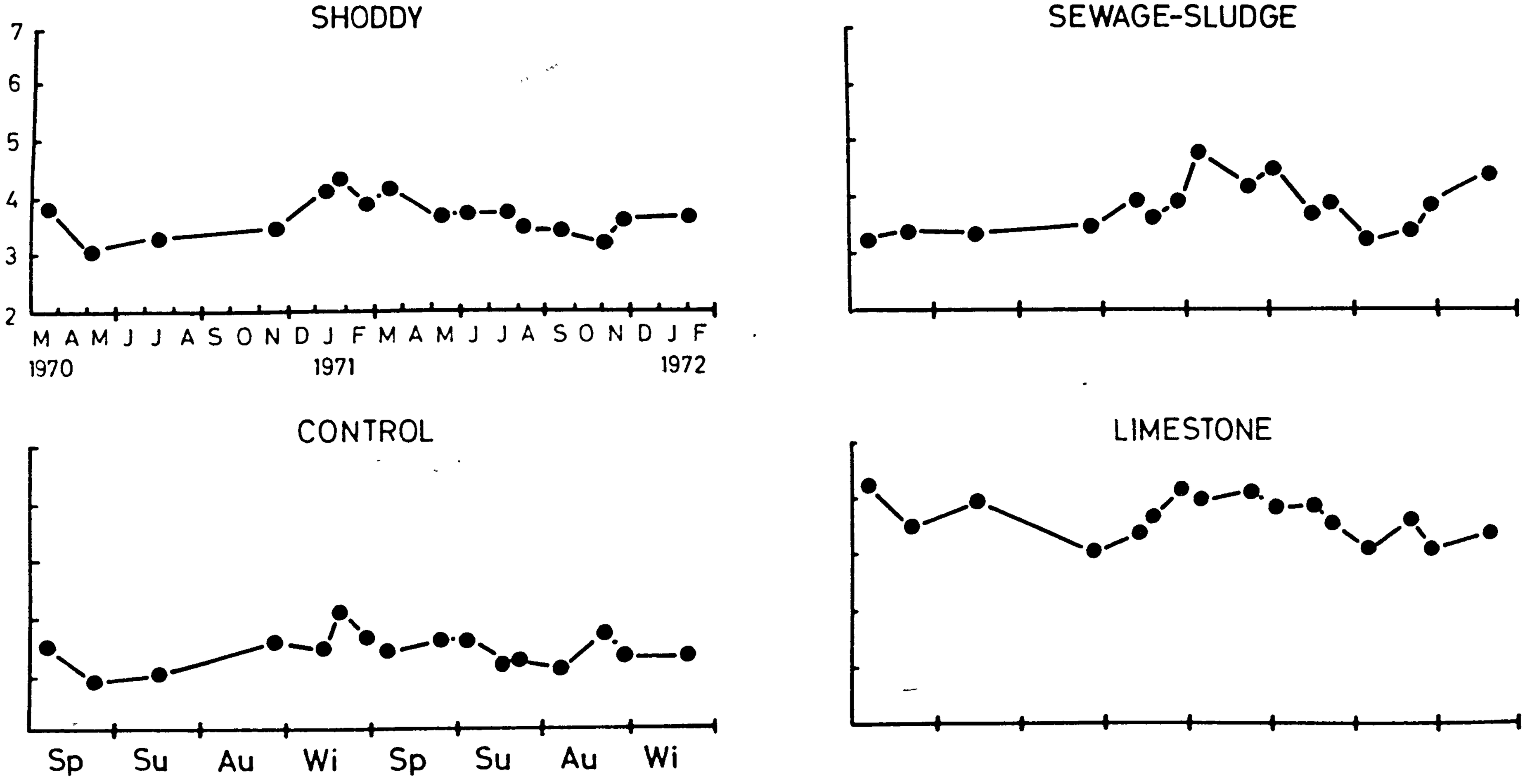


Fig. 3.1. Mitchell's Main. Seasonal variation of pH.

Table 3.1. Mitchell's Main. pH. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio	Significance
Main plots	143.586	3	47.862	12.705	***
Blocks	6.512	2	3.256	0.864	ns
Error a	22.603	6	3.767		
Total	172.701	11			
Sub-plots	12.463	15	0.831	3.763	***
Spring & Winter vs Summer & Autumn	1.756	1	1.756	7.951	**
Spring vs Winter	0.079	1	0.079	0.359	ns
Summer vs Autumn	1.949	1	1.949	8.826	**
Interaction	14.685	45	0.326	1.479	**
Error b	26.498	120	0.221		
Total	226.347	191			

MEANS TABLES

Main Plots

Shoddy	3.64
Sewage-sludge	3.79
Control	3.42
Limestone	5.59

LSD (p = 0.05)
0.97

Blocks

I	3.87
II	4.17
III	4.31

Sub-Plots

					Overall mean
Spring	4.12	3.67	4.56	4.36	4.18
Summer	3.84	4.38	4.35	4.06	4.16
Autumn	3.85	3.73	3.98	3.93	3.87
Winter	4.16	4.37	4.32	4.09	4.24

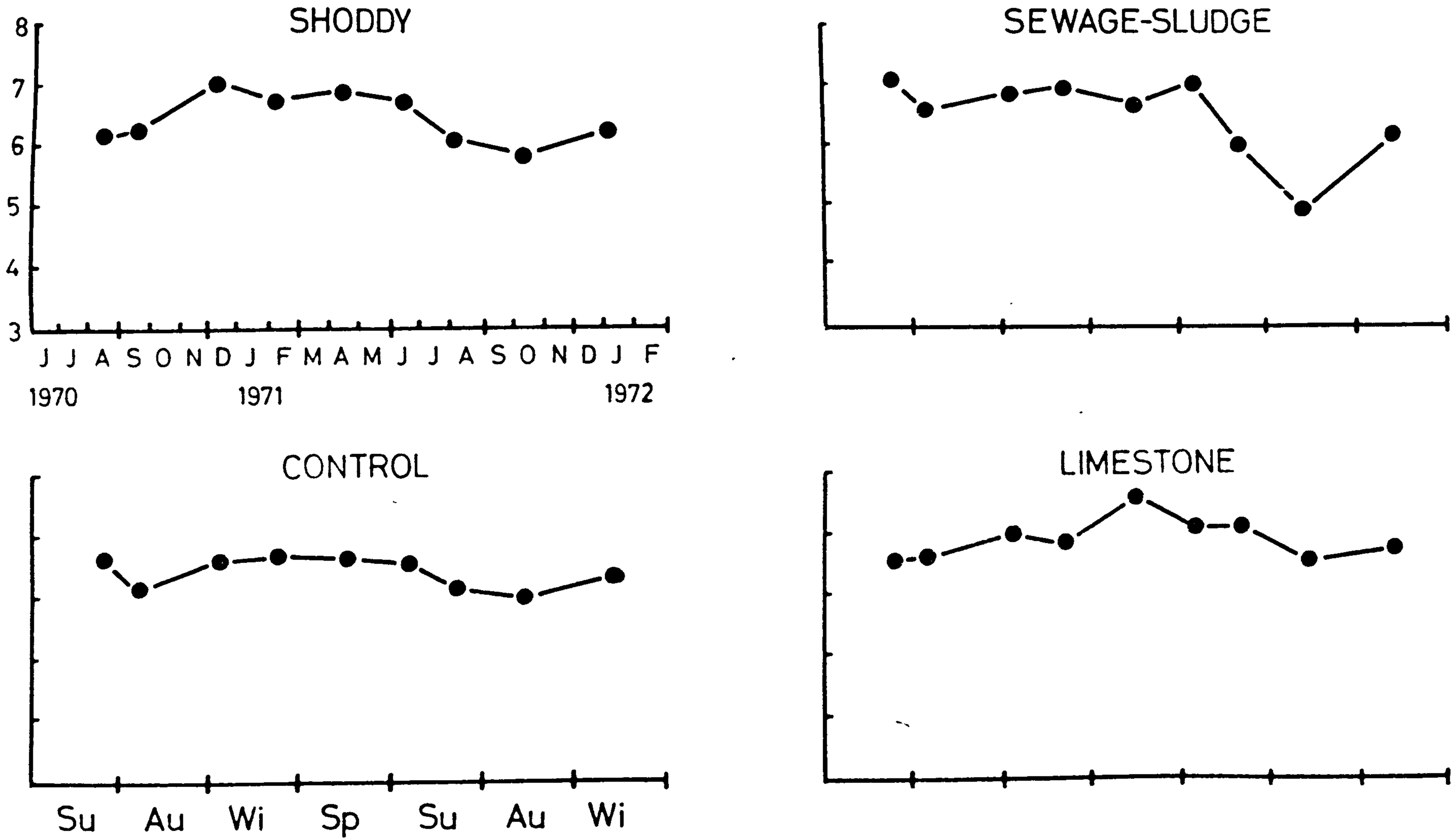


Fig. 3.2. Upton. Seasonal variation of pH.

Table 3.2. Upton. pH. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	4.3608	3	1.4536	0.862	ns
Blocks	16.6615	2	8.3308	4.938	ns
Error a	10.1234	6	1.6872		
Total	31.1457	11			
Sub-plots	13.2741	8	1.6593	11.514	***
Interaction	7.6980	24	0.3207	2.226	**
Error b	9.2233	64	0.1441		
Total	61.3411	107			

MEANS TABLES

Main Plots	
Shoddy	6.40
Sewage-sludge	6.40
Control	6.38
Limestone	6.86

Blocks	
I	5.98
II	6.64
III	6.91

Exp. No.	Sub-Plots	
5	Sp	6.90
3	Wi	6.83
6	Su	6.81
4	Wi	6.78
1	Su	6.59
2	Au	6.37
9	Wi	6.27
7	Su	6.26
8	Au	5.77

LSR (p = 0.05)
0.499

DISCUSSION

The acid reaction of the weathered colliery spoil at Mitchell's Main resulted from the oxidation of iron pyrites, a mineral found throughout the coal seams and associated shales. The reaction follows the sequence given by Brandt & Moulton (1960).

$$FeS_2 + 2O_2 \longrightarrow FeSO_4 + S$$

$$2FeS_2 + 2H_2O + 7O_2 \longrightarrow 2FeSO_4 + 2H_2SO_4$$

$$4FeSO_4 + 2H_2SO_4 + O_2 \longrightarrow 2Fe_2(SO_4)_3 + 2H_2O$$

$$Fe_2(SO_4)_3 + 6H_2O \longrightarrow 2Fe(OH)_3 + 3H_2SO_4$$

Thus, water soluble ferrous and ferric sulphates are formed and these hydrolyse to sulphuric acid and ferric hydroxide. These oxidation reactions begin as soon as the reactive pyrites is exposed to the atmosphere, the initial rate is slow but increases progressively with the extent of oxidation (Glover 1974). The primary oxidation products react with any carbonate mineral or clay present to form calcium, magnesium, manganese and aluminium sulphates (Glover 1974). If the spoil contains only small quantities of pyrite, enough neutralizing material may be present to counteract all the acid produced. In many spoils, however, there is a great excess of pyrites and as oxidation proceeds a zone of acidity forms around each oxidising pyrite particle and extends as the neutralizing materials are exhausted. These acid zones coalesce and may eventually cover the whole of the spoil surface. This has occurred at Mitchell's

Main.

The quantity of neutralising material present in a spoil is one of the two main factors that controls the rapidity with which initially neutral, unweathered pyritic colliery spoil becomes acid. The other factor is the physical form of the pyrites. Pyrites occurs in a number of forms ranging from large crystalline pieces weighing many grammes to small spheres of diameter 10μm called framboids. The rate of oxidation is related to the reactive surface area of the pyrites. Thus, brassy pyrites has a small surface to volume ratio and oxidises extremely slowly, shiny pieces of this material often remaining apparently unoxidised after many years exposure on the surface of a spoil heap. Framboidal pyrites on the other hand has an extremely large surface to volume ratio and oxidises very rapidly. Indeed, Caruccio (1974) has stated that framboidal pyrites is the most important form in colliery spoils since spoils that contain pyrites in other forms may not become acid on weathering. Framboidal pyrites has been found in acid sulphate soils (cat clays, Rickard 1973), and in these soils the very low pH values recorded have been attributed to the oxidation of this framboidal pyrites (van Breeman 1973).

The mechanism of pyrite oxidation has been studied for many years. It is thought that the actual electron transfer associated with the oxidation of the pyrite occurs on the surface of the mineral from either adsorbed oxygen or from ferric ion.

Since ferrous ion is oxidised at a negligible rate in acidic solution, certain acidophilic bacteria of the genus Thiobacillus have been implicated in this oxidation reaction. Although it has been shown innumerable times in laboratory studies that pyrite oxidation proceeds much more rapidly in the presence of these organisms and that they are present ubiquitously in coal mine working, their significance in pyrite oxidation in the field has not been established (Ashmead 1956; Baker & Wilshire 1970). Lau et al., (1970) have observed that the number of these bacteria in spoils is not usually great enough to account for the observed rate of pyrite oxidation.

Since both organic and inorganic oxidation processes are ultimately dependant upon a supply of oxygen, pyritic oxidation is restricted to the surface layers of the spoil heap. The acid layer does not usually extend deeper than about 25 cms and may be much less. As yet there is no method for preventing the oxidation of pyrites other than preventing the contact of pyrites and oxygen. This is not a practical proposition for reclamation work.

The acid reaction of spoil like Mitchell's Main is important not because of any direct effect of hydrogen ions since it has been shown that hydrogen ions per se are not usually toxic to plants (Arnon & Johnson 1942). The importance lies in the profound effect that low pH has on a great variety of chemical and biological processes that occur in a soil or spoil.

These effects will become apparent as individual nutrient elements are discussed.

2. ALUMINIUM

INTRODUCTION

Although aluminium is one of the most abundant elements present in soils, appreciable quantities of aluminium ions are only found in the soil solution if the pH is below 4.5 where they are a major soil toxin (Rorison 1973).

Aluminium is not usually considered to be one of the essentail elements for most plant species, however stimulations of growth have sometimes been found to result from exposure to low levels. Thus, Sommer (1926) noted an increase in seed production in millet and Lipman (1938) found enhanced growth of corn when supplied with 1ppm. Jackson (1967) has stated that such stimulations cannot be taken to indicate a requirement for aluminium since the effects may be due to an indirect effect of the uptake of other toxic ions such as copper, zinc and manganese.

The quantity of plant available aluminium is, therefore, only considered to be important in relation to its possible toxic effects. Since the solubility of aluminium increases with decreasing pH, aluminium toxicity is only associated with acid soils.

RESULTS AND INTERPRETATION

The results and statistical analyses for Mitchell's Main are given in Fig. 3.3. & Table 3.3. Detectable levels (i.e. > 1ppm) of aluminium were not found in the Upton plots.

Mitchell's Main

The statistical analysis shows that the limed plots contained significantly less aluminium than the others which were not significantly different, one from another. This was obviously a reflection of the higher pH in the limed plots.

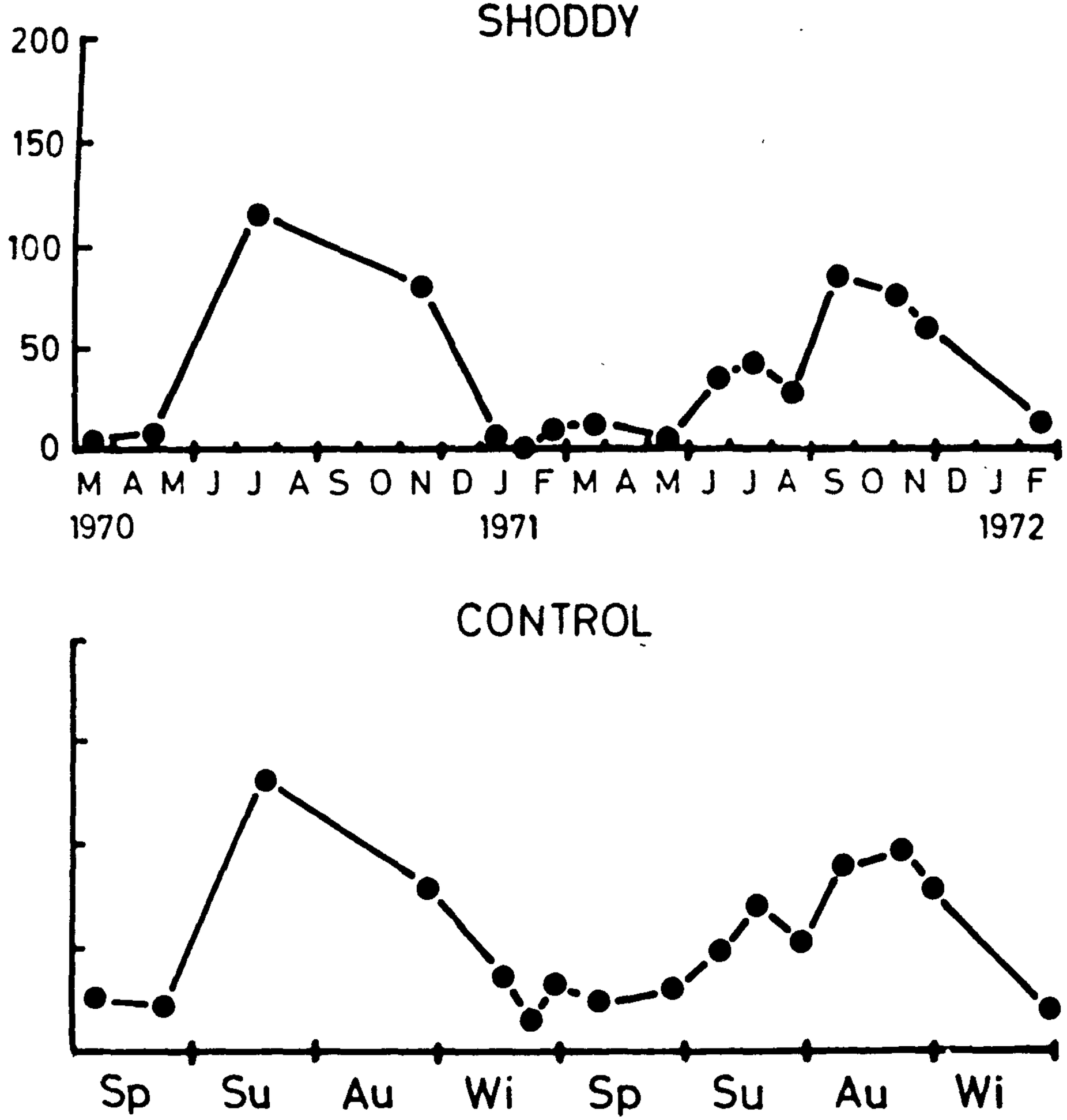
A very well marked seasonal variation was observed.in the shoddy, sewage-sludge and control plots where the levels of aluminium were significantly higher in the summer and autumn periods than in winter and spring. The summer vs autumn orthogonal comparison is significant showing that the levels were significantly higher in autumn than in summer. The significant interaction variance ratio results from the difference between the limestone plots and the others in respect of the level of aluminium and seasonal variation.

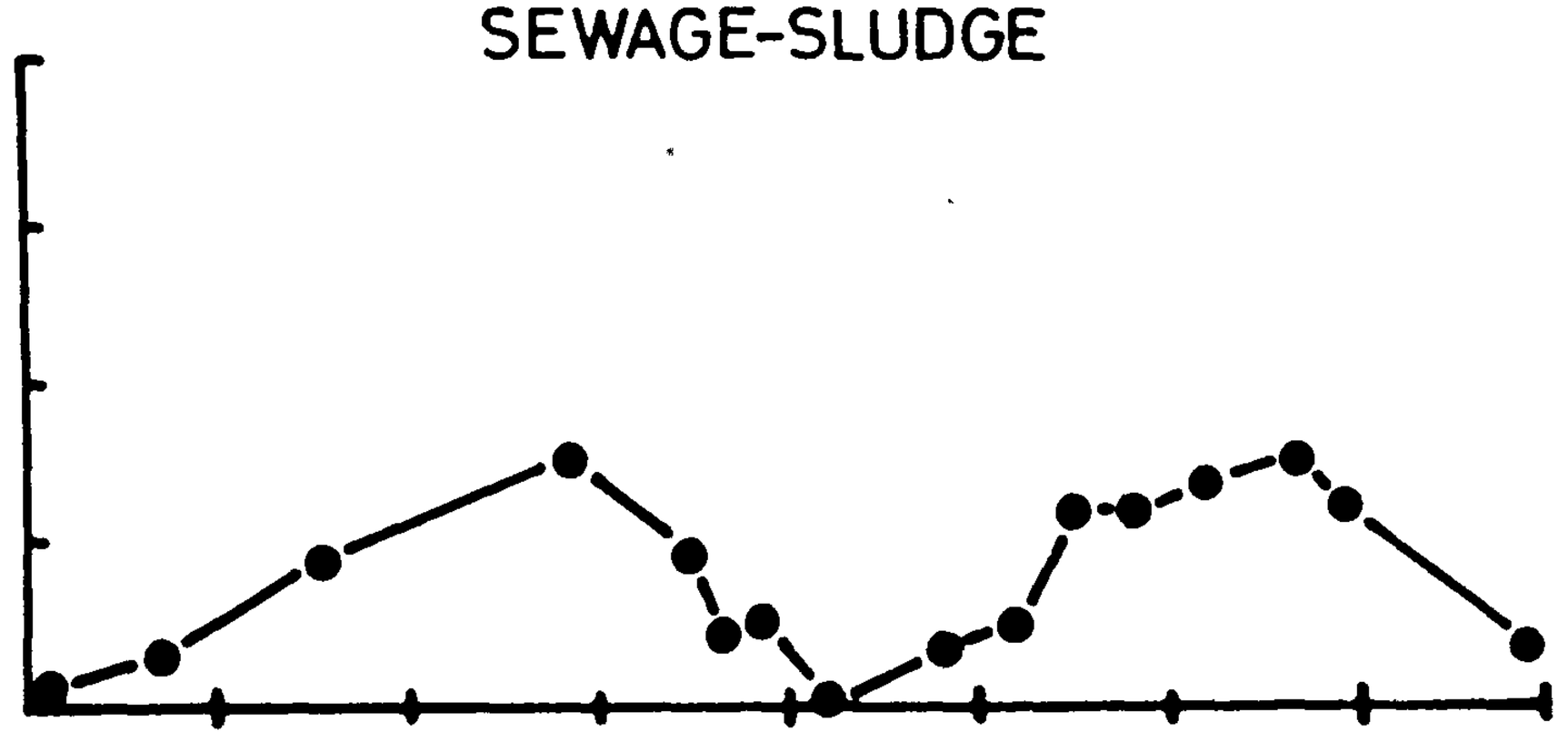
Upton

Detectable levels of aluminium were not found in the Upton plots because of the neutral reaction of the spoils.

Mitchell's Main and Upton comparison

The difference between the two sites is very obvious and could be predicted from the pH data. Aluminium toxicity is certainly of no importance at Upton.





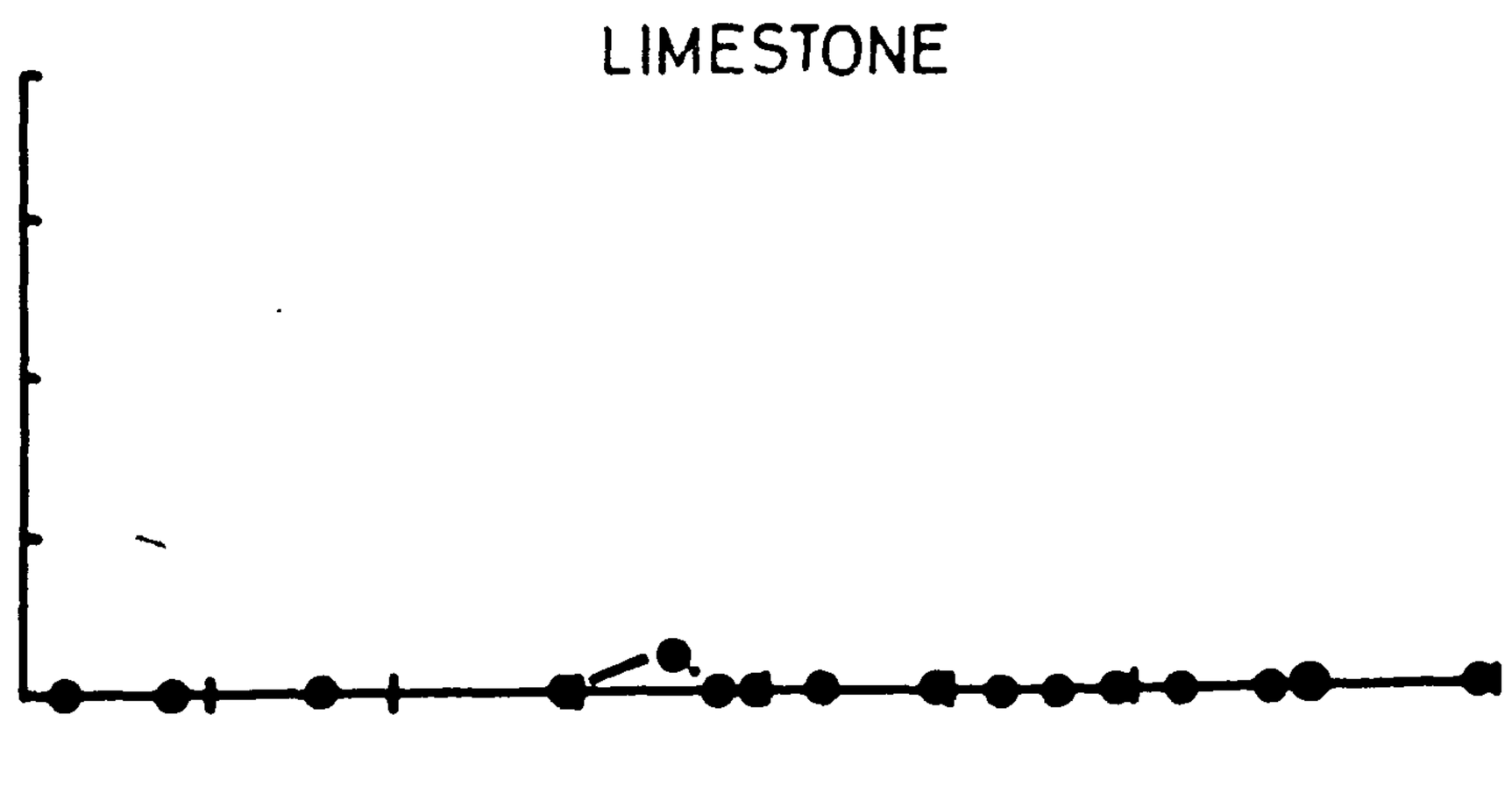


Fig. 3.3. Mitchell's Main. Seasonal variation of aluminium.

Table 3.3. Mitchell's Main. Aluminium. Statistical analysis.

VARIANCE TABLE (log_{10} transformation)

Source	ss	df	ms	vr	sig.
Main plots	108.5808	3	36.1936	7.948	*
Blocks	22.1589	2	11.0794	2.433	ns
Error a	27.3232	6	4.5539		
Total	158.0628	11			
Sub-plots	69.4827	15	4.6322	9.598	***
Spring & Winter vs Summer & Autumn	24.8477	1	24.8477	51.487	***
Spring vs Winter	0.0002	1	0.0002	0.0004	ns
Summer vs Autumn	3.192	1	3.192	6.614	*
Interaction	44.8543	45	0.9968	2.065	*
Error b	57.9164	120	0.4826		
Total	330.31617	191			

MEANS TABLES

Main Plots

Shoddy	0.802 (34.4)
Sewage-sludge	0.656 (38.8)
Control	1.091 (52.3)
Limestone	-0.849 (0.71)

LSD (p = 0.05) = 1.066

Blocks

I	0.879 (56.1)
II	0.333 (22.5)
III	0.062 (16.0)

Sub-Plots

					Overall mean
Spring	-0.329	0.148	-0.109	-0.226	-0.129 (9.7)
Summer	1.095	0.515	0.742	0.842	0.799(44.2)
Autumn	1.165	1.130	1.143	1.215	1.163(58.1)
Winter	0.227	-0.449	-0.310	0.003	-0.132(14.1)

DISCUSSION

Species vary considerably in susceptibility to aluminium toxicity. Those that normally grow in acid soils, calcifuges, are generally more resistant than those that only grow in neutral soils, calcicoles. Thus Clarkson (1966) showed that the calcifuge Agrostis tenuis, one of the two species sown on the trial plots, was intermediate in tolerance between the strongly calcifugeous A. setacea and the strongly calcicoleous A. stolonifera. Festuca rubra the other sown species is not a strongly calcifugeous species but rather one with a wide range that can tolerate fairly acid conditions. This species would also be expected to show aluminium tolerance. The degree of tolerance possessed by these species is not, however, very great when compared with the quantities of aluminium in the acid plots at Mitchell's Main. In a saturated paste extract <0.5ppm aluminium is usually regarded as being harmless, 0.5 - 1.0 ppm as toxic and greater than 1.0ppm highly toxic (Pratt 1966). The levels found in all but the limed plot were very much higher than even the highly toxic value quoted above especially in the summer and autumn months when this value was sometimes exceeded one hundred times. Chadwick & Salt (1969) found that 2.7ppm aluminium in a culture solution experiment depressed the root growth of A. tenuis strains isolated from an acidic colliery spoil heap. Harding (1970), however, reported that the response of A. tenuis

strain, again isolated from spoil heaps, to aluminium was dependant on the composition of the nutrient solution in which the plants were grown. Thus, in a single salt solution ($CaNO_3$) root length was reduced by 50% in the presence of 0.5ppm aluminium whilst in a weak nutrient solution (1/5 Long Ashton) including phosphorus, 27ppm had no effect.

Despite the mitigating effect of other cations in the spoil solution on aluminium toxicity, the levels found in the shoddy, sewage-sludge and control plots would certainly appear high enough to be phytotoxic at some times of the year. Symptoms of aluminium toxicity, characterised by stubby brown roots and small dark green leaves (Jackson 1967) were looked for but were not found. This is probably because the toxicity symptoms briefly summarised above usually only appear when plants are grown in culture media in the laboratory. In the fields, such symptoms are not often seen and Pratt (1966) has concluded that aluminium toxicity cannot be diagnosed from visual symptoms because the growth depression that occurs is non specific. This may result from the indirect effect of high aluminium concentrations on the availability of other nutrient elements, especially phosphorus, calcium, potassium and manganese. The interactions between aluminium and these other nutrients will be discussed when each element is described separately.

3. MANGANESE

INTRODUCTION

Manganese is an essential constituent of a large number of enzyme systems and is associated with the stability of subcellular organelles (Possingham et al., 1964).

Manganese toxicity can occur in acid soils and spoils (Berg & Vogel 1968) and Rorison (1971) has found that manganese can be more toxic than equivalent strengths of aluminium.

Under neutral or alkaline conditions manganese deficiencies can occur and the symptoms are typically those of chlorotic mottling and necrotic spotting (Hewitt 1963).

RESULTS AND INTERPRETATION

The results are shown in Figs. 3.4 & 3.5 and statistical analyses in Tables 3.4 & 3.5.

Mitchell's Main

The limed plots contained significantly less manganese than did the others. As with aluminium the difference is a reflection of the pH dependant solubility of manganese. The concentration of manganese showed a marked seasonal variation. Significantly higher levels occurred in summer and autumn than in spring and winter.

A very significant block difference is found for this element and it can be seen that this was due to the much higher levels that occurred in block I.

Upton

No significant main plot effects are observed. Since the treatments were applied to neutral spoils this would be the expected result.

A significant sub-plot effect is observed but Table 3.6 indicates that the difference could not be certainly attributed to seasonal effects because although the levels were generally higher in summer and autumn, application of the LSR did not produce good separation of the means. Further, there was no progressive increase or decrease with time, indicating that initial weathering reactions were not important in determining the level of water soluble manganese in the spoil.

A significant block difference is observed and as at Mitchell's Main, this was due to the higher levels found in block I.

Mitchell's Main and Upton comparison

The levels of manganese on all but the limed plots at Mitchell's Main were generally higher than those at Upton. The mean values are 13.1 (excluding limed plots) and 1.48 ppm respectively. The range of manganese concentrations at Mitchell's Main was, however, very large (see page 294), indicating the variability of the spoil at this site. The general difference between the two sites was obviously a reflection of the different pH's of the spoils.

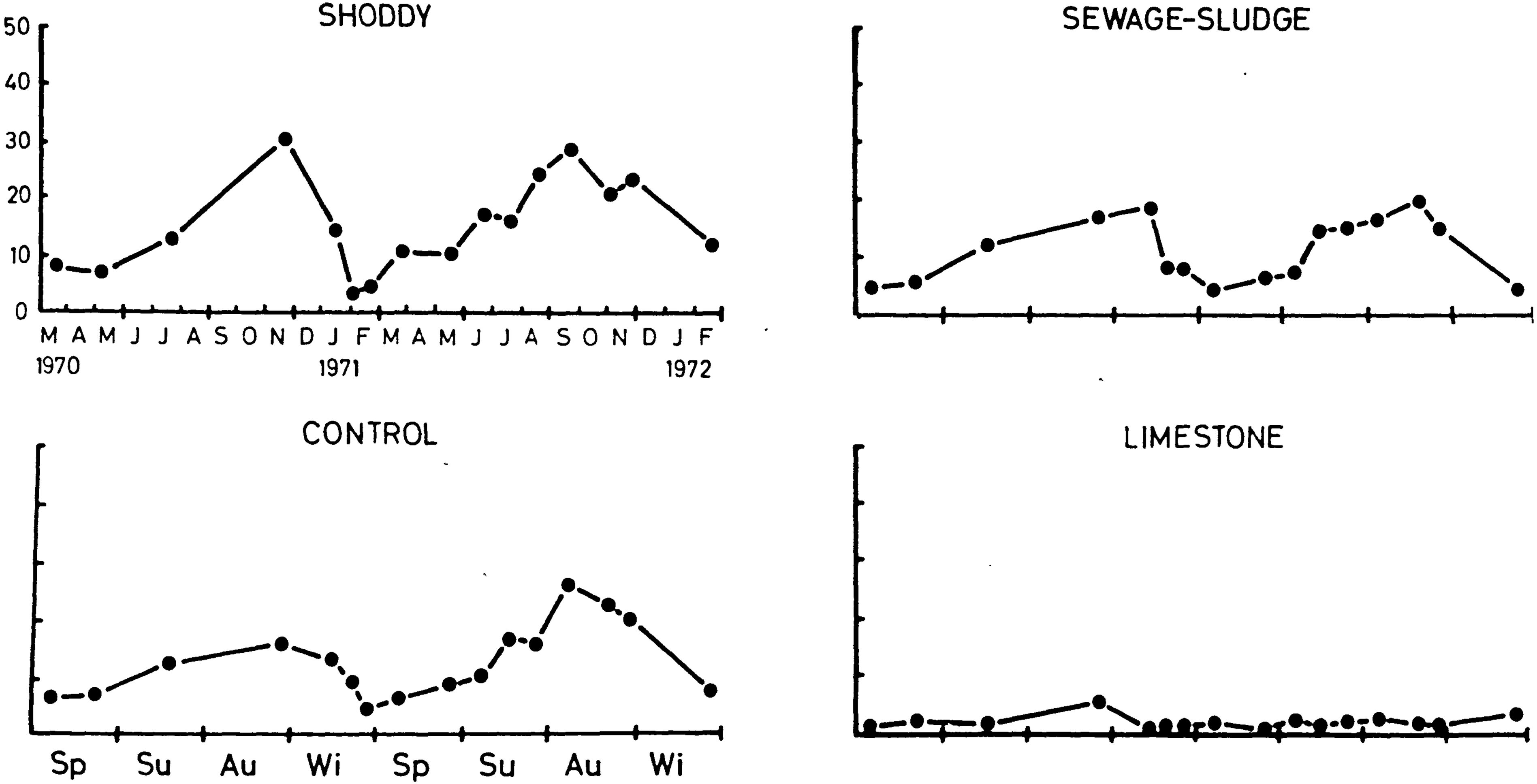


Fig. 3.4. Mitchell's Main. Seasonal variation of manganese.

Table 3.4. Mitchell's Main. Manganese. Statistical analysis.

VARIANCE TABLE (log_{10} transformation)

Source	ss	df	ms	vr	sig.
Main plots	83.0688	3	27.6896	33.041	***
Blocks	53.1050	2	26.5525	31.684	***
Error a	5.0282	6	0.8380		
Total	141.2021	11			
Sub-plots	16.1785	15	1.0786	3.928	***
Spring & Winter vs Summer & Autumn	5.0155	1	5.0155	18.265	***
Spring vs Winter	0.8039	1	0.8039	2.928	ns
Summer vs Autumn	0.6958	1	0.6958	2.534	ns
Interaction	15.2690	45	0.3393	1.236	ns
Error b	32.9501	120	0.2746		
Total	205.5997	191			

MEANS TABLES

Main Plots

Shoddy	0.836 (15.45)
Sewage-sludge	0.782 (11.05)
Control	0.719 (12.87)
Limestone	-0.737 (1.48)

LSD (p = 0.05)
0.457

Blocks

I	1.136 (23.72)
II	0.080 (3.79)
III	0.003 (3.14)

LSD (p = 0.05)
0.423

Sub-Plots

					Overall mean
Spring	0.433	0.517	0.050	0.320	0.330 (5.55)
Summer	0.515	0.364	0.544	0.483	0.477 (11.30)
Autumn	0.502	0.759	0.560	0.767	0.647 (16.70)
Winter	-0.508	0.438	0.446	0.212	0.147 (7.31)

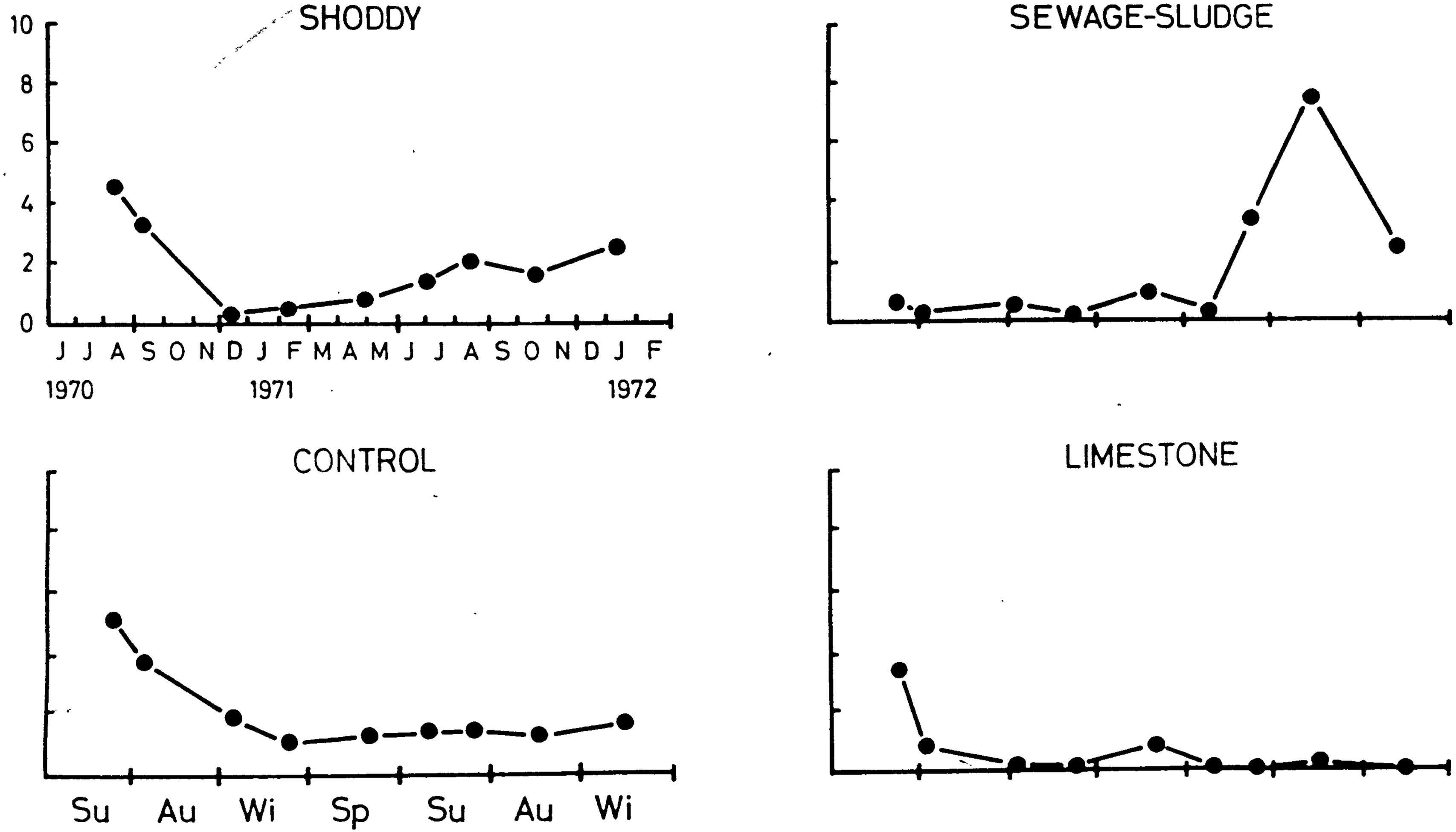


Fig. 3.5. Upton. Seasonal variation of manganese.

Table 3.5. Upton. Manganese. Statistical analysis.

VARIANCE TABLE (square root +2)

Source	ss	df	ms	vr	sig.
Main plots	2.2458	3	0.7486	1.286	ns
Blocks	14.7030	2	7.3515	12.627	**
Error a	3.4933	6	0.5822		
Total	20.4421	11			
Sub-plots	4.7473	8	0.5934	2.859	**
Interaction	8.7155	24	0.3631	1.750	*
Error b	13.2826	64	0.2075		
Total	47.1874	107			

MEANS TABLES

Main Plots

Shoddy	1.56 (1.82)
Sewage-sludge	1.52 (1.83)
Control	1.60 (2.12)
Limestone	1.23 (0.74)

Blocks

I	2.00 (3.62)
II	1.25 (0.77)
III	1.18 (0.49)

LSD (p = 0.05)
0.762

Exp. No.		Sub-plots	
1	Su	1.81	(3.44)
8	Au	1.77	(2.70)
7	Su	1.58	(1.81)
2	Au	1.56	(2.02)
9	Wi	1.49	(1.67)
5	Sp	1.36	(0.96)
6	Su	1.33	(0.89)
3	Wi	1.26	(0.75)
4	Wi	1.16	(0.40)

LSR (p = 0.05)
0.598

DISCUSSION

The literature on manganese toxicity demonstrates the effect that nutrients, other than manganese can have on the appearance and severity of manganese toxicity. Thus Berg & Vogel (1968) found that Lespedeza stipulacea showed manganese toxicity symptoms when grown in certain Kentucky strip-mine spoils containing as little as 1ppm of water soluble manganese whilst in other spoils no symptoms appeared at 30ppm. They concluded that the different levels of aluminium and calcium in the spoil were responsible for the variable response.

The calcium levels at both sites were very high and this would be beneficial since it has often been shown that calcium can reduce manganese toxicity effects.

The mean levels of manganese found in the plots at both sites would not appear to have been high enough to be directly toxic. The finding that individual bulked spoil samples from Mitchell's Main (especially in the main plots of block I) often contained very high levels of manganese (see Table 5.1), strongly suggests that localised areas occurred in which the manganese levels were directly phytotoxic.

4. COPPER

INTRODUCTION

The essentiality of copper lies in the fact that it is a constituent of certain oxidising-reducing enzymes.

Copper deficiencies occur frequently in crops growing on newly reclaimed peats in Europe causing the so called "Reclamation Diseases" (Russell 1961).

Phytotoxic levels of copper have been found in smelter wastes (Weston *et al.*, 1965), and heavy metal ore spoil heaps (Bradshaw *et al.*, 1965).

RESULTS AND INTERPRETATION

The results and statistical analyses are given in Figs. 3.6 & 3.7 and Tables 3.6 & 3.7.

Mitchell's Main

The limestone plots contained less copper than the others but the difference was not large enough to be statistically significant ($p = 0.05$).

A significant sub-plot difference is found and the orthogonal comparisons demonstrate that this was related to the higher levels of copper in the summer and autumn months.

Upton

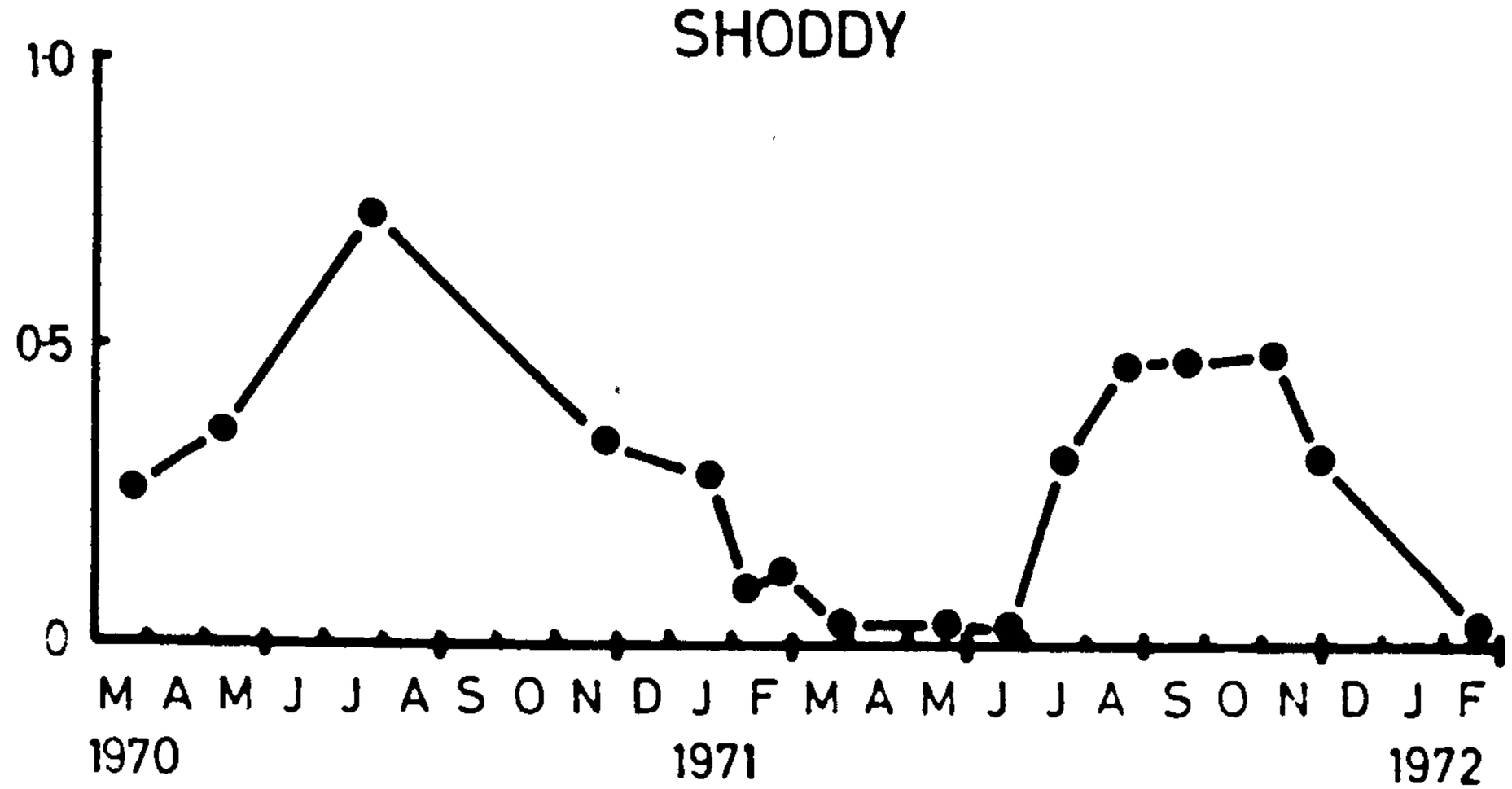
A significant main plot variance ratio is observed. The calculated LSD value indicates that the shoddy plots contained significantly higher levels of copper than limestone plots. Since, however, the control and

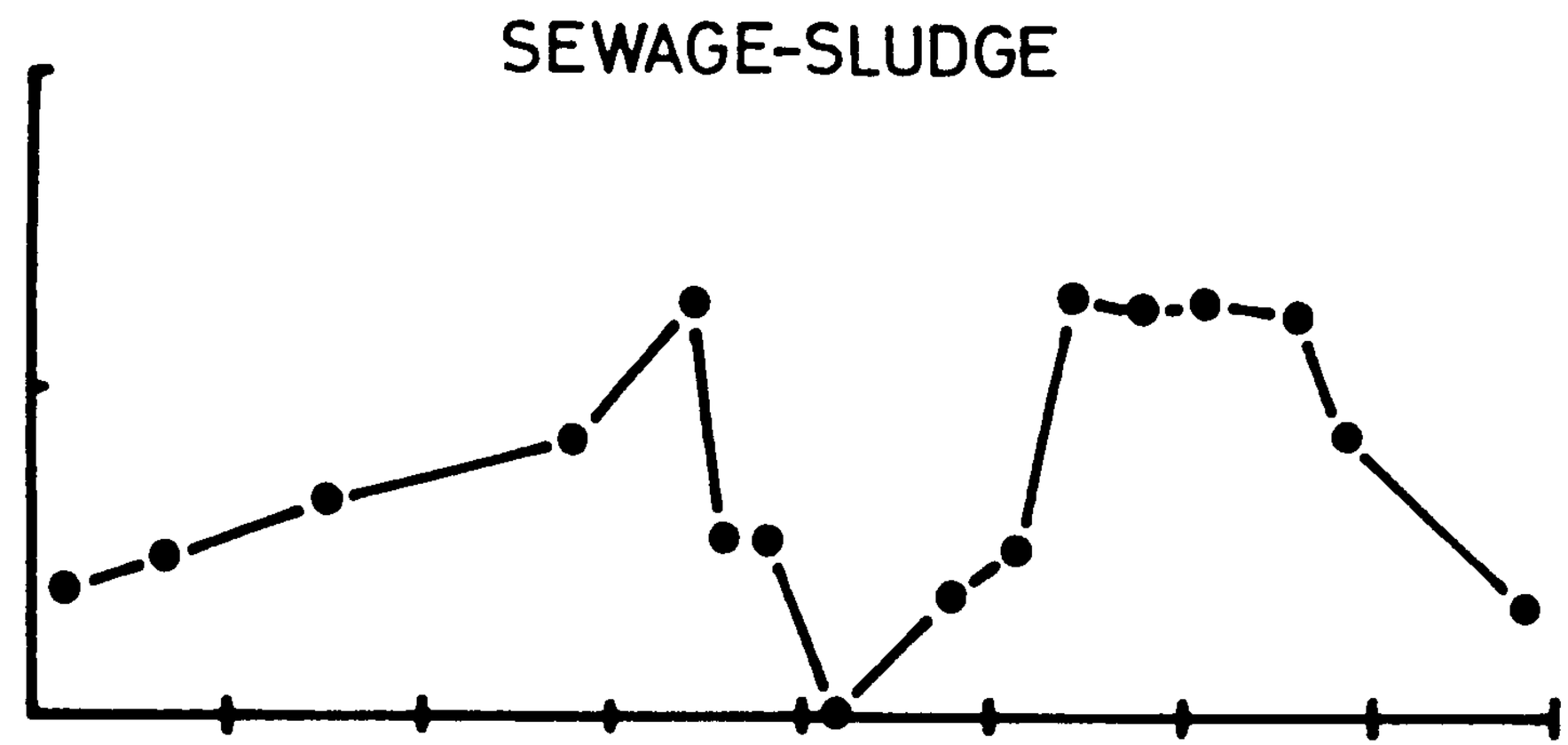
shoddy plots contained insignificantly different levels of copper, it cannot be concluded that the extra copper arrived in the shoddy.

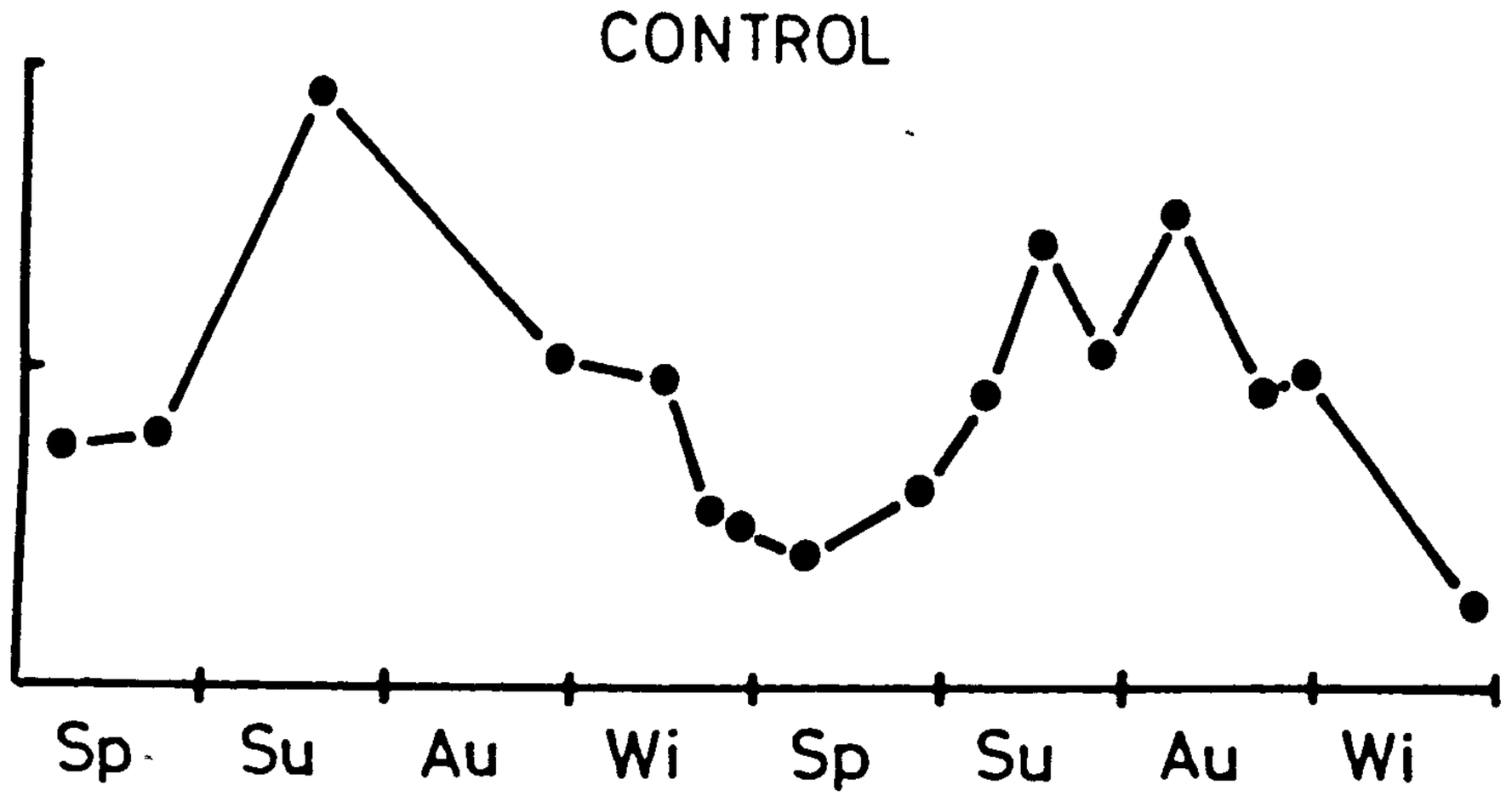
A significant sub-plot variance ratio is observed, but the variation is not apparently induced by weathering or seasonal changes.

Mitchell's Main and Upton comparison

The levels of copper at Mitchell's Main were generally higher than those at Upton but the difference was not very great. Since the pH at the two sites was very different this result indicates that the level of water extractable copper was not well related to spoil reaction.







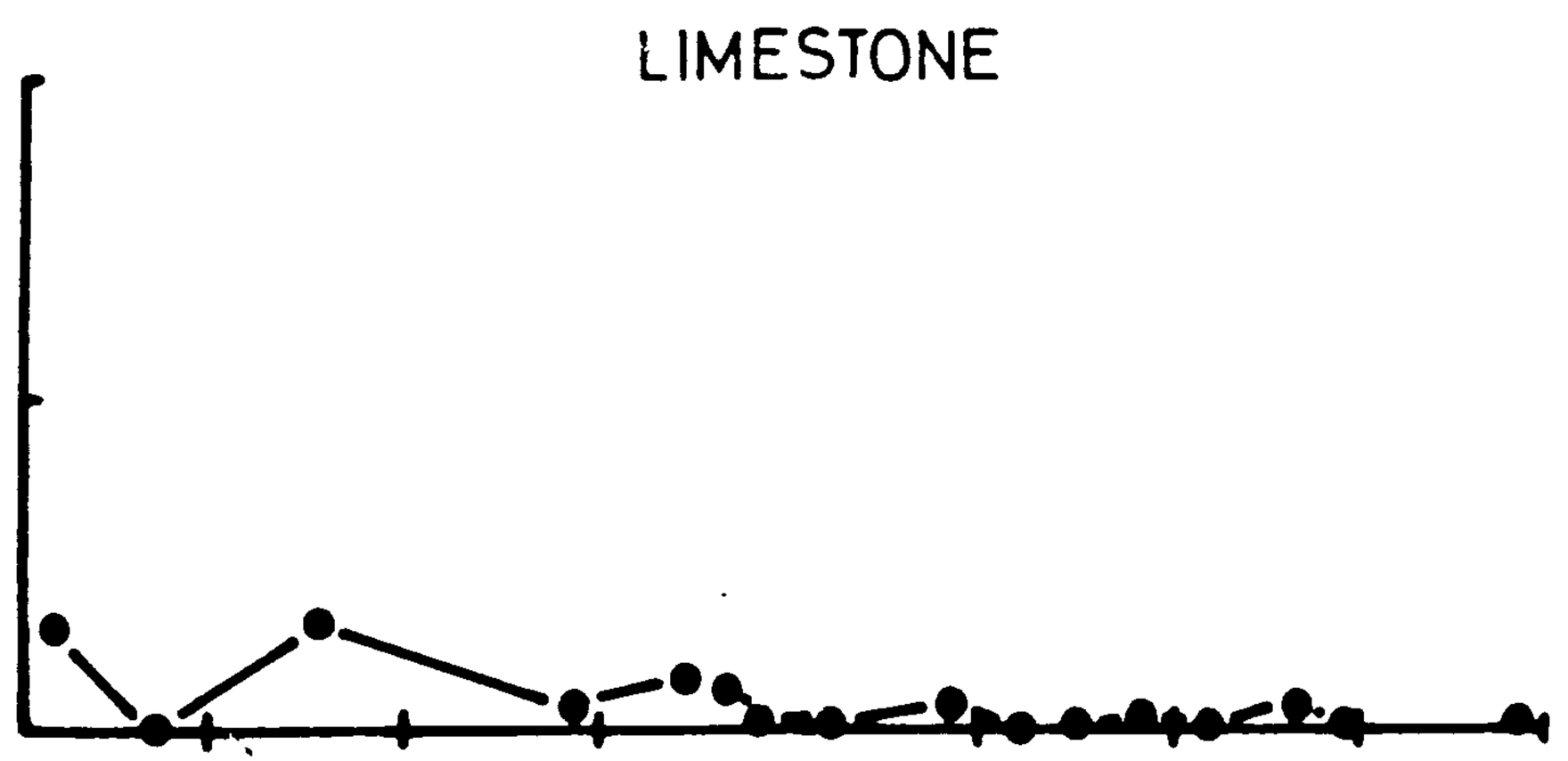


Fig. 3.6. Mitchell's Main. Seasonal variation of copper.

Table 3.6. Mitchell's Main. Copper. Statistical analysis.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots	0.8767	3	0.2922	1.611	ns
Blocks	0.7258	2	0.3629	2.000	ns
Error a	1.0886	6	0.1814		
Total	2.6912	11			
Sub-plots	0.6638	15	0.0443	9.785	***
Spring & Winter vs Summer & Autumn	0.3329	1	0.3329	73.978	***
Spring vs Winter	0.0015	1	0.0015	0.331	ns
Summer vs Autumn	0.0001	1	0.0001	0.022	ns
Interaction	0.3917	45	0.0087	1.925	**
Error b	0.5427	120	0.0045		
Total	4.2894	191			

MEANS TABLES

Main Plots

Shoddy	1.13 (0.28)
Sewage-sludge	1.16 (0.38)
Control	1.20 (0.47)
Limestone	1.02 (0.04)

Blocks

I	1.212 (0.51)
II	1.099 (0.22)
III	1.070 (0.15)

Sub-Plots

					Overall mean
Spring	1.12	1.12	1.03	1.07	1.09 (0.18)
Summer	1.23	1.09	1.18	1.18	1.17 (0.40)
Autumn	1.15	1.21	1.18	1.14	1.17 (0.39)
Winter	1.16	1.08	1.07	1.04	1.09 (0.20)

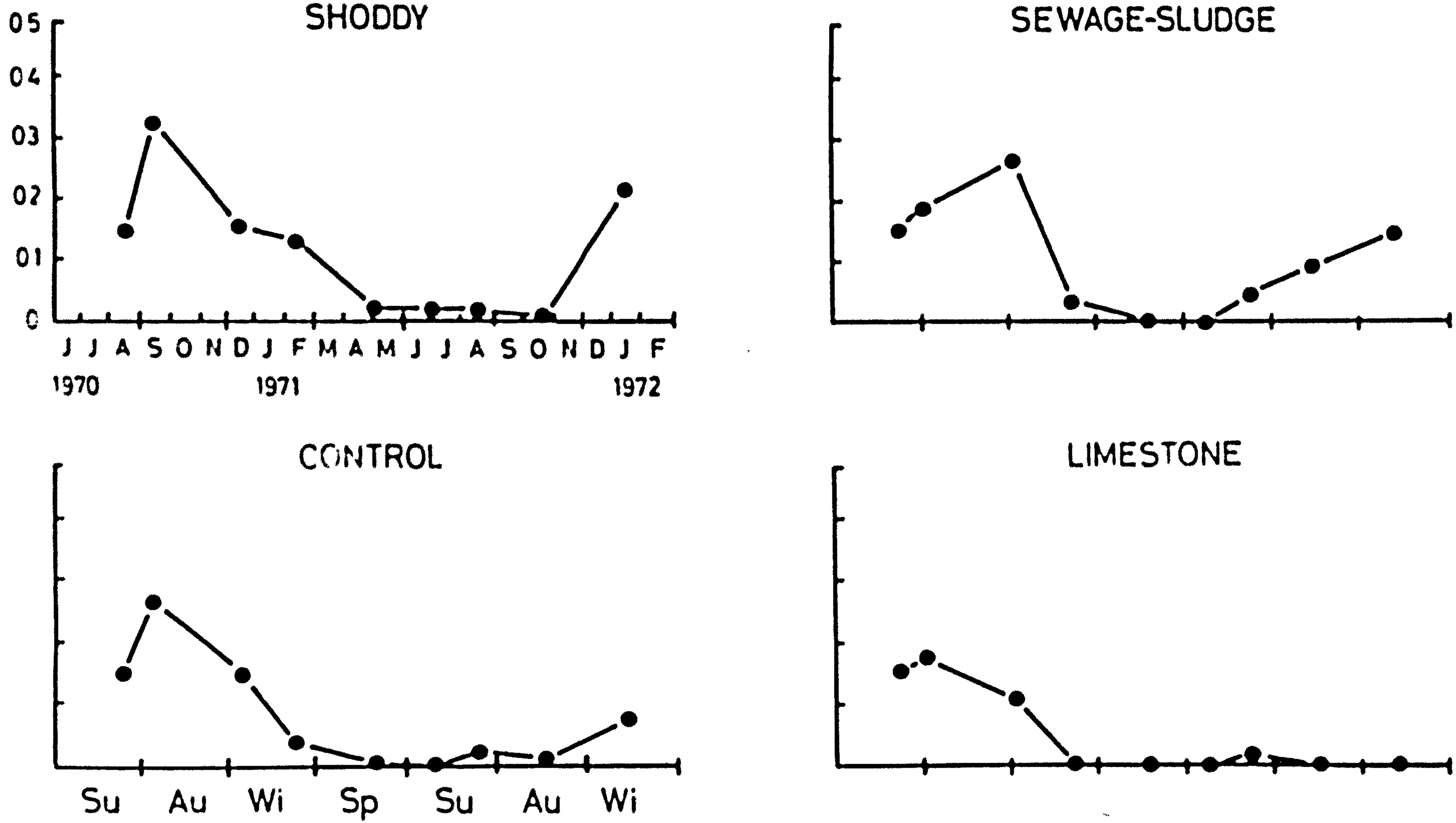


Fig. 3.7. Upton. Seasonal variation of copper.

Table 3.7. Unton. Conrer. Statistical analysis.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots	0.01400	3	0.00466	5.219	*
Blocks	0.00644	2	0.00322	3.608	ns
Error a	0.00536	6	0.00089		
Total	0.02578	11			
Sub-plots	0.138144	8	0.01726	15.411	***
Interaction	0.03024	24	0.00126	1.125	ns
Error b	0.0717	64	0.00112		
Total	0.26588	107			

MEANS TABLES

Main Plots

Shoddy	1.06 (0.12)
Sewage-sludge	1.05 (0.11)
Control	1.04 (0.09)
Limestone	1.03 (0.06)

LSD (p = 0.05)
0.020

Blocks

I	1.05 (0.11)
II	1.04 (0.08)
III	1.04 (0.08)

Exp. No.			
2	Au	1.11	(0.24)
3	Wi	1.08	(0.17)
1	Su	1.07	(0.15)
9	Wi	1.06	(0.12)
4	Wi	1.03	(0.05)
7	Su	1.02	(0.04)
8	Au	1.02	(0.04)
5	Sp	1.01	(0.02)
6	Su	1.01	(0.02)

Sub-Plots

LSR (p = 0.05)
0.044

DISCUSSION

Harding (1970) found that the water extractable copper in an acid soil of pH 3.3 was equivalent to 0.58ppm; almost the same level was found in a commercially prepared soil (John Innes). The mean values for Mitchell's Main and Upton were 0.29 and .09ppm respectively.

Copper toxicities would, therefore, not appear to be an important factor at either site. The low levels found in the Upton plots and the limed plots at Mitchell's Main (0.04ppm) might suggest that copper deficiencies might be of importance. Such deficiencies only appear to occur, however, in soils that contain a high level of organic matter, for example peaty soils. Since colliery spoils contain little soil-like organic matter copper deficiencies seem unlikely even on neutral or limed acid spoils.

5. ZINC

INTRODUCTION

Like copper, zinc is essential to plants but only very small quantities are required for satisfactory growth. If these are exceeded, toxicities can occur.

Toxic levels of zinc have been reported in heavy metal ore heaps (Bradshaw et al., 1965), and smelter wastes (Weston et al., 1965).

RESULTS AND INTERPRETATION

The results and statistical analyses are shown in Figs. and Tables 3.8 & 3.9.

Mitchell's Main

A significant main plot variance ratio is observed and this is shown to be attributable to a difference between the limestone plots and the others; the levels of zinc being significantly lower in the limestone plots.

The significant sub-plot variance ratio is shown to be largely attributable to the significantly higher levels of zinc in the summer and autumn months than the winter and spring ones. Thus a very definite seasonal pattern was observed.

Block I contained significantly higher levels of zinc than the other blocks.

Upton

A significant main plot variance ratio is not observed. The sub-plot variance ratio is highly significant but the variation appears to be of a random

nature.

Mitchell's Main and Upton comparison

The levels of zinc were generally higher at Mitchell's Main than Upton, and the significant depressive effect of the limestone applications on the levels of water extractable zinc at Mitchell's Main indicates that the between site difference was related to the different pH status of the two spoils.

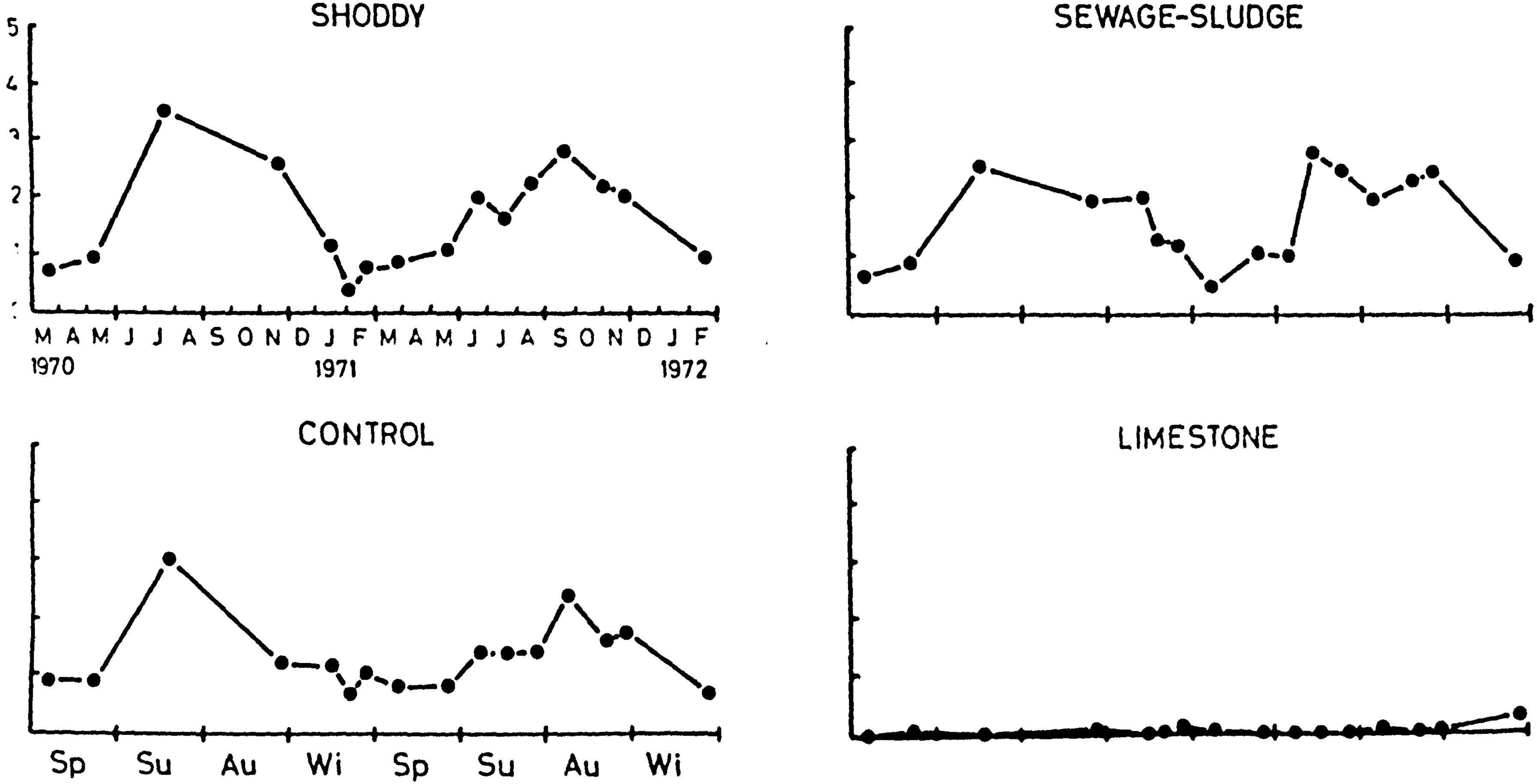


Fig. 3.8. Mitchell's Main. Seasonal variation of zinc.

Table 3.8. Mitchell's Main. Zinc. Statistical analysis.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots	10.5620	3	3.5207	11.475	**
Blocks	5.2471	2	2.6235	8.551	*
Error a	1.8409	6	0.3068		
Total	17.6500	11			
Sub-plots	4.4807	15	0.2987	9.253	***
Spring & Winter vs Summer & Autumn	3.4262	1	3.4262	106.127	***
Spring vs Winter	0.0565	1	0.0565	1.751	ns
Summer vs Autumn	0.0020	1	0.0020	0.062	ns
Interaction	2.8611	45	0.0636	1.969	**
Error b	3.8741	120	0.0323		
Total	28.8660	191			

MEANS TABLES

Main Plots

Shoddy	1.593 (1.65)
Sewage-sludge	1.575 (1.63)
Control	1.499 (1.36)
Limestone	1.020 (0.04)

LSD ($p = 0.05$) 0.277

Blocks

I	1.655 (1.94)
II	1.310 (0.81)
III	1.295 (0.77)

LSD ($p = 0.05$) 0.240

Sub-Plots

					Overall mean
Spring	1.242	1.286	1.232	1.294	1.264 (0.65)
Summer	1.752	1.422	1.550	1.478	1.551 (1.59)
Autumn	1.518	1.627	1.543	1.549	1.559 (1.64)
Winter	1.396	1.251	1.309	1.291	1.312 (0.82)

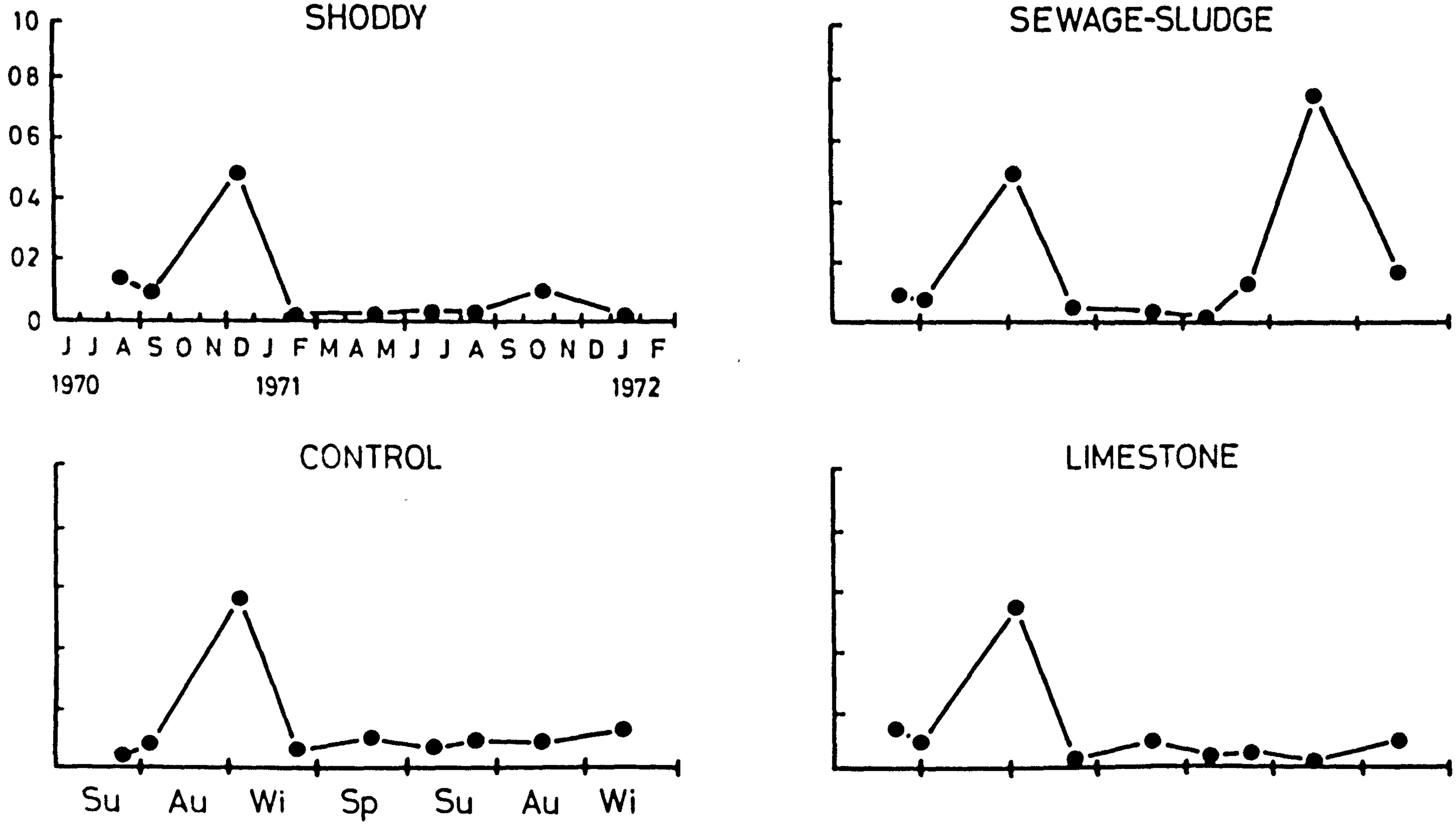


Fig. 3.9. Upton. Seasonal variation of zinc .

Table 3.9. Upton. Zinc. Statistical analysis.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots	0.03320	3	0.01107	1.858	ns
Blocks	0.03195	2	0.01598	2.682	ns
Error a	0.03574	6	0.00596		
Total	0.10090	11			
Sub-plots	0.46057	8	0.05757	26.967	***
Interaction	0.18100	24	0.00754	3.533	***
Error b	0.13663	64	0.00213		
Total	0.87910	107			

MEANS TABLES

Main Plots		Exp. No.		Sub-Plots	
Shoddy	1.05 (0.11)	3	Wi	1.23	(0.53)
Sewage-sludge	1.09 (0.21)	8	Au	1.11	(0.25)
Control	1.07 (0.15)	1	Su	1.07	(0.15)
Limestone	1.05 (0.11)	9	Wi	1.05	(0.11)
		2	Au	1.04	(0.08)
		5	Sp	1.03	(0.06)
		7	Su	1.03	(0.07)
Blocks		6	Su	1.02	(0.04)
I	1.09 (0.20)	4	Wi	1.01	(0.02)
II	1.06 (0.13)				
III	1.04 (0.11)	LSR ($p = 0.05$) 0.061			

DISCUSSION

Street & Goodman (1967) stated that 5ppm of water soluble zinc could be toxic to vegetation. Harding (1970) found that the concentration of zinc in a water extract of a commercially prepared soil (John Innes) was 0.176ppm as compared with 0.335ppm in an acid moorland soil. The mean value for Mitchell's Main spoil (excluding the limestone treatments) was 1.55ppm and Upton spoil was 0.15ppm. The Mitchell's Main samples therefore contained higher levels of zinc than a very acid soil whilst those from Upton contained slightly less than a good soil. Whilst the mean value for Mitchell's Main was higher than would normally be expected in soil, no value was found to be as high as 5ppm (see page 294), the level quoted above as being toxic. Occasionally, however, values of 4ppm were recorded and this indicates that whilst zinc toxicity may not have been of major importance at Mitchell's Main, it may have been a contributory cause of poor plant growth.

Zinc deficiency seems unlikely to have been of importance in the neutral Upton spoils because, like copper deficiency, that of zinc is usually associated only with soils of high organic content.

Sewage-sludge is often held to contain appreciable quantities of zinc and it has been suggested that repeated heavy applications of sewage-sludge can lead to zinc toxicity problems (Russell 1961). The present investigations gave no indication that the levels of

zinc in the spoils receiving sewage-sludge were higher than those not receiving this amendment.

6. IRON

INTRODUCTION

Iron is involved in the synthesis of chlorophyll and is, therefore, an essential micronutrient. Deficiencies of iron lead to the production of characteristic chloroses. Iron toxicity sometimes occurs in acid or waterlogged soils and Warwick (1958) considered that ferrous iron was an important toxic factor in colliery spoil.

RESULTS AND INTERPRETATION

The results are shown in Figs. 3.10 & 3.11 and the statistical analyses in Tables 3.10 & 3.11.

Mitchell's Main

The only significant variance ratio found is that for the sub-plots. The orthogonal comparisons show that the levels of iron were higher in the summer and autumn months than in the spring and winter ones, and further, that the spring levels were lower than the winter ones. A well marked seasonal pattern was, therefore, apparent.

Upton

As at Mitchell's Main the only significant variance ratio is the sub-plot one. As previously noted for this site, this difference did not appear to be related to progressive weathering or seasonal variation.

Mitchell's Main and Upton comparison

The levels of iron at Mitchell's Main were higher than those at Upton.

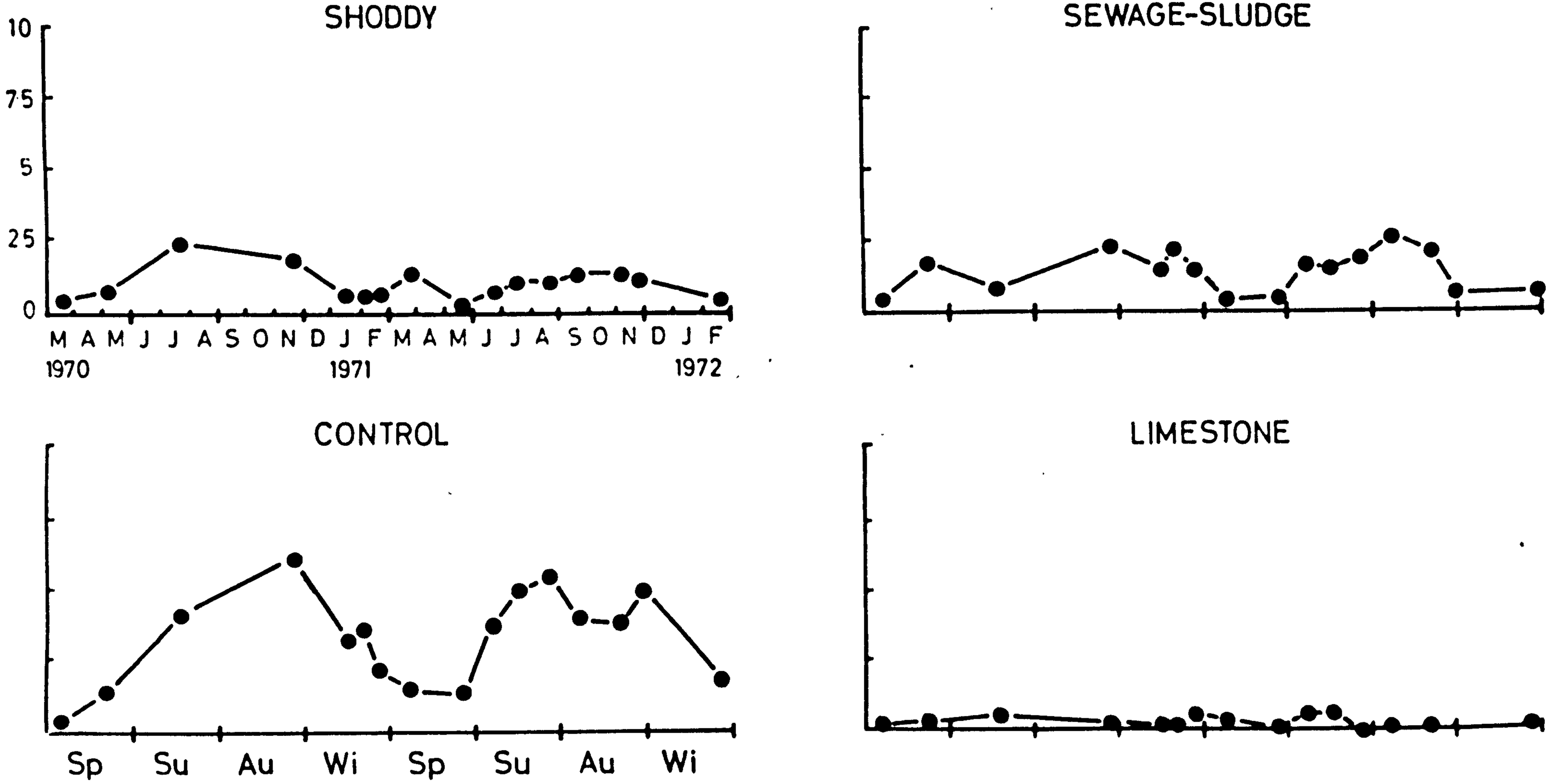


Fig. 3.10. Mitchell's Main. Seasonal variation of iron.

Table 3.10. Mitchell's Main. Iron. Statistical analysis.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots	12.4113	3	4.1371	0.990	ns
Blocks	12.1710	2	6.0855	1.456	ns
Error a	25.0716	6	4.1786		
Total	49.6539	11			
Sub-plots	4.7235	15	0.3149	3.445	***
Spring & Winter vs Summer & Autumn	3.3549	1	3.3549	36.700	***
Spring vs Winter	0.5046	1	0.5046	5.520	*
Summer vs Autumn	0.0094	1	0.0094	0.102	ns
Interaction	4.6075	45	0.1024	1.120	ns
Error b	10.9698	120	0.0914		
Total	69.9548	191			

MEANS TABLES

Main Plots

Shoddy	1.345 (0.90)
Sewage-sludge	1.493 (1.42)
Control	1.806 (3.15)
Limestone	1.104 (0.23)

Blocks

I	1.755 (2.98)
II	1.291 (0.77)
III	1.221 (0.54)

Sub-Plots

				Overall mean
Spring	1.113	1.342	1.287	1.187	1.232 (0.59)
Summer	1.595	1.523	1.596	1.523	1.559 (1.89)
Autumn	1.671	1.611	1.537	1.497	1.579 (2.02)
Winter	1.389	1.455	1.386	1.279	1.377 (1.20)

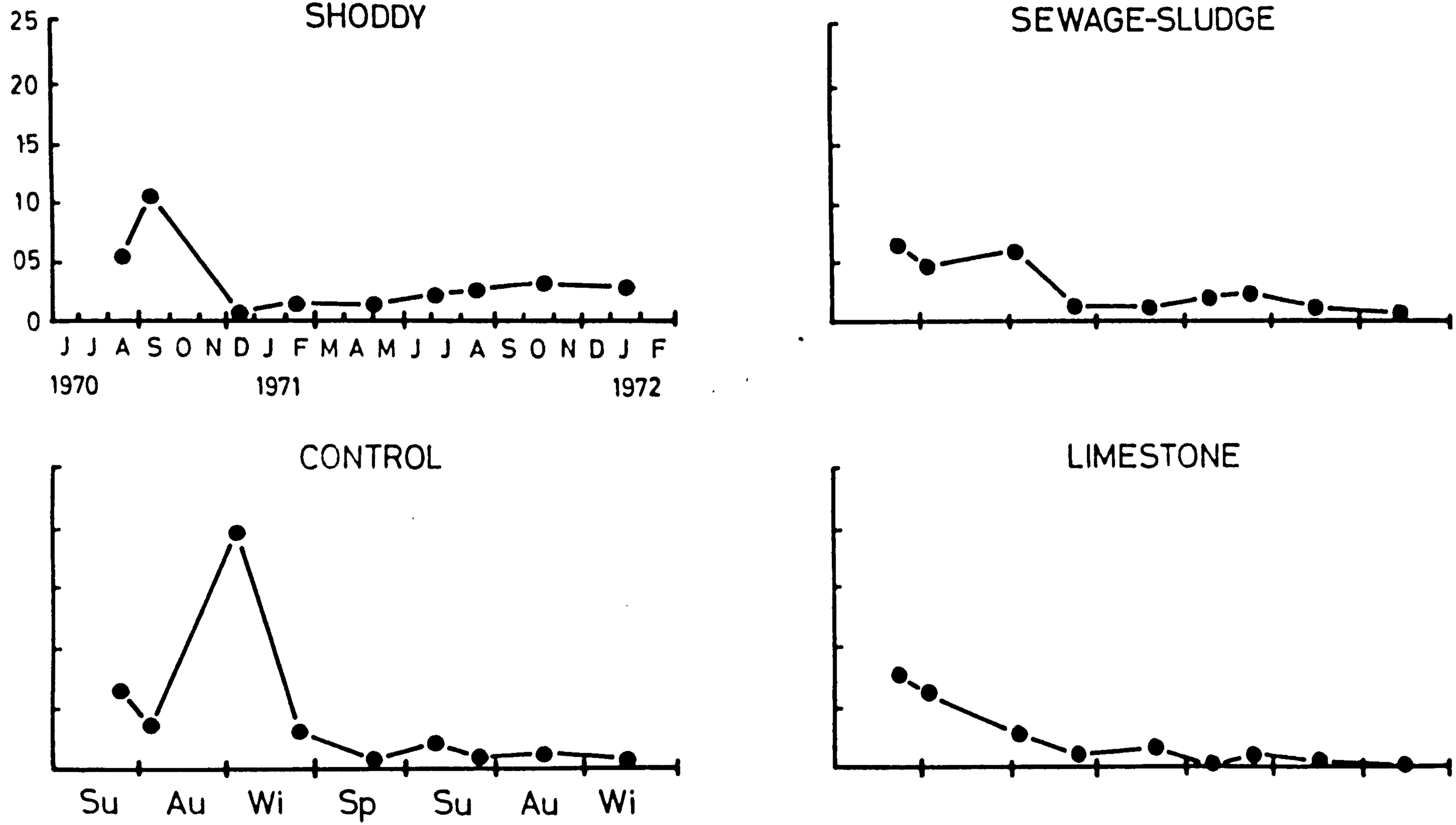


Fig. 3.11. Upton. Seasonal variation of iron.

Table 3.11. Upton. Iron. Statistical analysis.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots	0.4660	3	0.0155	0.448	ns
Blocks	0.5217	2	0.0261	0.753	ns
Error a	0.2080	6	0.0347		
Total	0.3067	11			
Sub-plots	0.9146	8	0.1143	4.281	***
Interaction	0.7400	24	0.0308	1.155	ns
Error b	1.7090	64	0.0267		
Total	3.6703	107			

MEANS TABLES

Main Plots		Exp. No.	Sub Plots	
Shoddy	1.13 (0.30)	1	Su	1.29 (0.66)
Sewage-sludge	1.15 (0.33)	2	Au	1.26 (0.64)
Control	1.17 (0.44)	3	Wi	1.25 (0.72)
Limestone	1.11 (0.25)	8	Au	1.09 (0.20)
		7	Su	1.09 (0.18)
		6	Su	1.08 (0.17)
Blocks		4	Wi	1.07 (0.15)
I	1.16 (0.42)	5	Sp	1.06 (0.12)
II	1.14 (0.33)	9	Wi	1.05 (0.12)
III	1.11 (0.24)			

LSR ($p = 0.05$)
0.215

DISCUSSION

The form in which iron occurs is important in relation to its possible toxicity. Thus ferric iron is not as toxic as ferrous iron, Harding (1970). Because of the weathering reactions of colliery spoil ferrous iron is likely to exist in colliery spoil Palmer (personal communication).

Martin (1968) showed that for some plants 10ppm of iron was very toxic. Harding (1970) found that the levels of iron in saturated paste extracts of an acid moorland and a commercial soil (John Innes) were equivalent to 0.44 and 0.30ppm respectively. The mean values for the two sites were Mitchell's Main 1.43ppm and Upton 0.33ppm. The level of iron at Upton was certainly not toxic or deficient. Although the level at Mitchell's Main was higher than that recorded in the two soil types, it was not particularly high, and the high levels of calcium in the spoil probably prevented the development of iron toxicity (Olsen 1958).

7. SODIUM

INTRODUCTION

Sodium is not usually considered to be an essential plant nutrient although some plants grow more vigorously in its presence (Russell 1961). It is considered to be important because it is a major constituent of the soluble salts of saline soils and salinity has been considered to be an important factor in the infertility of colliery spoil (Knabe 1965).

RESULTS AND INTERPRETATION

The results and statistical analyses are shown in Figs. and Tables 3.12 & 3.13.

Mitchell's Main

A significant main plot variance ratio is not observed. The significant block variance ratio is due to the higher levels of sodium in samples from the plots of block I.

The orthogonal comparisons indicate that the levels of sodium were higher in summer and autumn than in spring and winter. Although this shows that there was some seasonal variation, the proportion of the sub-plot sum of squares represented by this comparison is much smaller than occurred for many of the elements previously discussed and hence indicates that the seasonal variation was not particularly obvious.

Upton

The main plot and block variance ratios are not large enough to be significant.

The highly significant sub-plot variance ratio results largely from the differences between the first few samplings and subsequent ones. The level of sodium was initially very high but dropped rapidly in all treatments over a relatively short period of time.

Mitchell's Main and Upton comparison

After the initial decreases the levels of sodium remained higher at Upton than at Mitchell's Main.

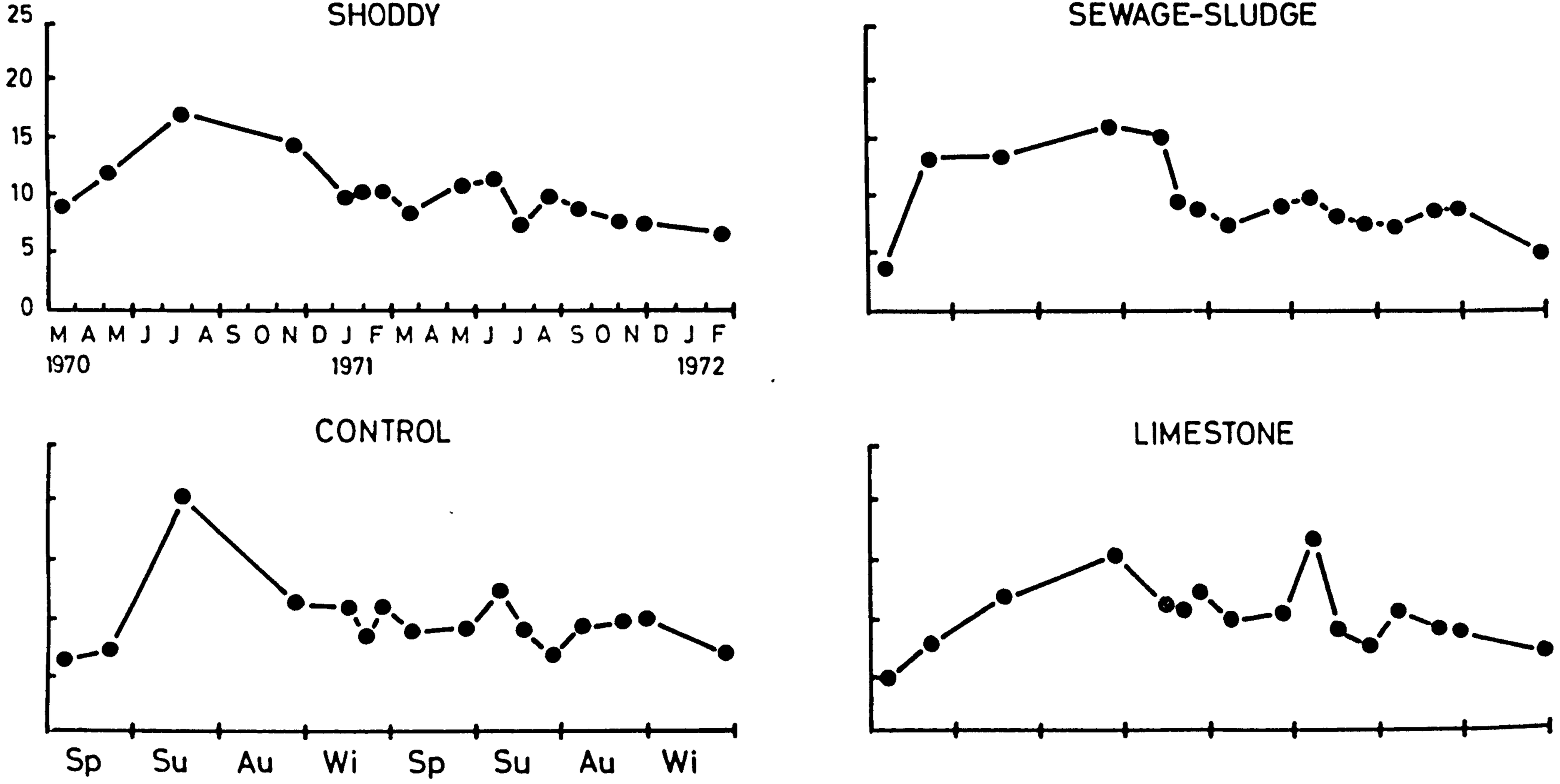


Fig. 3.12. Mitchell's Main. Seasonal variation of sodium.

Table 3.12. Mitchell's Main. Sodium. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	18.124	3	6.041	0.435	ns
Blocks	425.585	2	212.793	15.338	**
Error a	83.241	6	13.874		
Total	526.951	11			
Sub-plots	1219.956	15	81.330	8.434	***
Spring & Winter vs Summer & Autumn	139.33	1	139.33	14.448	***
Spring vs Winter	30.1056	1	30.1056	3.122	ns
Summer vs Autumn	19.984	1	19.984	2.072	ns
Interaction	525.698	45	11.682	1.211	ns
Error b	1157.214	120	9.643		
Total	3429.819	191			

MEANS TABLES

Main Plots

Shoddy	10.00
Sewage-sludge	9.87
Control	9.68
Limestone	10.50

Blocks

I	1.12
II	9.34
III	9.00

LSD (p = 0.05) 1.61

Sub-Plots

					Overall mean
Spring	6.15	9.33	8.84	10.10	8.61
Summer	15.80	12.80	8.52	8.18	11.33
Autumn	14.40	9.11	9.03	9.11	10.41
Winter	11.80	9.77	10.80	6.53	9.73

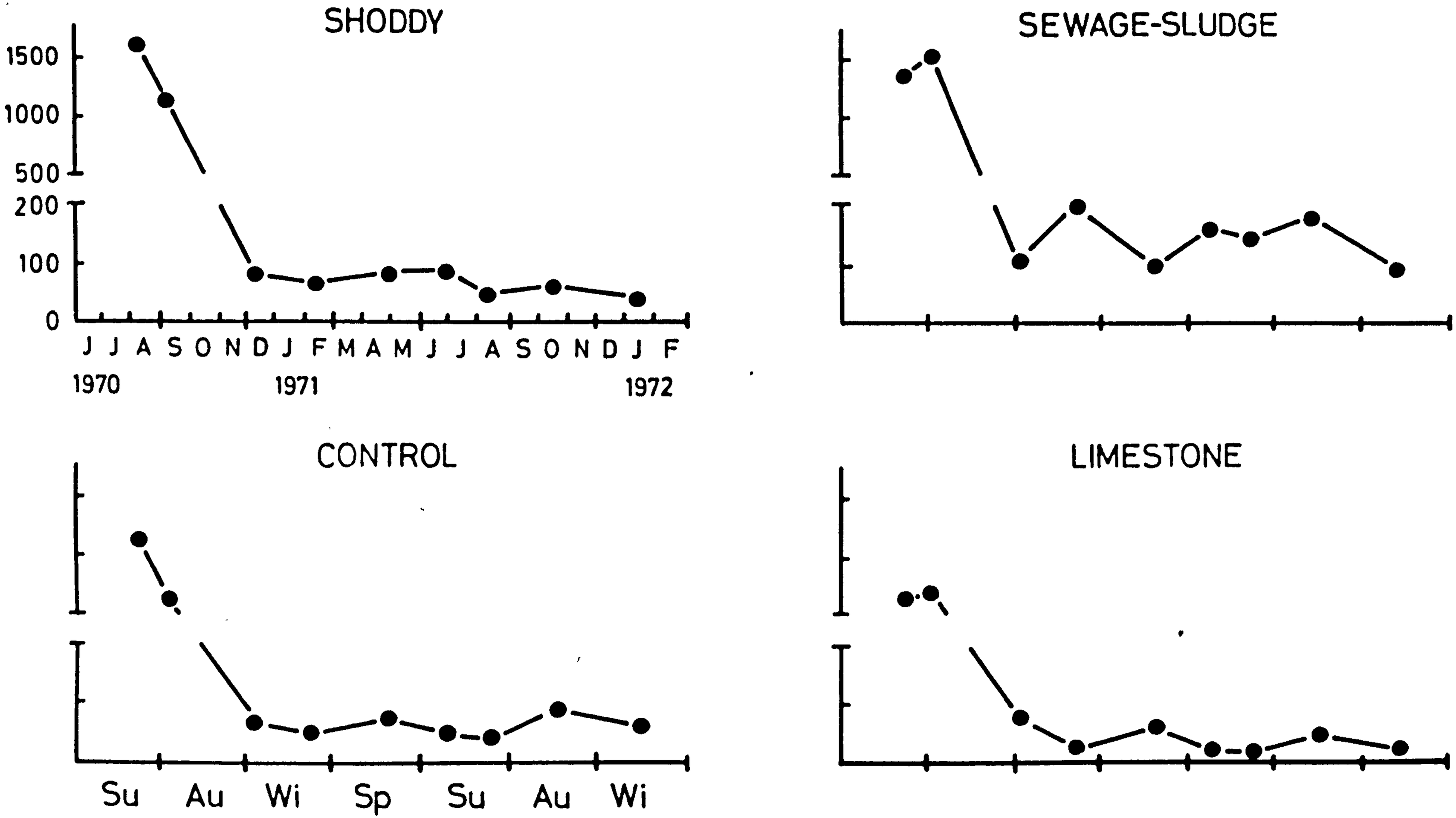


Fig. 3.13. Upton. Seasonal variation of sodium.

Table 3.13. Upton. Sodium. Statistical analysis.

VARIANCE TABLE (log_{10} transformation)

Source	ss	df	ms	vr	sig.
Main plots	3.8670	3	1.2890	3.437	ns
Blocks	2.6800	2	1.3400	3.573	ns
Error a	2.2503	6	0.3750		
Total	8.7972	11			
Sub-plots	25.8296	8	3.2287	81.473	***
Interaction	1.8026	24	0.0751	1.895	*
Error b	2.5362	64	0.0396		
Total	38.9657	107			

MEANS TABLES

Main Plots		Exp No.	Sub-Plots		
Shoddy	2.01 (345.7)	1	Su	2.93	(1162.0)
Sewage-sludge	2.27 (409.8)	2	Au	2.83	(972.7)
Control	1.86 (241.6)	3	Wi	1.88	(78.0)
Limestone	1.77 (175.9)	8	Au	1.81	(86.5)
		4	Wi	1.73	(80.1)
Blocks		5	Sp	1.72	(75.1)
I	1.81 (126.3)	6	Su	1.72	(74.2)
II	1.93 (255.3)	7	Su	1.60	(60.0)
III	2.19 (498.3)	9	Wi	1.50	(50.2)

LSR ($p = 0.05$)
0.261

DISCUSSION

More than 345ppm of sodium in a saturated paste extract is considered to be excessive for plant growth (Chapman & Pratt 1961). At Mitchell's Main the mean value was only of the order of 10ppm and therefore unimportant.

At Upton, the initial values were approximately 1000ppm and had these very high levels remained they would certainly have presented a threat to the vegetation. The levels did, however, fall rapidly and become stable at about 50ppm, an insignificant level.

It has often been observed that freshly deposited or newly exposed (i.e. unweathered) colliery spoil is highly saline. Since much of the salinity is attributable to sodium chloride (Palmer 1972) which is readily soluble and hence easily leached, the salinity usually decreases markedly over the first winter. This was the case at Upton. It is now common practice to allow saline spoils to overwinter before attempts are made to establish a cover of vegetation on them in reclamation schemes.

It is interesting to note that in the case of the field trial at Upton, no harmful effects of high sodium levels were observed even though the seeds were sown when the levels were in excess of 1000ppm. This was probably due to the fact that leaching was very rapid at this site and that the seeds were sown in autumn. If the field trials had been established in spring, detrimental effects may have occurred.

8. POTASSIUM

INTRODUCTION

Potassium is one of the three essential nutrients that plants require in fairly large quantities.

Doubleday (1972b) commented that "There is seldom any great lack of available potash (in colliery spoil) and additions occasionally depress plant growth". Coates (1964) however, found that most plant species growing on spoil heaps in Lancashire were suffering from potassium deficiency and other workers (Beyer & Hutnik 1969; Knabe 1965) have attributed poor plant growth to potassium deficiency.

RESULTS AND INTERPRETATION

The results and statistical analyses are shown in Figs. and Tables 3.14 & 3.15.

Mitchell's Main

Significant main plot and block variance ratios are not observed.

The orthogonal comparisons show that the levels of potassium were higher in spring and winter than summer and autumn and this was largely due to high levels recorded in the spring months.

Upton

The significant main plot variance ratio is attributable to the higher levels of potassium found in samples from the shoddy plots. The three other treatments contained insignificantly different levels of potassium.

The sub-plot variance ratio is highly significant and appears to result in part, from the gradual fall in potassium levels after the second sampling date. This decrease was probably the combined result of plant uptake and leaching losses.

The levels of potassium increased in all treatments between the first and second sampling dates. This suggests that initial weathering reactions may have lead to the release of potassium.

Mitchell's Main and Upton comparison

The levels of potassium at Upton were higher than those at Mitchell's Main. The apparent effect of shoddy on the potassium status of the spoil at Upton was not observed at Mitchell's Main.

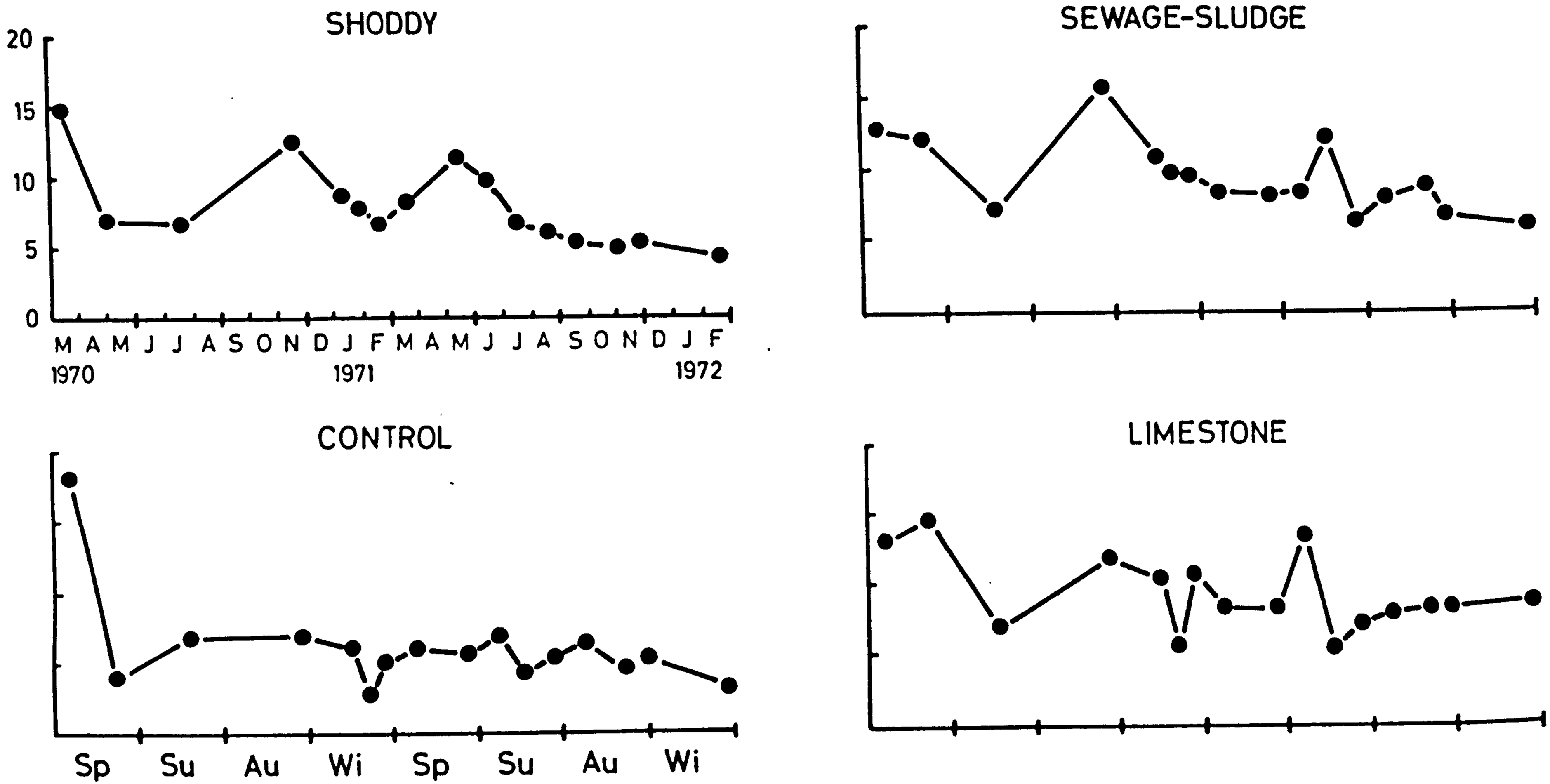


Fig. 3.14. Mitchell's Main. Seasonal variation of potassium.

Table 3.14. Mitchell's Main. Potassium. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	397.215	3	132.405	1.571	ns
Blocks	62.959	2	31.479	0.373	ns
Error a	505.714	6	84.286		
Total	965.888	11			
Sub-plots	979.301	15	65.287	8.407	***
Spring & Winter vs Summer & Autumn	33.768	1	33.768	4.348	*
Spring vs Winter	191.196	1	191.196	24.619	***
Summer vs Autumn	1.750	1	1.750	0.225	ns
Interaction	558.229	45	12.405	1.597	
Error b	931.940	120	7.766		
Total	3435.358	191			

MEANS TABLES

Main Plots

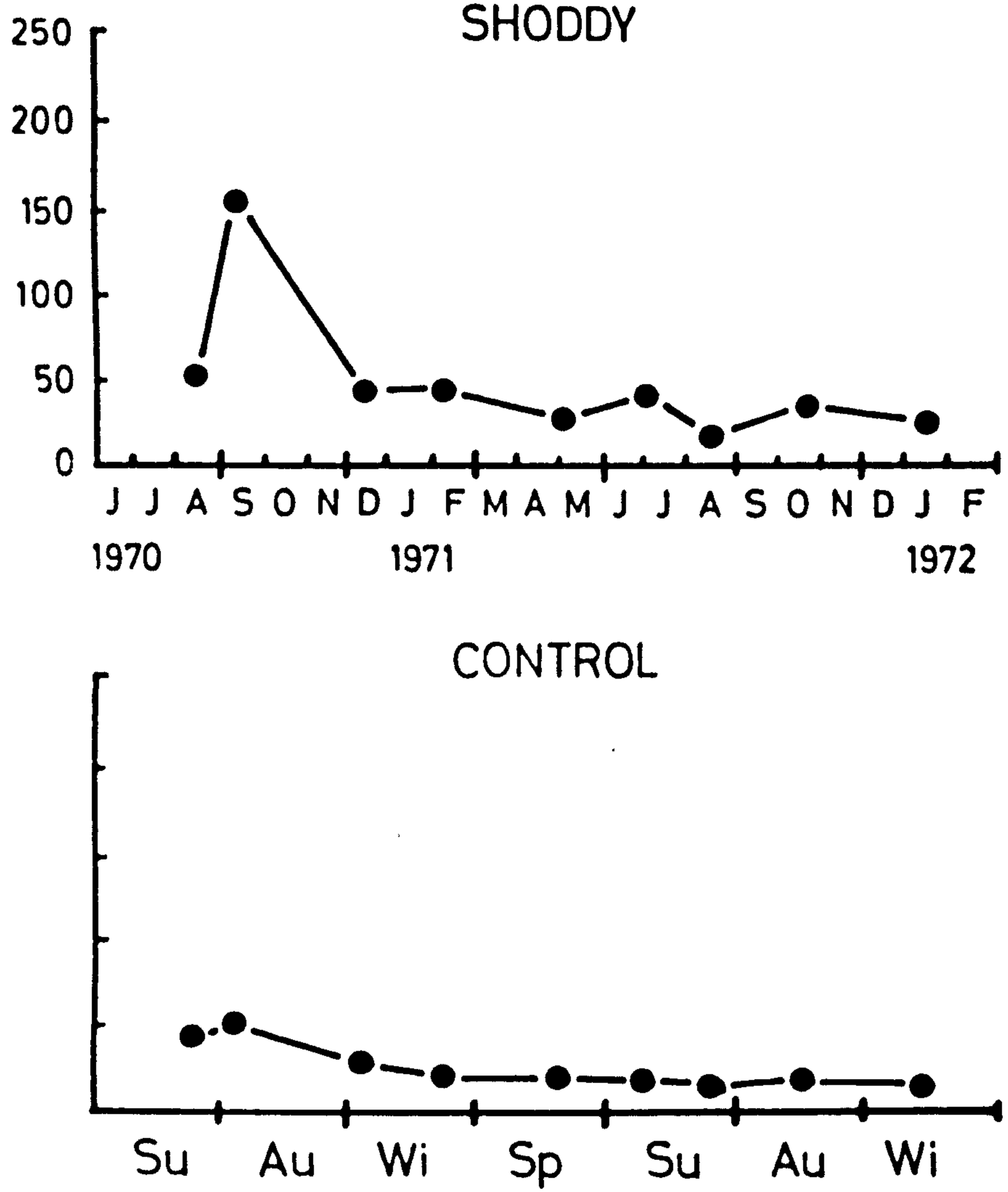
Shoddy	7.71
Sewage-sludge	9.47
Control	6.07
Limestone	9.58

Blocks

I	7.78
II	7.83
III	9.02

Sub-Plots

					Overall mean
Spring	14.70	9.33	7.85	8.28	10.04
Summer	7.07	9.51	7.78	6.26	7.66
Autumn	11.80	6.82	6.64	6.44	7.93
Winter	8.89	6.47	8.07	5.44	7.22



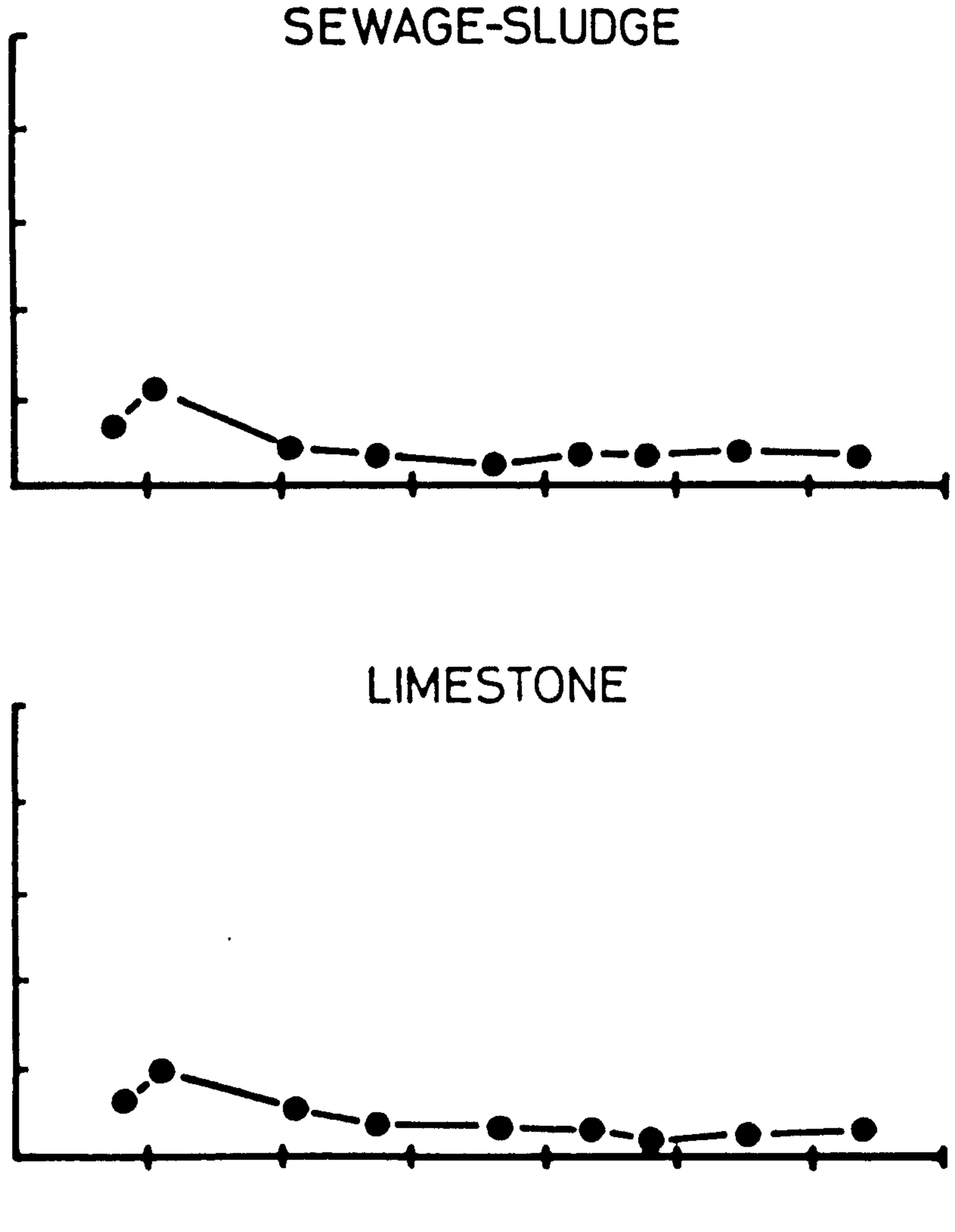


Fig. 3.15. Upton. Seasonal variation of potassium.

Table 3.15. Upton. Potassium. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main Plots	12244.5	3	4081.5	12.081	**
Blocks	729.642	2	364.82	1.080	ns
Error a	2027.139	6	337.857		
Total	15001.296	11			
Sub-plots	35468.0	8	4433.500	48.141	***
Interaction	16952.78	24	706.3659	7.670	***
Error b	5893.965	64	92.0932		
Total	73316.043	107			

MEANS TABLES

Main Plots	
Shoddy	48.8
Sewage-sludge	24.7
Control	26.8
Limestone	22.1

LSD (p = 0.05)
12.24

Blocks	
I	34.21
II	29.49
III	28.13

Exp. No.	Sub-Plots	
2	Au	77.7
1	Su	41.5
3	Wi	28.6
4	Wi	25.8
6	Su	24.5
8	Au	24.5
5	Sp	19.3
9	Wi	18.2
7	Su	15.4

LSR (p = 0.05)
12.60

DISCUSSION

In saturated paste extracts of soil, 40-200ppm is the acceptable concentration range for the growth of most plant species (Chapman & Pratt 1961). The mean value for Mitchell's Main was 8.2ppm and for Upton, excluding the shoddy plots, was 24.5ppm. The mean value for the shoddy plots was 48.8ppm. For all treatments at Upton, however, the levels of potassium decreased with time and towards the end of the sampling programme, the concentrations were much smaller than the mean values quoted above.

The low levels of potassium at both sites indicated that the unameliorated spoils could provide little of this nutrient for plant growth. It is not possible to state at what concentration potassium becomes growth limiting because this depends upon the availability of other nutrients, especially nitrogen and phosphorus.

Harding (1970) examined the potassium levels in saturated paste extracts of a number of spoils, including Mitchell's Main, and soils. This worker's results showed that the levels were lower in spoils than soils and this low availability was reflected by a low concentration of potassium in plants growing in the spoils. Further, Harding (1970) noted that the plant isolated from colliery spoil heaps, failed to respond to all but low concentrations of potassium in nutrient culture experiments and concluded from this that they were adapted to growth at low potassium levels.

The source of the potassium in the shoddy plots is of interest because shoddy itself contains negligible quantities of this nutrient (Analysis of the shoddy applied to the plots at Mitchell's Main indicated that the percentage of potassium in the material was only 0.6%, personal communication with the manufacturers, J.S. Hardy & Co., Morley). Potassium analyses were not performed on the shoddy samples used on the field at Upton but the manufacturers (personal communication with H.H. Haig, Springdale Mills, Huddersfield) knew of no reason why the levels should be higher than the values quoted above. It must, therefore, be assumed that either the shoddy became 'contaminated' with potassium during transportation or spreading on the trial or that the results were due to chance variation in the spoil at Upton.

It can be concluded that the availability of potassium was low at the two sites and that this may have contributed to the poor growth of the vegetation.

9. CALCIUM

INTRODUCTION

Calcium is a plant nutrient essential for the normal development of cellular membrane systems.

Deficiencies of this element are of frequent occurrence in leached acid soils and result in abnormal plant growth.

High calcium levels may have detrimental effects on the uptake of magnesium and potassium (Russell 1961) and iron, manganese, copper, boron and zinc (Bould 1963). Such effects are not, however, of common occurrence in neutral or acid soils.

RESULTS AND INTERPRETATION

The results and statistical analyses are shown in Figs. and Tables 3.16 & 3.17.

Mitchell's Main

Despite the fact that large quantities of calcium were added to the limestone plots, these plots did not contain significantly higher levels of calcium than the others. In fact, the main plot variance ratio is not significant.

A significant block variance ratio resulted from the high levels of calcium recorded in samples from block I.

The orthogonal comparisons show that the levels of calcium were higher in the summer and autumn than in winter and spring. The levels in winter were also significantly lower than those in spring. Since the sums of squares for the three comparisons represented

only a relatively small proportion of the sub-plot sum of square, factors in addition to seasonal variation were important in producing the very significant sub-plot variance ratio.

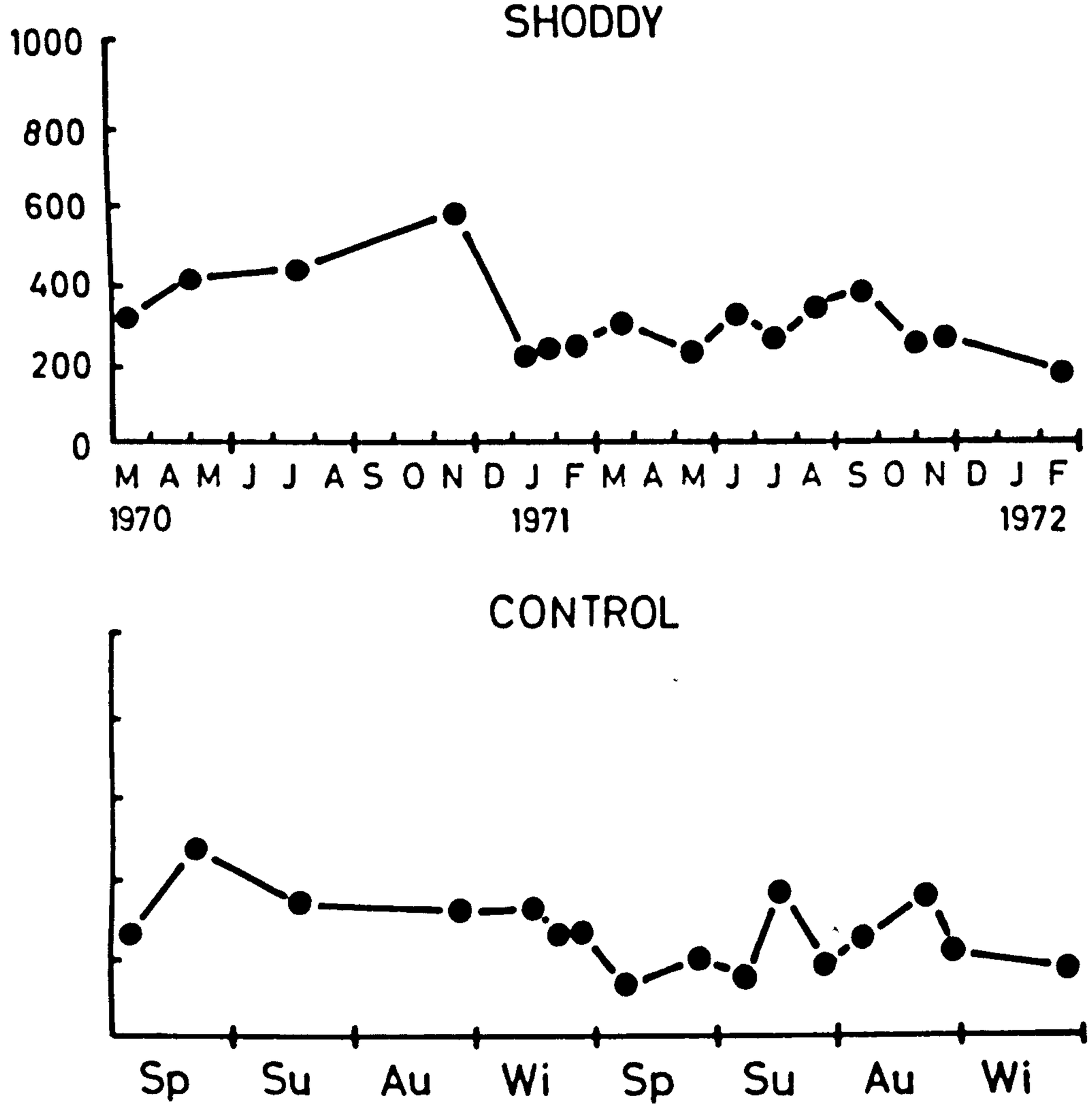
Upton

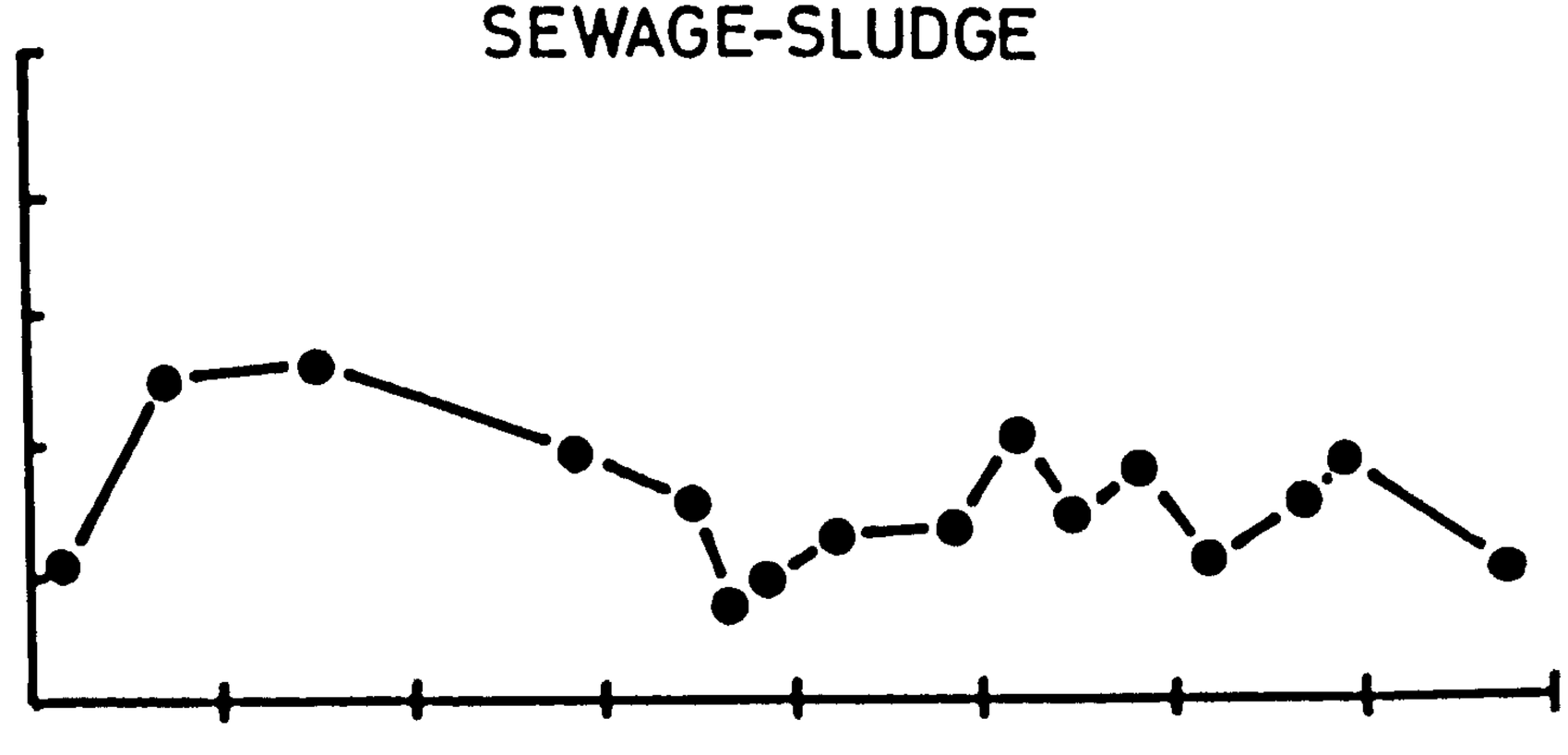
A significant main plot variance ratio is not observed and the significant block variance ratio is attributable to the high levels of calcium in block I samples.

As for many of the elements discussed at this site, the variation between sub-plots that resulted in the production of a significant sub-plot variance ratio was not related either to initial weathering or to seasonal effects and appeared to be of a random nature.

Mitchell's Main and Upton comparison

The levels of calcium at Upton were approximately double those at Mitchell's Main.





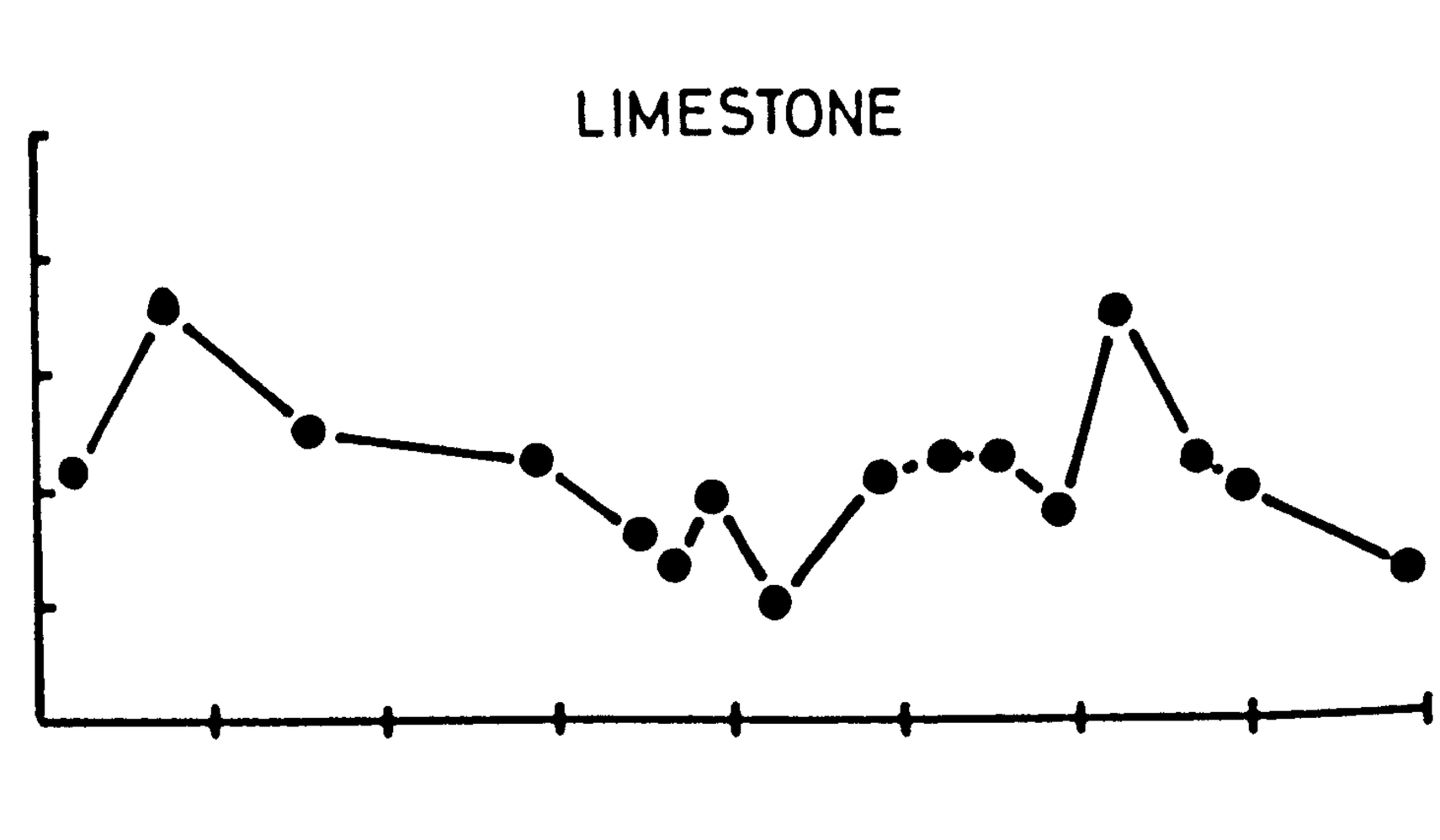


Fig. 3.16. Mitchell's Main. Seasonal variation of calcium.

Table 3.16. Mitchell's Main. Calcium. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	397483.6	3	132494.5	1.260	ns
Blocks	1578568.2	2	789284.08	7.508	*
Error a	630784.6	6	105130.8		
Total	2606836.3	11			
Sub-plots	924941.0	15	61662.7	5.899	***
Spring & Winter vs Summer & Autumn	179646.8	1	170646.8	16.327	***
Spring vs Winter	112614.0	1	112614.0	10.774	**
Summer vs Autumn	1633.5	1	1633.5	0.156	ns
Interaction	614133.9	45	13647.4	1.306	**
Error b	1254300.6	120	10452.5		
Total	5400211.8	191			

MEANS TABLES

Main Plots

Shoddy	258
Sewage-sludge	254
Control	215
Limestone	340

Blocks

I	395
II	206
III	200

LSD (p = 0.05) 140.3

Sub-Plots

					Overall mean
Spring	249	427	182	227	271
Summer	365	272	279	254	293
Autumn	355	318	279	251	301
Winter	253	182	218	158	203

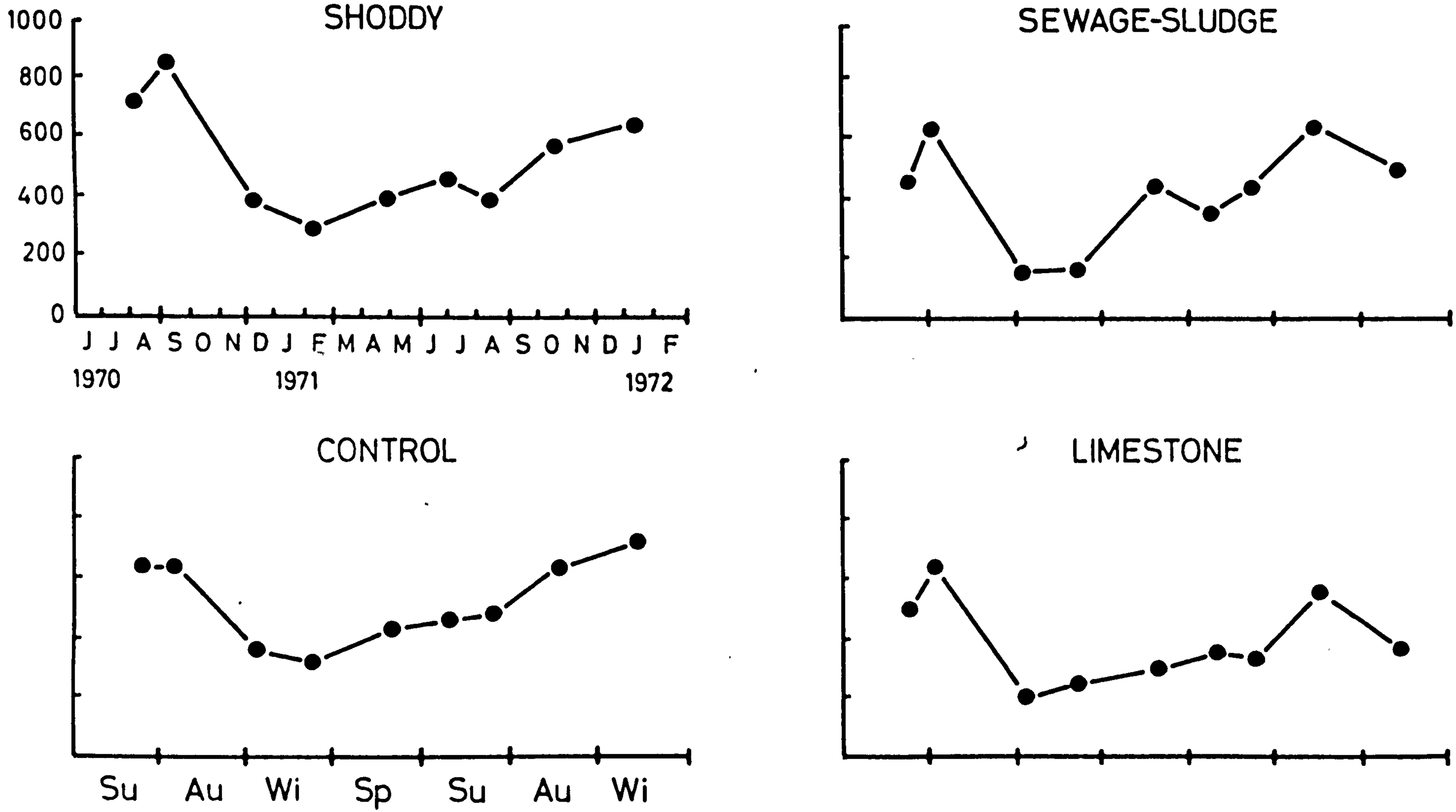


Fig. 3.17. Upton. Seasonal variation of calcium.

Table 3.17. Upton. Calcium. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	331844.96	3	110615.00	2.576	ns
Blocks	1149753.60	2	574876.79	13.390	*
Error a	257607.76	6	42934.63		
Total	1739206.30	11			
Sub-plots	2115238.00	8	264404.75	16.267	***
Interaction	313488.70	24	13062.03	0.804	ns
Error b	1040288.70	64	16254.51		
Total	5208221.6	107			

MEANS TABLES

Main Plots	
Shoddy	523
Sewage-sludge	420
Control	500
Limestone	388

Blocks	
I	564
II	490
III	318

LSD (p = 0.05) 119.5

Exp. No.	Sub-Plots	
2	Au	687
8	Au	605
1	Su	581
9	Wi	509
7	Su	414
6	Su	406
5	Sp	389
3	Wi	273
4	Wi	256

LSR (p = 0.05) 167.5

DISCUSSION

The levels of calcium in saturated paste extracts of soils vary between 2-200ppm; the satisfactory range for most plants being between 20-200ppm (Chapman & Pratt 1961). The mean values for the two sites were: Mitchell's Main 267ppm, and Upton 457ppm.

Since it is well known that high calcium levels can alleviate the injurious effects of low pH (Arnon & Johnson 1942, Raine *et al.* 1964, Sutton & Hallsworth 1958), the high levels of calcium at Mitchell's Main may have been beneficial. It is interesting to note that the highest levels of calcium were found in block I, where it was previously shown that manganese concentrations were also highest. Calcium may therefore have reduced the development or severity of manganese toxicity.

At Upton, the levels of calcium were generally outside the range quoted by Chapman & Pratt (1961) as being suitable for the growth of most plant species. This is probably not of major importance because the spoils were of neutral rather than alkaline reaction and hence interferences by calcium on the uptake of other nutrients would not be important. Further, although the high levels of calcium would contribute to the total soluble salt content of the spoil, the levels of the other salts that could cause a salinity problem, namely, sodium and magnesium were not particularly high (except the initial sodium levels) and salinity does not appear to have been a major factor

at Upton.

The situation at Mitchell's Main where the spoil was acid but still contained very high levels of calcium contrasts sharply with the normal situation in acid soils where the calcium levels may be very low and deficient.

10. MAGNESIUM

INTRODUCTION

Magnesium is the only mineral constituent of the chlorophyll molecule and is, therefore, essential for all green plants.

Deficiencies of this nutrient commonly occur in acid soils where the situation may be accentuated if ammonium is the predominant form of mineral nitrogen (Mulder 1956).

RESULTS AND INTERPRETATION

The results and statistical analyses are shown in Figs. and Tables 3.18 & 3.19.

Mitchell's Main

A significant main plot variance ratio is not observed and the higher levels of magnesium in samples from block I were largely responsible for the significant sub-plot variance ratio.

The orthogonal comparisons indicate that the levels of magnesium were significantly higher in the summer and autumn months than the spring and winter ones. In this case these comparisons account for nearly 70% of the sub-plot sum of squares showing that seasonal variation was the main factor involved in the production of the significant sub-plot variance ratio.

Upton

The main plot variance ratio was insignificant and the significant block variance ratio represented a difference between blocks I and III, the former having the highest concentrations of magnesium.

The significant sub-plot variance ratio once again did not indicate a definite seasonal variation or progressive weathering reactions.

Mitchell's Main and Upton comparison

In terms of levels of magnesium, the two sites were similar. Seasonal variation was, however, well marked at Mitchell's Main but not at Upton.

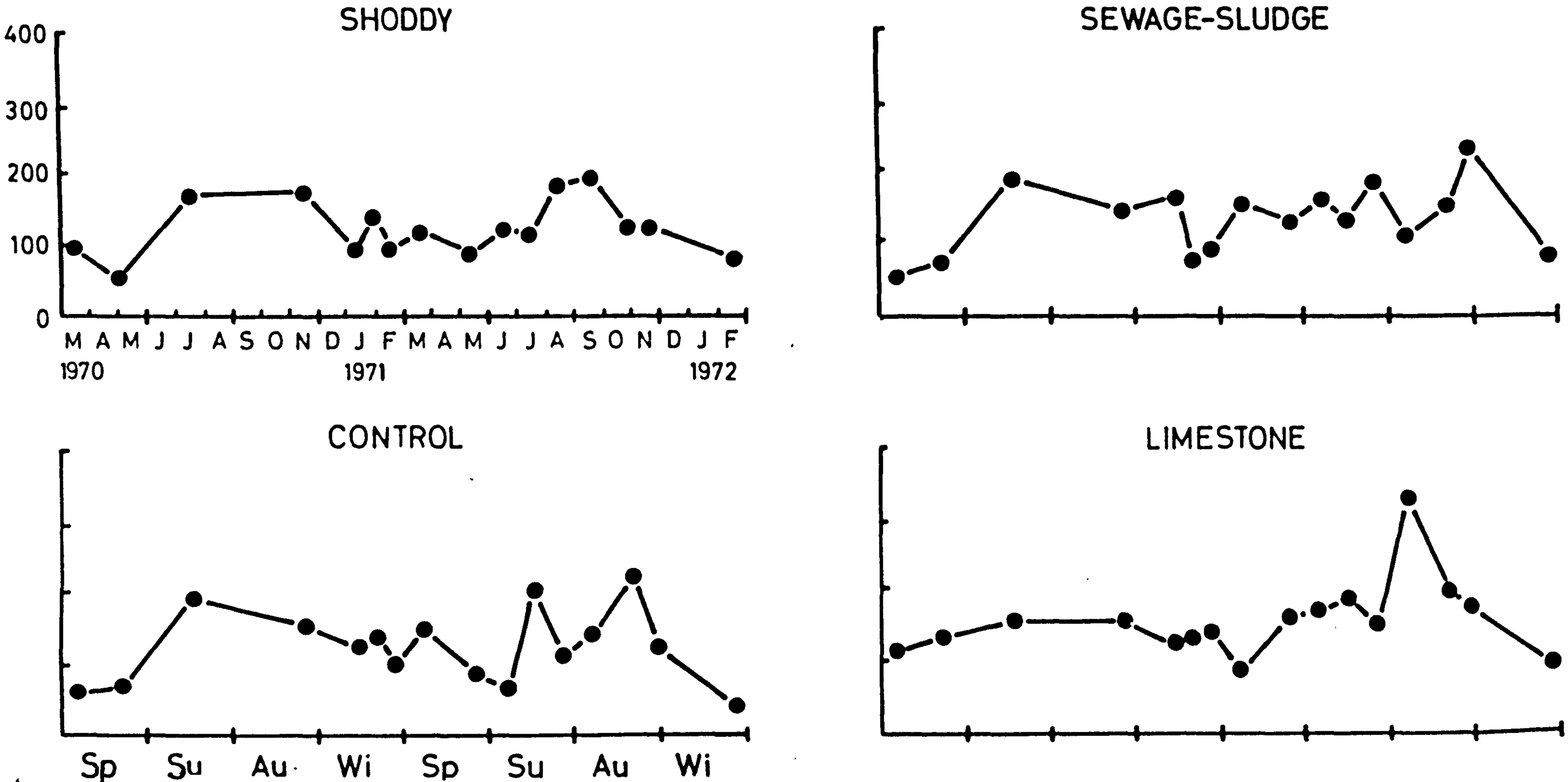


Fig. 3.18. Mitchell's Main. Seasonal variation of magnesium.

Table 3.18. Mitchell's Main. Magnesium. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	23587.8	3	7862.6	0.284	ns
Blocks	314741.6	2	157370.8	5.685	*
Error a	166096.7	6	27682.8		
Total	504426.1	11			
Sub-plots	255445.1	15	17029.7	5.286	***
Spring & Winter vs Summer & Autumn	163987.3	1	163987.3	50.900	***
Spring vs Winter	5364.1	1	5364.1	1.66	ns
Summer vs Autumn	937.5	1	937.5	0.29	ns
Interaction	265517.4	45	5900.4	1.831	*
Error b	386604.4	120	3221.7		
Total	1411993.0	191			

MEANS TABLES

Main Plots

Shoddy	124
Sewage-sludge	131
Control	117
Limestone	147

Blocks

I	187
II	105
III	98

LSD ($p = 0.05$) 72

Sub-Plots

					Overall mean
Spring	71.0	83.8	103.0	114.0	93.0
Summer	177.0	129.0	159.0	158.0	155.8
Autumn	121.0	194.0	172.0	161.0	162.0
Winter	131.0	122.0	107.0	71.6	107.9

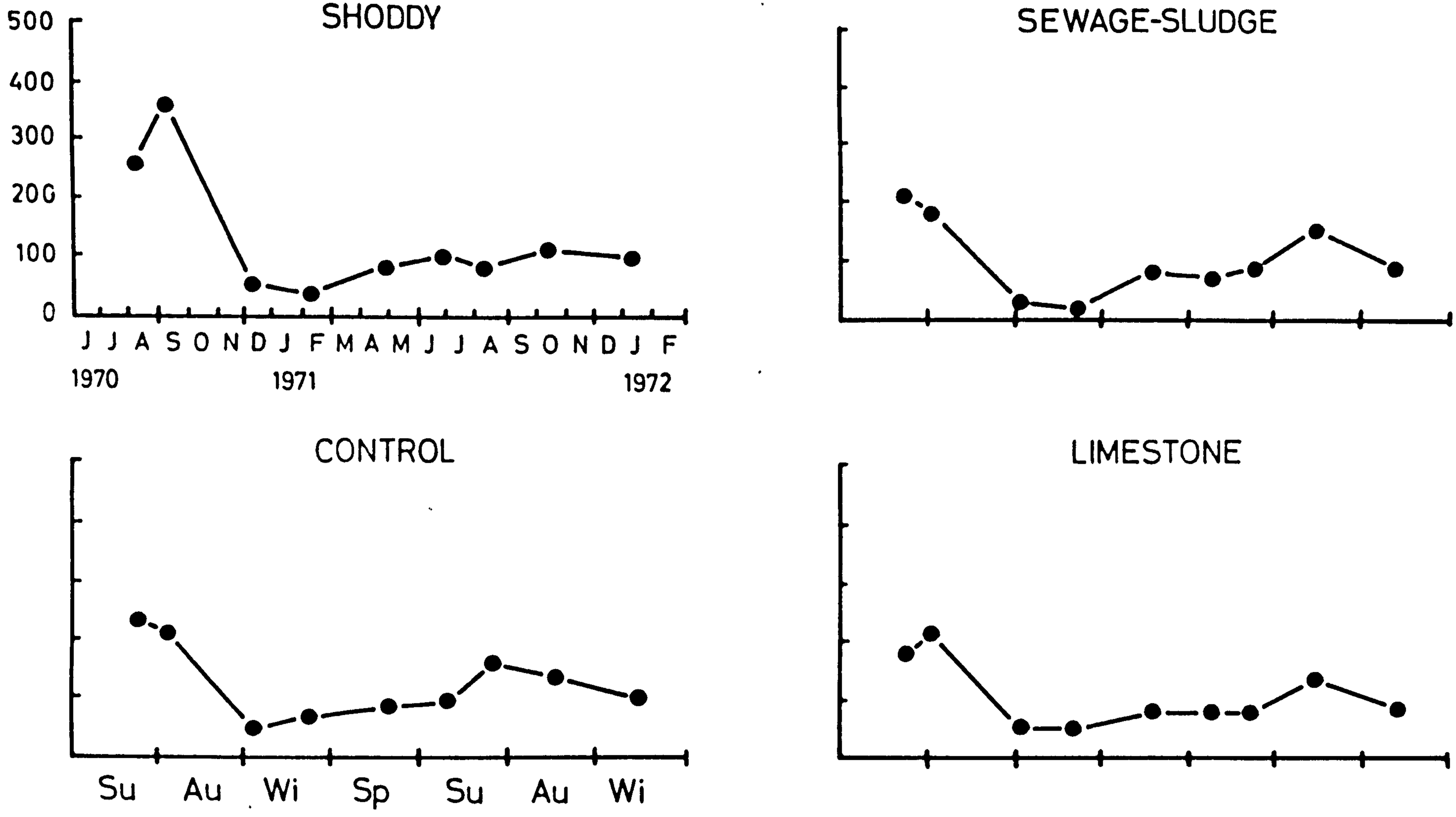


Fig. 3.19. Upton. Seasonal variation of magnesium.

Table 3.19. Upton. Magnesium. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig
Main plots	13432.4	3	4477.5	0.809	ns
Blocks	62735.4	2	31367.7	5.67	*
Error a	33209.1	6	5534.9		
Total	109376.9	11			
Sub-plots	457053.3	8	57131.7	11.653	***
Interaction	72967.2	24	3040.3	0.620	ns
Error b	313786.8	64	4902.9		
Total	953184.3	107			

MEANS TABLES

Main Plots		Exp. No.	Sub-Plots	
Shoddy	133	2	Au	239
Sewage-sludge	104	1	Su	216
Control	120	8	Au	134
Limestone	109	7	Su	104
		9	Wi	93
		6	Su	87
		5	Sp	83
Blocks		4	Wi	47
I	146	3	Wi	45
II	116			
III	87			

LSD (p_{43} = 0.05)

LSR (p_{92} = 0.05)

DISCUSSION

In saturation extracts of soils, 25-60ppm is the satisfactory range of magnesium for most plants and concentrations in excess of 360ppm are considered toxic (Chapman & Pratt 1961). The mean values for the sites studied were: Mitchell's Main 130ppm and Upton 116ppm.

These results indicate that whilst the levels were higher than those found in normal soils they were not high enough to be directly toxic to plants.

11. SPOIL MOISTURE

INTRODUCTION

The availability of water in a soil or spoil is of fundamental importance to both the micro-, and macroflora. Cornwell (1971) and Richardson (1958) have indicated that the unavailability of water in spoils during the warmer periods of the year can result in poor plant growth and even death.

In the present investigations gravimetric determinations of spoil moisture were routinely made. Since, however, the proportion of the total water present in a spoil or soil that is available depends upon the nature of the spoil or soil (i.e. sandy or clayey), the relationship between gravimetric determinations and availability must be demonstrated. This was achieved by plotting the drying curves for the two spoil types and relating the percentage moisture to pF.

RESULTS AND INTERPRETATION

The results and statistical analyses are shown in Figs. and Tables 3.20 & 3.21. The data used in the statistical analysis was percentage water content. The drying curves are shown in Fig. 3.22.

Mitchell's Main

The drying curve indicates that the spoil at this site demonstrated drying characteristics intermediate between those possessed by sandy and loam soils.

Significant variance ratios are only associated with the sub-plot comparisons and indicate, as would be

expected that the spoil was wettest in the winter and driest in the summer months. Whilst the overall mean (14.5) indicates that the spoil was generally very near field capacity (14.8) individual values recorded in the summer and autumn periods showed that the spoil occasionally became very dry.

Upton

In the analysis of variance, significant main plot, sub-plot and block variance ratios are observed.

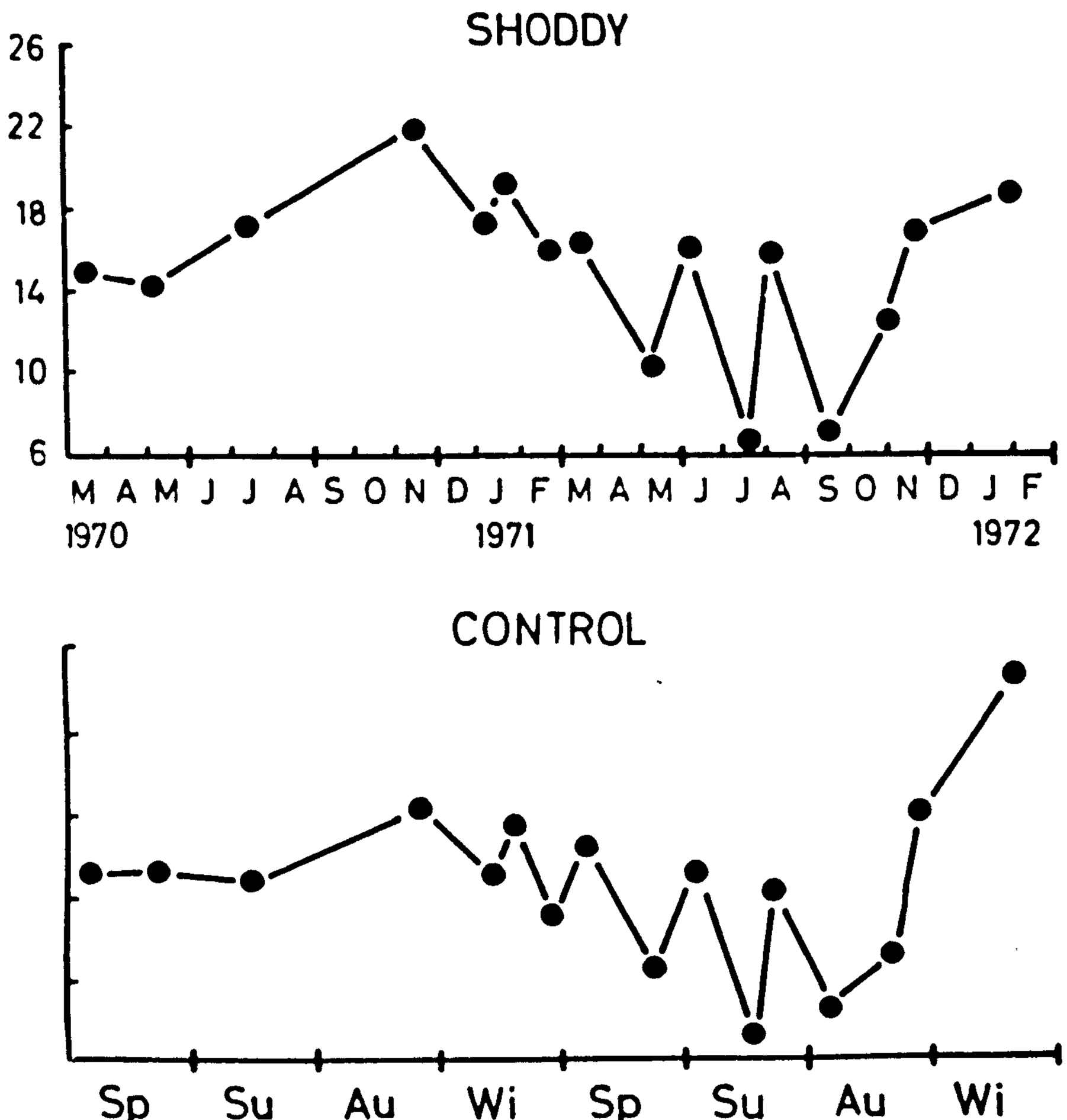
The significant main plot variance ratio is shown to represent a difference between the shoddy plots and the limestone ones, the former containing the higher percentage of water. This may have been related to the abundance of vegetation on the plots (see pages 255-273) since the shoddy plots supported significantly more vegetation than the others. Thus, although transpiration losses may have been greater on the shoddy plots, the greater insulation afforded by the vegetation would have reduced water loss. This is not a completely satisfactory argument, however, because the moisture regime of all three blocks was significantly different although the density of vegetation was similar. This suggests that the spoil was of variable physical composition over the trial area.

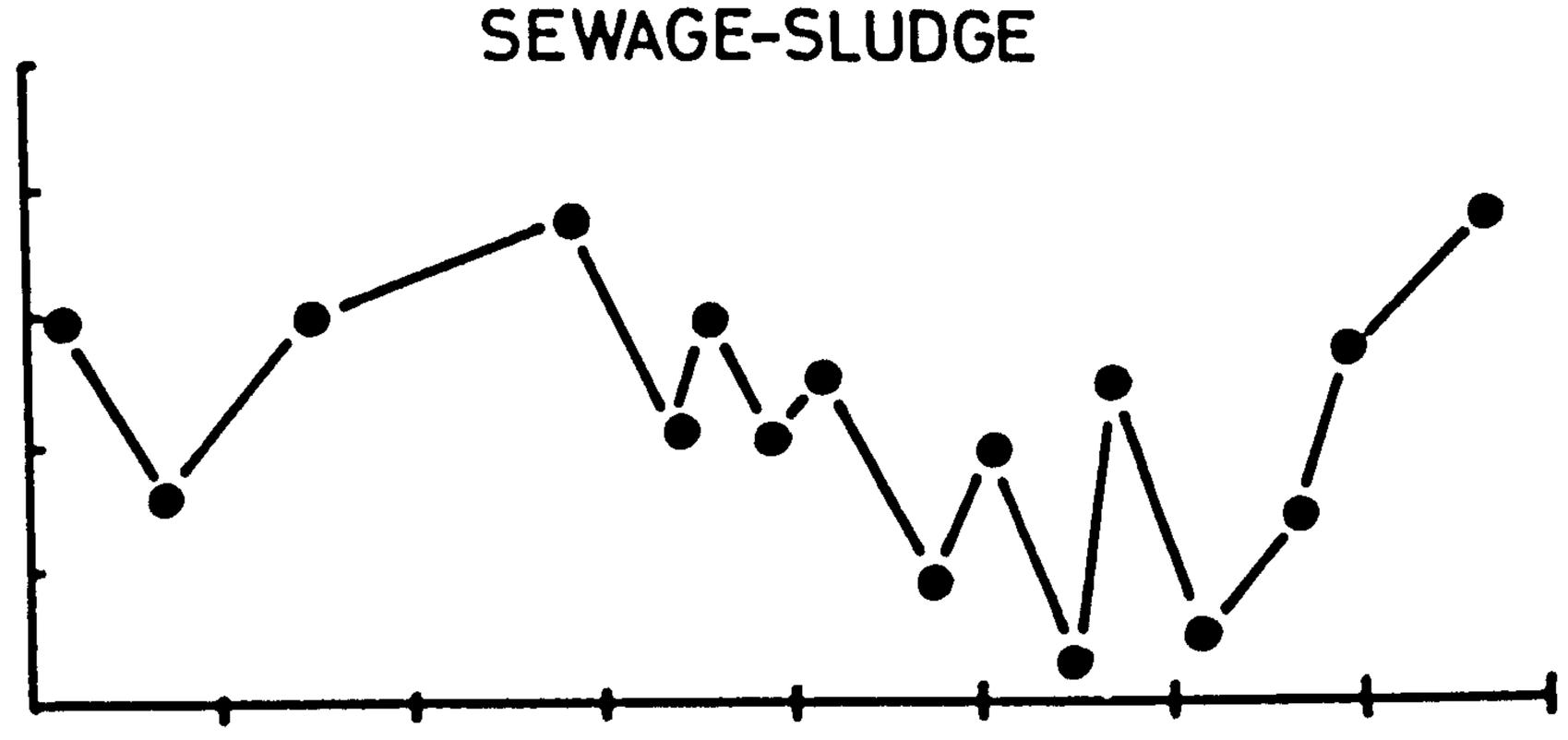
The significant sub-plot variance ratio indicates that the spoil samples taken in winter contained more water than those taken at other times. This seasonal variation would be expected.

The overall mean for this site (10.4) indicates that the spoil was generally below field capacity (13.5).

Mitchell's Main and Upton comparison

Although both spoils were similar and intermediate between a sandy and a loamy soil, Upton spoil was somewhat more sandy than Mitchell's Main, the percentage water contents at field capacity being 13.5 and 14.8 respectively.





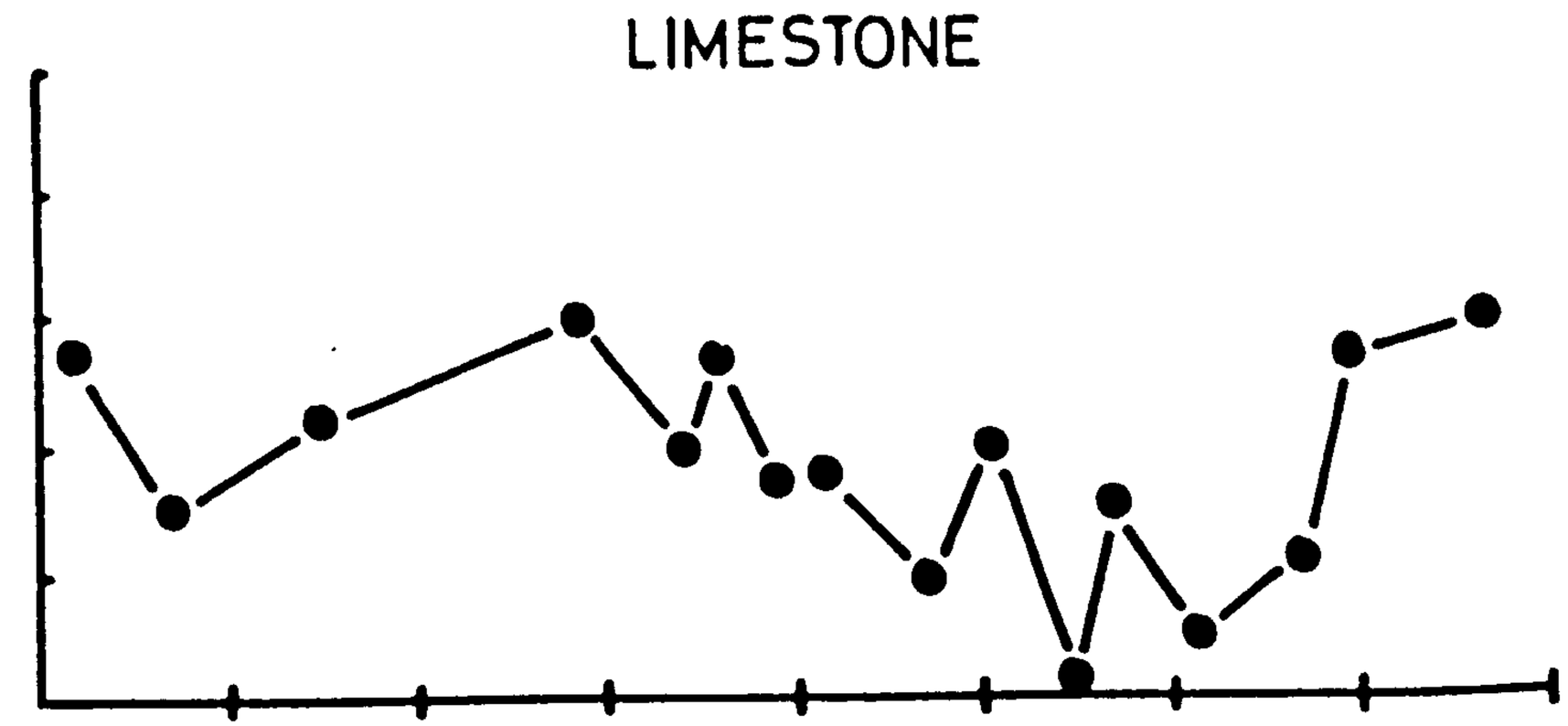


Fig. 3.20. Mitchell's Main. Seasonal variation of spoil moisture.

Table 3.20. Mitchell's Main. Spoil Moisture. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	55.8279	3	18.6093	0.868	ns
Blocks	156.9632	2	78.4816	3.659	ns
Error a	128.7030	6	21.4505		
Total	341.4942	11			
Sub-plots	2611.0283	15	174.0686	35.875	***
Spring & Winter vs Summer & Autumn	116.8752	1	116.8752	24.088	***
Spring vs Winter	194.9400	1	194.9400	40.176	***
Summer vs Autumn	22.0033	1	22.0033	4.535	ns
Interaction	189.4471	45	4.2099	0.8677	ns
Error b	582.2471	120	4.8521		
Total	3724.2167	191			

MEANS TABLES

Main Plots

Shoddy	15.0
Sewage-sludge	14.8
Control	14.6
Limestone	13.6

Blocks

I	15.8
II	14.5
III	13.9

Sub-Plots

					Overall mean
Spring	16.2	13.6	15.3	10.4	13.9
Summer	16.5	14.8	6.8	14.9	13.3
Autumn	8.1	19.9	11.5	17.3	14.2
Winter	15.0	17.6	13.9	20.4	16.7

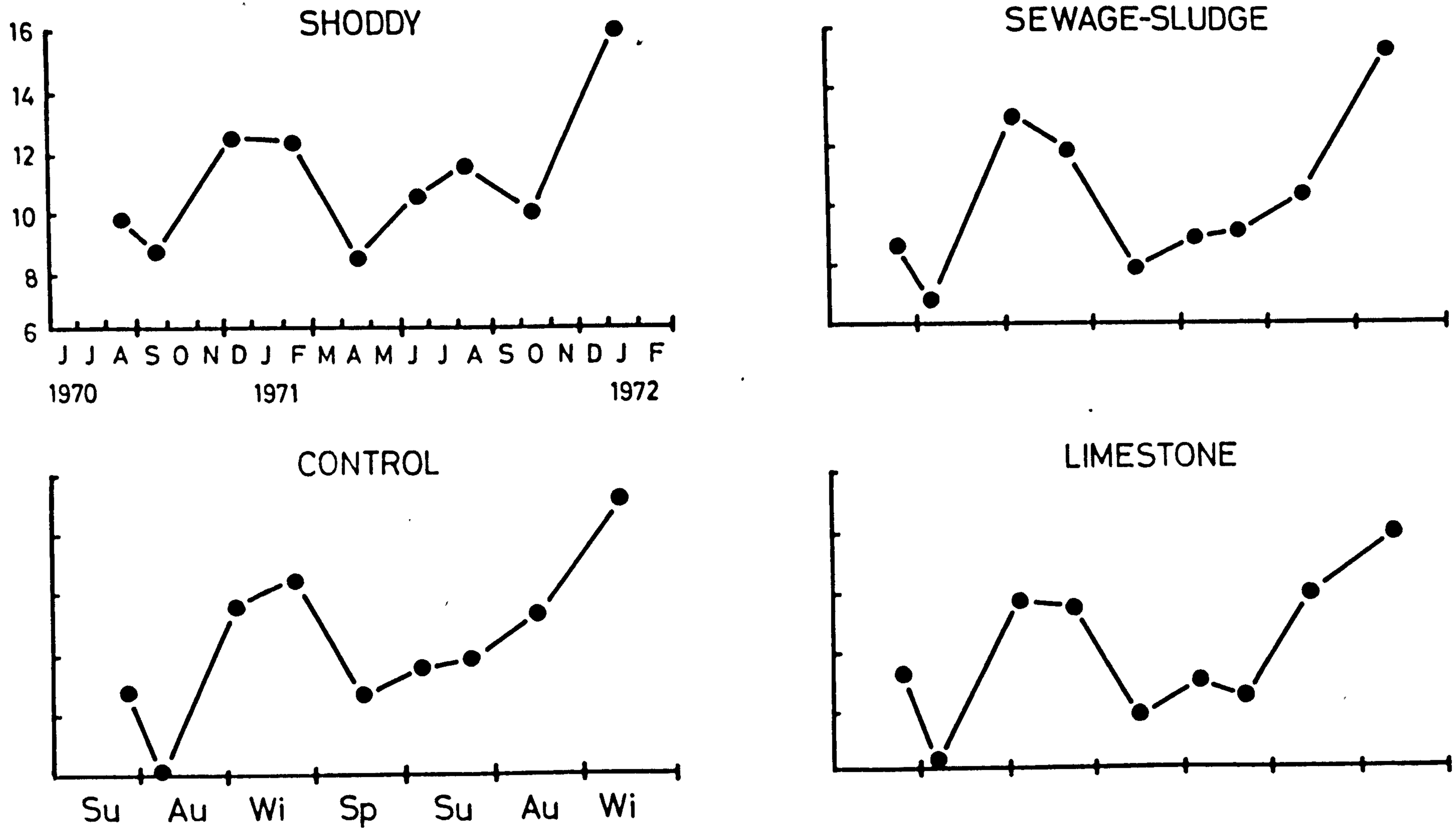


Fig. 3.21. Upton. Seasonal variation of spoil moisture.

Table 3.21. Upton. Spoil Moisture. Statistical analysis.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	16.5441	3	5.5147	6.378	*
Blocks	57.2600	2	28.6300	33.110	***
Error a	5.1881	6	0.8647		
Total	78.9922	11			
Sub-plots	573.0633	8	71.6329	46.153	***
Interaction	38.9493	24	1.6229	1.0456	ns
Error b	99.3319	64	1.5521		
Total	790.3367	107			

MEANS TABLES

Main Plots		Exp No.	Sub-Plots	
Shoddy	11.0	9	Wi	15.1
Sewage-sludge	10.2	3	Wi	12.1
		4	Wi	12.0
Control	10.4	8	Au	10.9
Limestone	9.9	7	Su	9.8
		6	Su	9.4
LSD ($p = 0.05$) 0.6		1	Su	9.0
		5	Sp	8.2
Blocks		2	Au	7.0
I	11.3	LSR ($p = 0.05$) 1.6		
II	9.5			
III	10.4			
LSD ($p = 0.05$) 0.5				

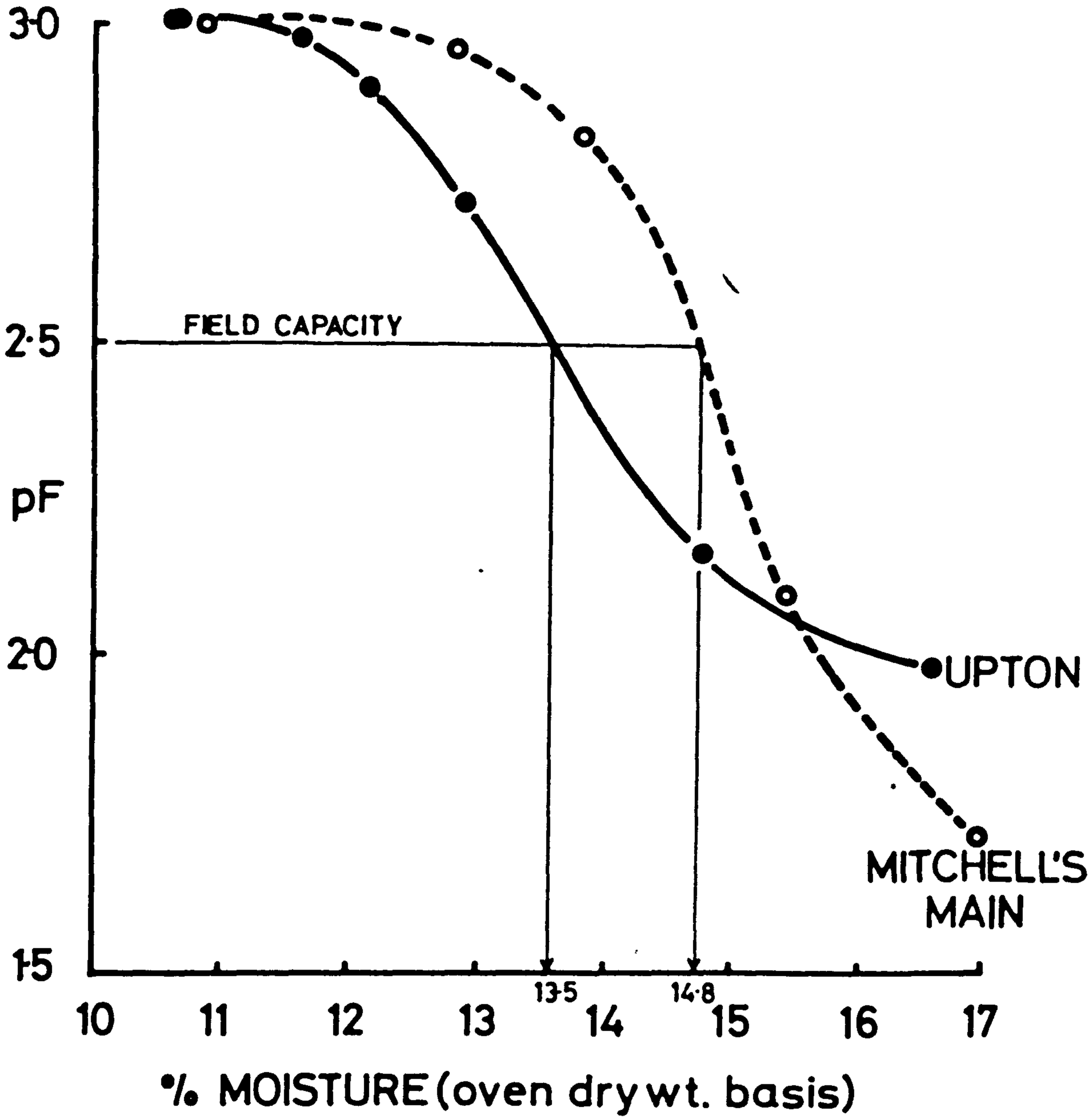


Fig. 3.22. Drying curves for Mitchell's Main and Upton spoils.

DISCUSSION

The results demonstrate that the spoils at both sites became very dry during the summer and autumn periods. Because drying curves produced with data obtained from tensiometers cannot be extended beyond approximately pF 3, it is not known whether the percentage moisture of the spoils ever became low enough to induce wilting (pF 4.2). Air dry spoil (pF 4.5) at Mitchell's Main and Upton contained 6.0 and 5.0% moisture respectively (based on the results of the air drying experiment, p.28). Some of the values recorded for both sites came close to these air dry states.

The actual percentage of moisture in the spoil is perhaps not as important as the period of time over which the dry conditions prevail. This information could not be obtained from the data collected from the sampling programme carried out and could only be obtained by continuously monitoring the spoil moisture. Further, other factors, especially spoil temperature, are important in relation to the effect of low spoil moisture on plants and this will be discussed in the next section.

Despite the limitations that apply to the drying curve and spoil moisture data, the results obtained do indicate that the spoils at both sites could dry out rapidly and that water was in very short supply at certain times during the summer and autumn months.

12. SPOIL TEMPERATURE

INTRODUCTION

Richardson (1958) showed that during the summer months the surface layers of dark coloured, south facing slopes of colliery spoil heaps in Co. Durham could reach temperatures in excess of 40°C. These high temperatures were maintained for periods of several hours and resulted in the death of unshaded seedlings. Similar findings were reported by Schraam (1966) for spoil banks in Pennsylvania.

The spoil at Mitchell's Main was dark coloured and the trial plots faced south. High summer temperatures would, therefore, be expected to occur at this site and have been measured. At Upton, the spoil was somewhat lighter coloured because of the presence of a greater proportion of burnt material and the trials faced west. The temperatures would not, therefore, be expected to reach such high levels at this site. Measurements were not made because a suitable vandal-proof storage place was not available at this site.

RESULTS AND INTERPRETATION

The seasonal variation in spoil temperature is shown in Fig. 3.23. The plotted values are the weekly mean (i.e. mean of seven days observation) maximum and minimum temperature 1 cm below the spoil surface. It can be seen that the spoil became very hot during the summer months. The values on individual days were often considerably higher than the mean values shown in Fig. 3.23, this is demonstrated in Fig. 3.24.

This shows the diurnal temperature changes during the first three days in July 1971 (based on hourly observations), at three depths, 1, 5 and 10cm below the surface (The plotted values are the mean of the three replicate thermocouples at each depth).

The temperature 1cm below the surface showed the greatest fluctuations with the spoil heating and cooling rapidly. This was obviously related to the fact that the dark coloured spoil surface was a good absorber and emitter of radiant energy. The amplitude of the temperature fluctuations decreased with depth; and the time of day at which the maximum temperature was recorded also varied with depth. Thus at 1 cm, the maximum was reached in the early afternoon, whilst at 10 cms, it did not occur until the middle of the evening. This resulted from the slow rate of heat conduction through the surface layers and gave rise to the steep temperatures gradient. This indicates that the temperature on the spoil surface could have been considerably higher than that at 1 cm.

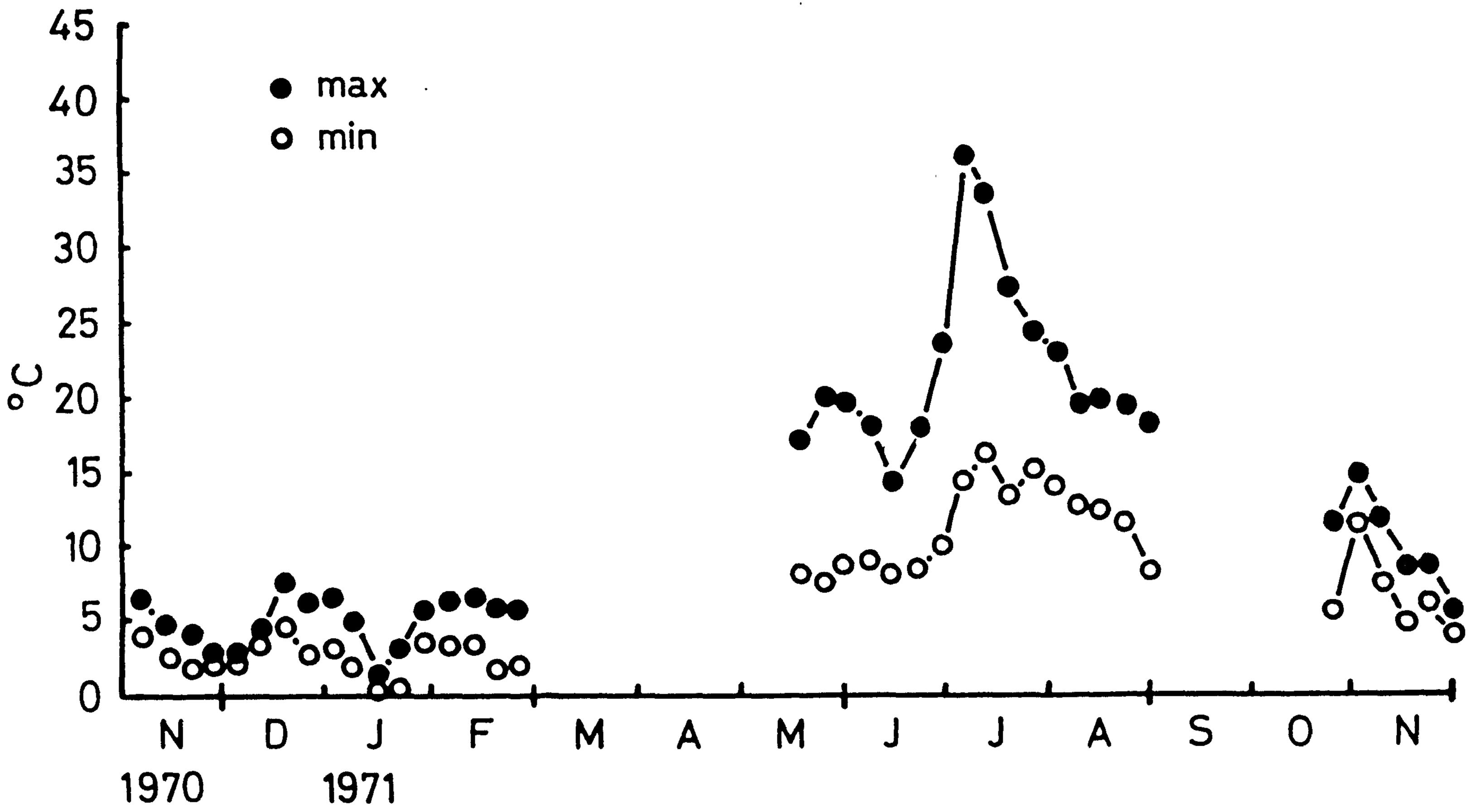


Fig. 3.23. Weekly maximum and minimum temperatures 1cm below a bare spoil surface at Mitchell's Main.

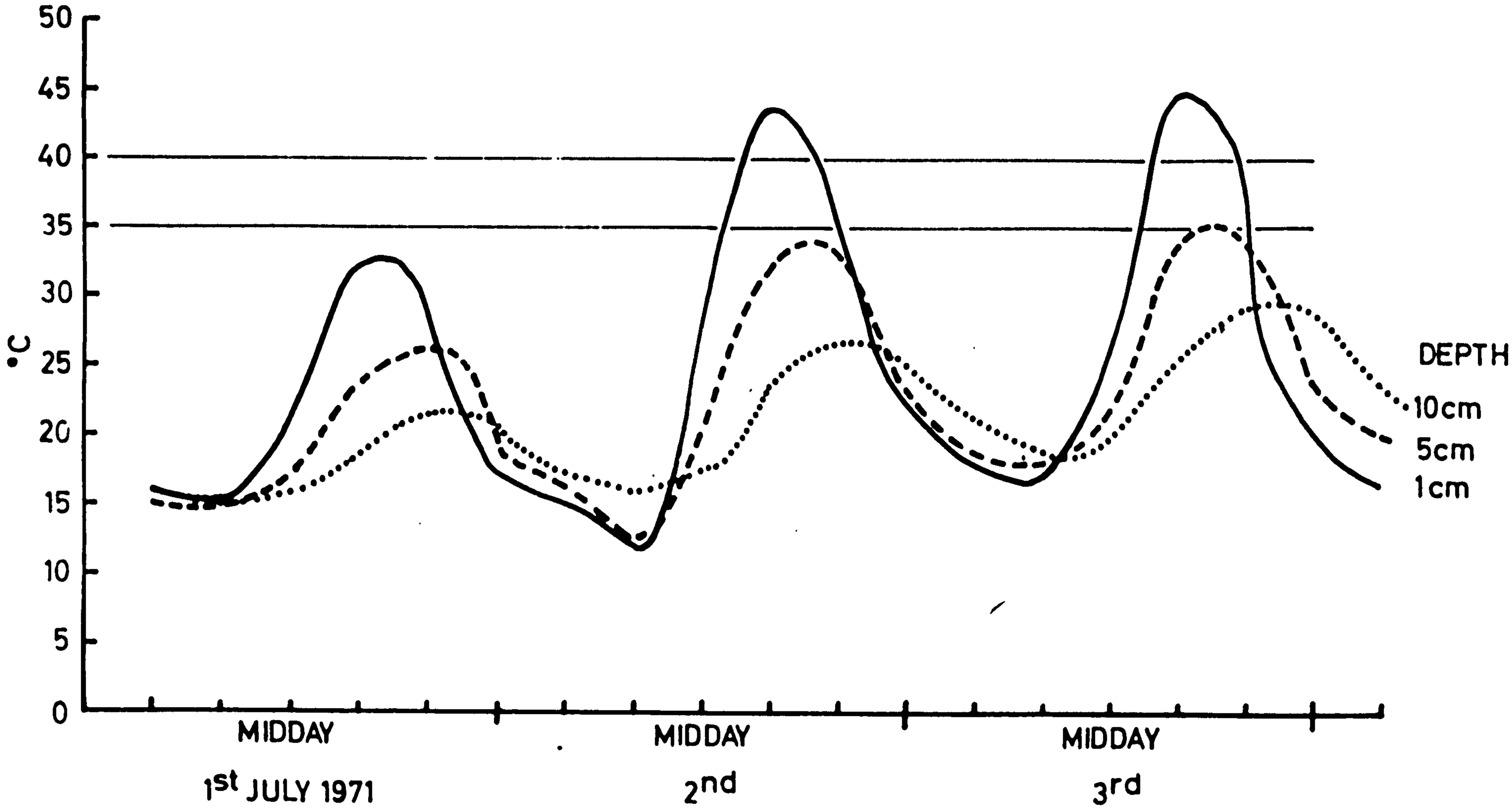


Fig. 3.24. Diurnal temperature fluctuations at three depths in bare spoil at Mitchell's Main.

DISCUSSION

The pattern observed in Fig. 3.24 was repeated throughout the year with the amplitude of the temperature fluctuations being greater in the summer and smallest in the winter. The days chosen for the preparation of Fig. 3.24 were not unique, and higher temperatures were, in fact, recorded on a number of days during June, July and August.

The temperature of the spoil was not as important as the exposure. On the 2nd and 3rd July, temperatures in excess of 40°C were maintained for approximately four hours, and in excess of 35°C for seven hours. Since it was previously shown that the spoil at Mitchell's Main could rapidly dry out, these prolonged periods of high temperature would have a desiccating effect that could result in very unfavourable conditions for seedlings or other plants with poorly developed root systems.

The values shown in Figs. 3.23 & 3.24 were recorded under a bare spoil surface. Richardson (1958) showed that the presence of a cover of vegetation reduced the temperature at the spoil surface. Thus whilst a bare surface reached 40°C, that beneath a cover of Agrostis tenuis rose no higher than 26°C.

The importance of spoil temperature is therefore somewhat reduced once vegetation has become established. In the situation described by Richardson (1958) establishment began in shaded hollows and progressed outwards. The surface of a newly regraded spoil heap is,

however, smooth and therefore provides no shade for developing seedlings. Adverse effects of high summer temperatures would therefore be expected, especially in the case of seed sown in late spring.

GENERAL DISCUSSION

1. Seasonal variation in the levels of plant nutrients Mitchell's Main

a) The nature and cause of the seasonal variation

The results indicated that the levels of all elements except potassium were generally higher in summer and autumn than in winter or spring. The most pronounced seasonal variation was shown by the elements, aluminium, manganese, copper, zinc and iron. Since the solubility of these elements is related to pH, it is interesting to find that the levels appeared to be highest when the spoil pH was lowest. In order to examine this relationship and that between the elements in general, a correlation matrix has been prepared. This is shown in Table 3.22. It can be seen that pH is highly negative correlated with aluminium, manganese, copper, zinc and iron, each of which is high positively correlated with every other. This clearly indicates the relationship between pH and the levels of these elements.

In soils, pH values obtained during the summer months when warm dry conditions prevail are generally lower than those obtained in the colder and wetter seasons, thus indicating the importance of soil moisture and temperature on soil pH. Measurements of both these parameters were made at Mitchell's Main but only spoil moisture data could meaningfully be put into the correlation matrix. From Table 3.22 it can be deduced that

Table 3.22. Mitchell's Main. Correlation matrix.

	%H_2O	Mg	Ca	K	Na	Fe	Zn	Cu	Mn	Al
pH	.127 ns	.226 *	.244 *	.259 **	-.055 ns	.380 ***	.599 ***	.564 ***	.366 ***	.513 ***
Al	-.076 ns	.304 **	.114 ns	-.257 **	.334 ***	.686 ***	.813 ***	.866 ***	.632 ***	
Mn	-.135 ns	.421 ***	.377 **	-.053 ns	.280 **	.564 ***	.791 ***	.596 ***		
Cu	.117 ns	.199 *	.072 ns	-.237 *	.281 **	.787 ***	.756 ***			
Zn	-.021 ns	.265 **	.162 ns	-.104 ns	.348 ***	.524 ***				
Fe	-.018 ns	.255 **	.111 ns	-.264 **	.249 *					
Na	.147 ns	.354 ***	.443 ***	.230 *						
K	.174 ns	-.084 ns	.174 ns							
Ca	.079 ns	.756 ***								
Mg	-.052 ns									

Each value is based on 192 observations

the seasonal changes were independant of the spoil moisture levels recorded because neither pH nor any element was correlated with this factor. Since the spoil moisture data gave no information on the magnitude or frequency of rainfall, it cannot be concluded that the seasonal patterns were independant of rainfall.

The fall in pH in the warmer months was probably a reflection of the interaction of high spoil temperatures, which would increase the rate at which the pyrites oxidised, and low rainfall, which would result in the accumulation of reaction products. This could explain the well marked seasonal variation of those elements whose solubilites are pH dependant.

For the other elements (sodium, calcium and magnesium) that showed similar, but not so pronounced summer/autumn increases, and whose solubilities are not greatly affected by small pH changes, the seasonal pattern may have been simply related to a reduced rate of leaching.

In the correlation matrix, calcium, magnesium and manganese are well correlated and this may be due to the fact that all three have a common mineralogical origin in ankerite. Many of the other significant correlation coefficients are probably fortuitous since causal relationships between the correlated pairs are not known.

The seasonal pattern shown by the spoil at Mitchell's Main differs from that shown by soils because in soils the levels of nutrients commonly fall during the growing season as a result of plant uptake,

and increase as decomposition proceeds during the late winter and early spring.

b) The significance of seasonal variation.

The general increases found to occur for many of the elements investigated as the growing season progressed must have had a very significant adverse effect on the establishment and growth of vegetation.

Aluminium was singularly the most important element because its level was high during the winter and spring months and rose to very high levels during the summer and autumn. This in itself would be considered adverse but the situation was made much worse by increases in the levels of potential phytotoxic elements and decreases in those of essential macronutrients. Thus, whilst it was previously indicated that the level of certain nutrients like, manganese, zinc and iron were not high enough to be directly harmful to plant growth, the combined effects of the seasonal increases to supra-optimal levels of these elements may have been critical. The levels of potassium which were low in the spring months fell as the growing season progressed and phosphorus levels may have shown a similar pattern (through complexing reactions with aluminium). Further, the availability of water was sometimes quite low and the surface of the spoil frequently became very warm. The general picture that emerged shows that the spoil represented a hostile environment for plant growth. This point is further discussed in Chapter Five after the nitrogen regime of the spoil has been described.

Upton

Seasonal variations were not apparent for any of the elements investigated. This was not unexpected because the sampling regime was begun immediately after the spoil heap had been reshaped and the field trial layed down. Initial changes in the levels of many of the nutrients were, however, expected but with the notable exception of sodium and possibly also of potassium these did not occur. Nevertheless, for every element investigated, significant sub-plot variations occurred. These appeared to be of a random nature and are discussed in the next section.

A correlation matrix was prepared and is shown in Table 3.23. Spoil moisture is not correlated with either pH or copper, iron, zinc and manganese. Sodium and potassium are, however, negatively correlated with spoil moisture.

Calcium, manganese and magnesium are correlated, again reflecting their common mineralogical origin.

The results for this site showed that with the exception of sodium, initial weathering reactions did not lead to large changes in the levels of plant nutrients or pH.

2. The variability of colliery spoil in relation to statistical interpretation

At Upton significant sub-plot differences were found that could not be related either to initial weathering reactions or seasonal change. The variation appeared to be of a random nature suggesting that

Table 3.23. Upton. Correlation matrix.

	%H_2O	Mg	Ca	K	Na	Fe	Zn	Cu	Mn
pH	-.162 ns	-.248 *	-.413 ***	-.077 ns	-.133 ns	-.181 ns	-.336 ***	-.179 ns	-.651 ***
Mn	-.040 ns	-.568 ***	-.413 ***	-.192 **	-.068 ns	-.220 *	-.437 ***	-.301 **	
Cu	-.019 ns	-.349 ***	-.371 ***	-.487 ***	-.355 ***	-.421 ***	-.342 ***		
Zn	-.173 ns	-.011 ns	-.002 ns	-.015 ns	-.081 ns	-.327 ***			
Fe	-.122 ns	-.180 ns	-.147 ns	-.279 **	-.193 ns				
Na	-.307 **	-.308 **	-.405 ***	-.452 ***					
K	-.236 *	-.605 ***	-.431 ***						
Ca	-.607 ns	-.578 ***							
Mg	-.291 **								

Each value is based on 108 observations

the observed differences may have been at least partially attributable to procedural errors.

The erros associated with analytical techniques are small but those incurred during sampling may be large. It will be recalled that the sampling procedure at Upton involved the preparation of a single bulked sample for each treatment plot. This was obtained by pooling three randomly selected cores. Since the area of each treatment plot was only $8y^2$(yards) the collection of three cores represented quite intensive sampling. For normal soils this would have been adequate to cope with the inherant variability of the substrate but this would not appear to be the case for colliery spoil (the variability of the spoils both at Upton and Mitchell's Main is indicated by Table 5.1 page 294).

The problem is not easily overcome by taking more samples because: a) this may not be practically possible, and b) sampling is a destructive procedure that can influence the nature of the substrate under investigation. Although the spoil variability will tend to interfere with the resolution of real differences attributable to say, ameliorants, it must be remembered that the analysis of variance technique has a high degree of resolution and small differences are detected. Thus the interference due to sampling error would not in the present situation be great enough to invalidate the rest of analysis of variance results, i.e. other than sub-plot differences where

the effects of sampling error would be greatest.

The spoil at Mitchell's Main was also of very variable composition and similar errors would be expected. For some elements at this site, however, a large proportion of the sub-plot variance could be attributed to the well defined seasonal variation and sampling errors appeared to be of less importance.

Despite the fact that sampling errors were responsible for some of the significant variance ratios at both sites, it was encouraging to find that general trends could still be observed. At Upton, for example, the level of potassium decreased with time and there was a general tendancy for the levels of calcium and magnesium to be higher in samples obtained from block I than block III. Similar effects were noted at Mitchell's Main where block I samples contained the highest level of manganese, zinc, sodium, calcium and magnesium. Again at Mitchell's Main the effect of liming was apparent, and elements that would be expected to behave similarly, were in fact, observed to do so.

The significant sub-plot effects at Upton and perhaps also those for elements like calcium, magnesium and sodium at Mitchell's Main that did not demonstrate a very clear seasonal pattern, can be taken as an indication of the variability of colliery spoil as a substrate for plant growth.

3. <u>The effect of the ameliorants on the levels of available nutrients</u>

The only significant treatment (main plot) effects noted at Mitchell's Main were those attributable to liming. Thus significantly less aluminium, manganese, copper and zinc were found in samples from the limestone plots. This is obviously related to the increase in pH that resulted from the application of limestone to an initially acid spoil. The results demonstrated clearly that limestone added in 1967 was not exhausted five years later. This shows that the addition of fairly large quantities of limestone to moderately acid sites can effectively suppress phytotoxic concentrations of plant nutrients for a number of years. Similar treatment effects, attributable to liming would not have been expected at Upton and were found not to occur.

Significant differences in the levels of plant nutrients in response to shoddy and sewage-sludge applications were not found at all at Mitchell's Main and only for potassium and perhaps copper at Upton. This is not surprising because these two ameliorants did not alter the pH of the substrate and were applied as sources of nitrogen.

An overall assessment of the suitability of the spoils as growth media cannot be made until the nitrogen status of the spoils has been described. This is done in the following chapter and the relationship between all the various elements is discussed in Chapter Five.

CHAPTER FOUR

CHAPTER FOUR

NITROGEN

Introduction

The ability of colliery spoils to provide nitrogen for plant growth has received less attention than other nutritional aspects. Several workers have commented upon the very low levels of available nitrogen present in spoils (Doubleday 1972b; Schramm 1966; Wilson 1965) and Davison & Jefferies (1966) obtained growth responses from plants to nitrogen added to spoil. Others, however, have postulated high levels of nitrogen in some spoils, derived from within the clay minerals and released by weathering reactions (Cornwell & Stone 1968, 1973).

The investigations detailed in the present chapter were undertaken to provide information on the factors affecting nitrogen availability and recycling processes in general in colliery spoils. This involved several lines of investigation and the nature and results of each are presented in separate sections. The following are included:

SECTION I MINERAL NITROGEN

SECTION II MICROBIAL ENUMERATIONS

SECTION III INCUBATION EXPERIMENT I

SECTION IV INCUBATION EXPERIMENT II

SECTION V YIELD AND NITROGEN STATUS OF THE VEGETATION ON THE TRIAL PLOTS

SECTION VI THE NITROGEN BUDGET OF SPOILS

SECTION VII SUMMARY

To maintain the continuity of the investigation as a whole the results of each section are discussed in relation to those obtained in the preceding sections.

Experimental procedures were detailed and discussed in Chapter Two, and are only outlined in the present chapter where they are necessary to allow a section to be read as a whole.

SECTION I MINERAL NITROGEN

INTRODUCTION

i) The importance of mineral nitrogen

Mineral nitrogen usually represents only a small proportion of the total nitrogen reserve of a soil. Since however, it is mineral nitrogen that is taken up by vegetation, this small proportion is very important. The situation in colliery spoils may be very different because a humus fraction, which acts as the nitrogen reserve in soils, is initially absent. In this situation, mineral nitrogen may represent a substantial proportion of the total potentially available nitrogen.

Mineral nitrogen generally occurs either as ammonium or nitrate; the former under acid, and the latter under neutral conditions. The intermediate, nitrite is found in trace quantities and only accumulates if the pH is raised above about pH 8 by heavy liming or the addition of urea (Chapman & Liebig 1952; Soulides & Clark 1958).

The form in which mineral nitrogen occurs in a soil or spoil is of importance to the vegetation for a number of reasons. These include direct affects attributable to the fact that some species of vegetation can utilize ammonium more readily than nitrate and vice versa, (Kirkby 1969) and indirect affects that result from the uptake of a particular form of mineral nitrogen. Thus, uptake of ammonium results in a fall in the pH in the vicinity of

the roots, and nitrate has the opposite effect (Jackson 1966). Since the solubility of elements like aluminium can be greatly altered by relatively small changes in pH, the absorption of ammonium might induce, or intensify, aluminium toxicity in acid environments.

The mineral form of nitrogen is also of importance with respect to losses of nitrogen from the soil or spoil. Thus, nitrate is much more soluble than ammonium and is, therefore, more easily leached. Nitrate nitrogen can also be converted into nitrogen gas by the process of denitrification. Under suitable conditions, large quantities of nitrate may be lost in these two ways. These losses may be very important in colliery spoils where it seems likely that mineral nitrogen may represent a large proportion of the potentially available nitrogen.

ii) Mineral nitrogen determinations

The analysis of the spoil samples for the three forms of mineral nitrogen commenced in January, 1971 for Mitchell's Main, and in December, 1970 for Upton. These determinations were not performed earlier because a suitable analytical procedure had not been found.

iii) Statistical interpretation

A split-split-plot analysis of variance design (Snedecor 1956) has been used to analyse the data. Ameliorative treatment and sampling time are taken as the main plots and sub-plots respectively. The form of mineral nitrogen, i.e. ammonium, nitrite or nitrate is

taken as the sub-sub-plot. The main plot comparisons represent total mineral nitrogen i.e. the total of the three forms added together.

RESULTS AND INTERPRETATION

The results are shown graphically in Figs. 4.1 & 4.2. All three forms of mineral nitrogen are shown together on each axis. Each plotted value represents the mean of the three field replicates. The results of the statistical analyses are shown in Tables 4.1 & 4.2.

Mitchell's Main

The total mineral nitrogen contents of the shoddy, sewage-sludge and control plots were similar. The sewage-sludge and limestone plots also contained insignificantly different quantities. The levels of mineral nitrogen in the shoddy and control plots were, however, significantly greater than in the limestone plots. It is very obvious from the results that the three forms of mineral nitrogen did not occur in equal abundance. In the shoddy, sewage-sludge and control plots, ammonium was the predominant mineral form; nitrate and nitrite occurred in smaller and approximately equal concentrations. In the limestone plots, no predominance of ammonium was observed. This treatment (main plot) difference in respect of mineral form of nitrogen (sub-sub-plot) resulted in the significant treatment x mineral form interaction. The calculated LSD for this interaction, indicates that the levels of ammonium in the spoils from

the different treatments were significantly different. The shoddy plots contained significantly higher levels of ammonium than the rest. The sewage-sludge and control plots were similar, and both contained more ammonium than the limestone plots. No differences between the treatments in respect of either nitrate or nitrite were observed.

An obvious seasonal pattern in the levels of mineral nitrogen was not observed. The significant sub-plot (time) variance ratio is probably not entirely due to sampling error because rapid changes in the levels of mineral nitrogen are known to occur (Harmsen & Kolenbrander 1965). These result from rapid uptake by the micro and macroflora, additions in the rainfall and losses through denitrification and leaching. Because of these rapid fluctuations meaningful interpretation of the significant sub-plot variance ratio cannot be made and interactions in which this factor occurs are not of interest.

No significant block differences were found to occur.

Upton

The levels of mineral nitrogen were insignificantly different for all spoil treatments and blocks. The significant sub-sub-plot variance ratio is shown to represent a difference between nitrate and the other two mineral forms; the level of nitrate being significantly lower than either nitrite or ammonium. The reason is likely to be greater leaching loss and denitrification of nitrate rather than selective uptake, because it has been known for many years that grasses can utilize both

forms equally well (Richardson 1938).

Whilst the significant sub-plot variance ratio indicates that the mineral nitrogen content of the spoils fluctuated significantly with time, no seasonal pattern was obvious.

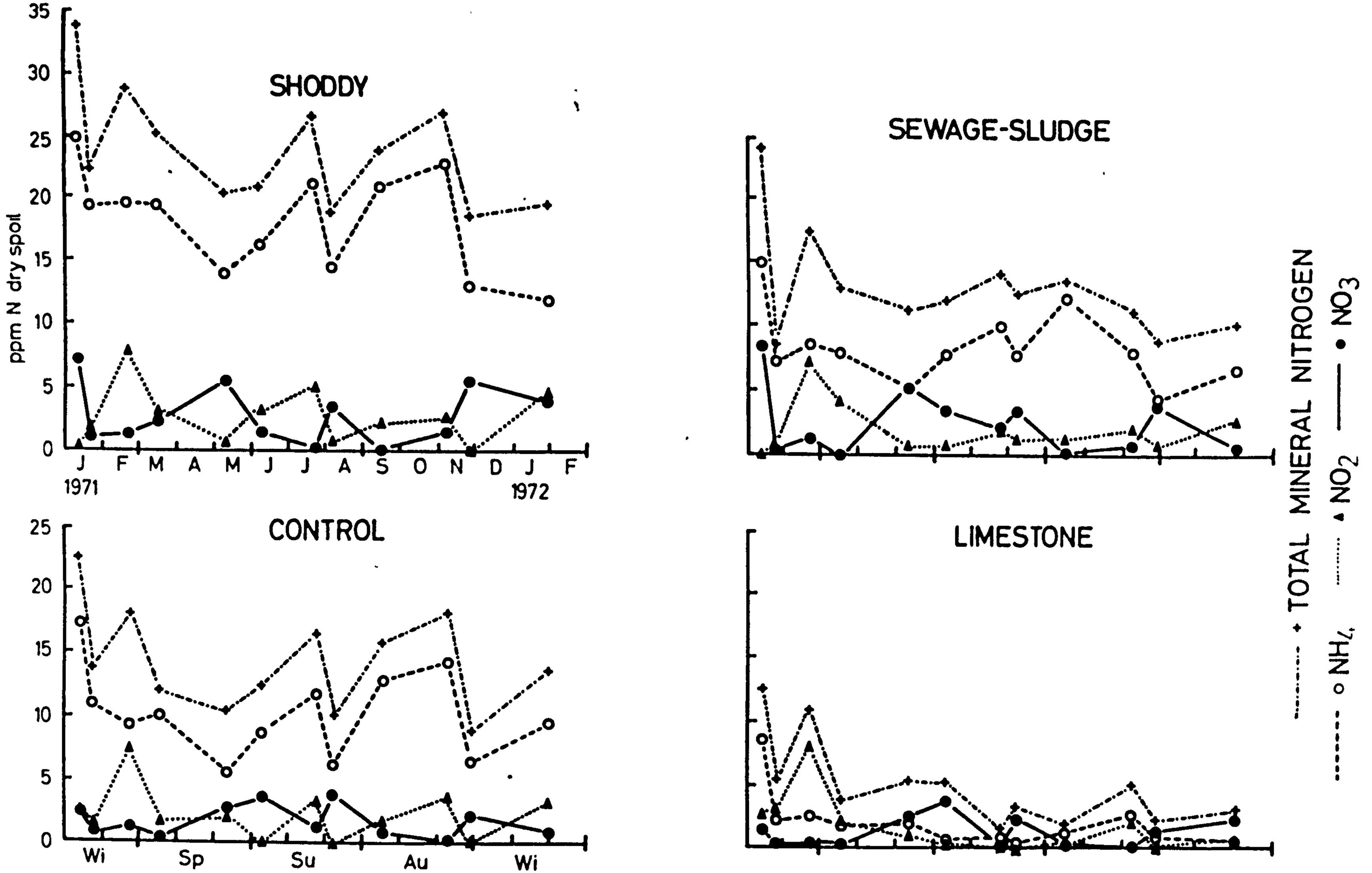


Fig. 4.1. Mitchell's Main. Seasonal variation of mineral nitrogen.

Table 4.1. Mitchell's Main. Statistical analysis.

Mineral nitrogen.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots (A)	68.5343	3	22.8448	6.743	*
Blocks	5.6731	2	2.8366	0.837	ns
Error a	20.3289	6	3.3882		
Total	94.5363	11			
Sub-plots (T)	18.9038	11	1.71852	12.748	***
A x T	5.1979	33	0.1575	1.169	ns
Error b	11.8627	88	0.1348		
Total	35.9644	132			
Sub-sub-plots (MF)	173.9476	2	86.9738	207.723	***
A x MF	71.1618	6	11.8603	28.326	***
T x MF	63.3649	22	2.8802	6.879	***
A x T x MF	22.7749	66	0.3451	0.824	ns
Error c	80.3908	192	0.419		
Total	542.1407	431			

MEANS TABLE

	Main Plots				
Sub-sub-plots	Shoddy	Sewage-sludge	Control	Lime-stone	Mean
Ammonium	4.31(18.14)	2.83(8.43)	3.26(10.27)	1.60(1.93)	3.00(9.69)
Nitrite	1.81(2.77)	1.65(2.09)	1.74(2.43)	1.53(1.73)	1.69(2.26)
Nitrate	1.78(2.75)	1.73(2.52)	1.58(1.76)	1.42(1.21)	1.63(2.06)
Mean	2.64(7.89)	2.07(4.35)	2.20(4.82)	1.52(1.62)	

LSD (p = 0.05)

Main plots = 0.613 Sub-sub-plots = 0.149

Interaction = 0.750

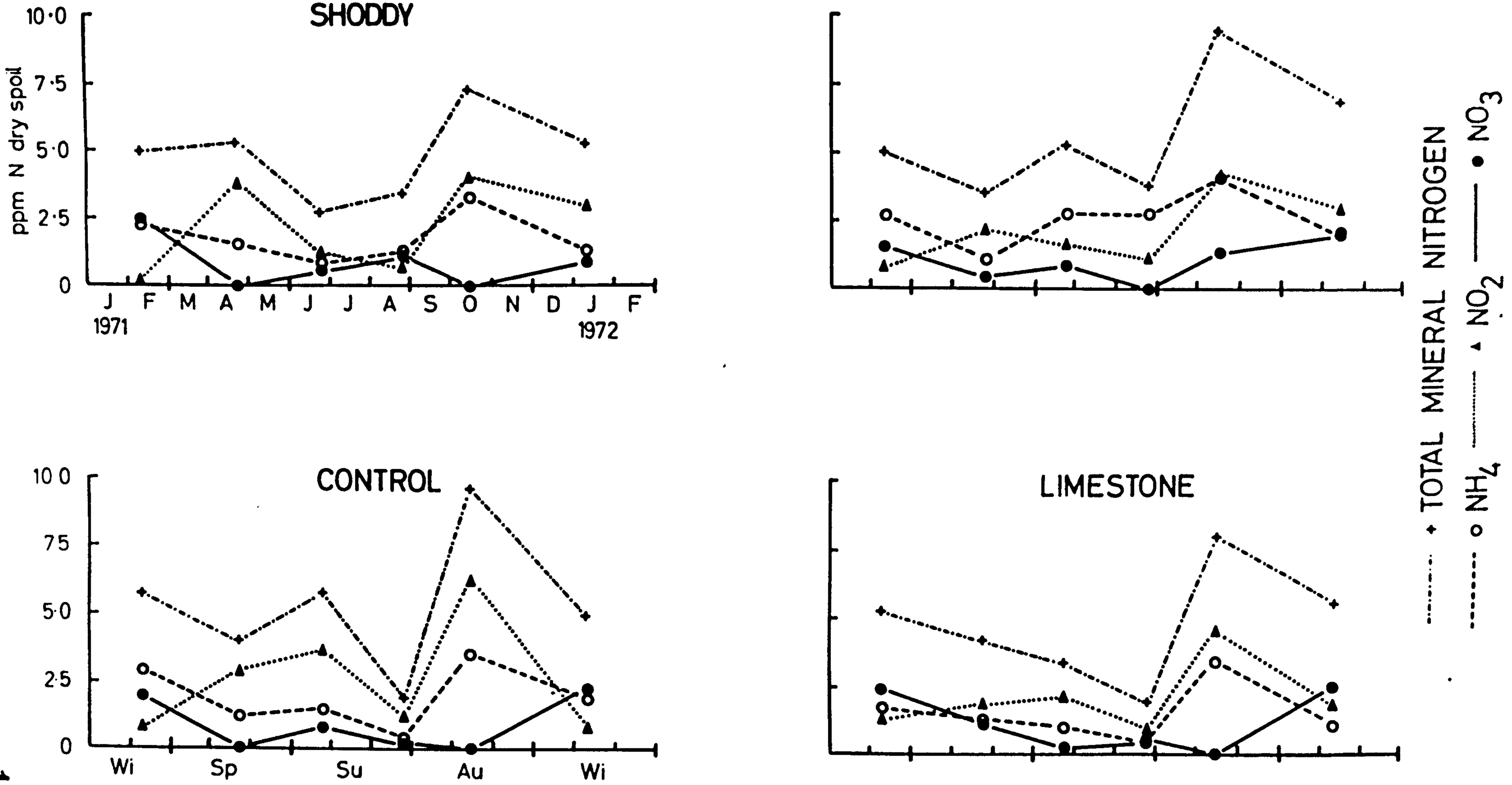


Fig. 4.2. Upton. Seasonal variation of mineral nitrogen.

Table 4.2. Upton. Statistical analysis. Mineral nitrogen.

VARIANCE TABLE (square root + 1 transformation)

Source	ss	df	ms	vr	sig.
Main plots (A)	0.3626	3	0.1208	0.7854	ns
Blocks	0.1186	2	0.0593	0.3855	ns
Error a	0.9229	6	0.1538		
Total	1.4041	11			
Sub-plots (T)	5.6012	5	1.1202	10.2865	***
A x T	1.1633	15	0.0775	0.7116	ns
Error b	4.3574	40	0.1089		
Total	11.1219	60			
Sub-sub-plots (MF)	6.2821	2	3.1410	18.8535	***
A x MF	0.8764	6	0.1460	0.8763	ns
T x MF	11.4920	10	1.1492	6.8979	***
A x T x MF	4.1906	30	0.1396	0.8379	ns
Error c	15.9955	96	0.1666		
Total	51.3626	215			

MEANS TABLE

Main plots

Sub-sub-plots	Shoddy	Sewage-sludge	Control	Lime-stone	Mean
Ammonium	1.62(1.72)	1.83(2.50)	1.74(2.13)	1.53(1.48)	1.68(1.96)
Nitrite	1.66(2.07)	1.69(2.13)	1.81(2.61)	1.70(2.16)	1.72(2.24)
Nitrate	1.31(0.85)	1.34(1.01)	1.30(0.81)	1.39(1.12)	1.34(0.96)
Mean	1.53(1.55)	1.62(1.88)	1.62(1.87)	1.54(1.59)	

LSD (p = 0.05)

Sub-sub-plots 0.135

DISCUSSION

The quantity of mineral nitrogen in a soil is a reflection of the interaction of all the processes of consumption and production. Agencies that increase the mineral nitrogen content include, mineralization of organic matter, input in rainfall and other natural agencies. Mineral nitrogen levels are decreased by plant and microbial uptake, leaching and denitrification. The relative proportions of ammonium to nitrate depends principally upon the rate of conversion of ammonium to nitrate by the nitrifying organisms and the rate of uptake by plants, together with losses of nitrate through leaching and denitrification. The heterogeneity of a soil allows all the processes to operate simultaneously and despite the complicated interactions that occur, particular soil types demonstrate characteristic mineral nitrogen regimes. Thus, the ammonium content of arable soils of neutral reaction remains at a fairly constant low level. The nitrate content is very variable and generally higher. Typical values would be 5 ppm for ammonium and between 2 and 20 ppm for nitrate (Russell 1961).

In grassland soils the mineral nitrogen level remains low throughout the year and rarely exceeds 5ppm (Cunningham & Cooke 1958; Richardson 1938; Soulides & Clark 1958; Theron 1951). Ammonium is the predominant form, and Soulides & Clark (1958) and Theron (1951) have suggested that this is due to the fact that grass roots excrete substances inhibitory

to the nitrifying organisms. Robinson (1963) took a different, and the more generally accepted view, suggesting that the low numbers of nitrifying organisms commonly found under grass covers was due, not to their inhibition by grass root exudates, but to low substrate availability, and/or, low pH.

The predominance of ammonium over nitrate in the acid spoils at Mitchell's Main and neutral spoils at Upton represents a situation not very different to that found in grassland soil. The levels of ammonium on all but the limed spoil treatments at Mitchell's Main appears, however, to be higher than those found in unfertilized soils. The presence of quantities of nitrite as great as those of nitrate is unusual because nitrite rarely accumulates in neutral or acid soils. This point will be discussed later, when the Nitrobacter populations are considered.

SECTION II MICROBIAL ENUMERATIONS

INTRODUCTION

i) The aim of the investigation

The investigations into the microbial populations of colliery spoil were not designed to provide extensive information on the whole spectrum of microbial activity. Rather, the intent was to examine representative groups of those microorganisms that were directly related to nitrogen transformations. This approach was adopted with the aim that the results obtained would, when taken together with mineral nitrogen and other data, allow an overall assessment to be made of the factors affecting the availability to plants of nitrogen in colliery spoil. The investigations involved examination of the population sizes of prominent members of three functional groups of microorganisms. These were, the decomposers, represented by the fungi; the nitrifyers, represented by Nitrosomonas and Nitrobacter; and the denitrifyers, represented by Denitrobacillus and Pseudomonas.

Since the Upton site was regraded only a matter of weeks before the first counts were performed, information was obtained on the rate of recolonization of spoil by microorganisms. The opportunity also occurred at this site to examine the population sizes of the microorganisms before ameliorant applications were made.

ii) Presentation of results

Each organism or pair of organisms is introduced before the results are presented and discussed. The actual results for each organism are expressed graphically. Each

plotted value represents the mean of the three field replicates at a single time. An arrow accompanying a point indicates that the true value was either "greater than", or "less than" the value actually plotted. The need for this convention has already been described in Chapter Two. For the Upton site, the enumeration performed before the ameliorants were applied is indicated by the symbol ✦ .

iii) Statistical interpretation

Microbial enumerative data are always rather unprecise because of the many sources of error involved in both plate count and most probable number techniques. In many instances in the enumerations considered here, the most probable number of organisms could not be evaluated and only "greater than" or "less than" values could be quoted. The elegant parametric analysis of variance technique was therefore not an appropriate statistical technique for interpretation of the results. Several non-parametric techniques have been devised to accommodate this sort of data and one such technique has been used in the present investigations. This is the two way analysis of variance method developed by Friedman (1937) and described by Siegel (1956). In the Friedman test, the data are cast in a two way table. The scores in each row are ranked separately and the test determines whether it is likely that the different columns of ranks come from the same population. This technique has been used to examine treatment differences by casting the data, summed over the blocks, into a two way table with the treatments (i.e.

shoddy, sewage-sludge, control and limestone) as the columns, and sampling time and the rows. Block differences have also been examined by casting the data, summed over treatments, into a table with blocks as the columns and sampling time as the rows. Analysis of the differences attributable to sampling time have not been examined because the observed large fluctuations in microbial populations would certainly give rise to significant differences.

In comparison with parametric analysis of variance techniques, non-parametric ones yield less information. Significant differences between a number of treatments can be indicated but LSD values cannot be calculated. However, by comparison of the rank total of the different treatments some indication of which treatment or treatments are responsible for a significant result can be obtained.

RESULTS

1. FUNGI

INTRODUCTION

The fungi play a vital role in the decomposition of organic matter in soils. In neutral soils this activity is aided, and shared by diverse groups of heterotrophic bacteria, but as the pH falls, the bacterial contribution decreases and fungal decomposition becomes increasingly important.

Quantitative estimation of fungal activity in soil is problematical since no quick, effective methods have been developed. Enumeration of fungal propagules, using plate counting techniques are simple, but interpretation

of the results in term of activity are problematical. Difficulties arise because fungi exist in soil both as active mycelia and dormant spores, and both may grow on the nutrient agar plates used in the enumeration technique. It is not, therefore, possible to say whether the colonies that develop came from active or quiescent propagules. Further, since the fungi includes many diverse groups of organisms, some of which require specific growth factors, no single medium and set of incubation conditions will be suitable for all.

Despite the various shortcomings of plate counts as indecies of fungal activity, this technique has been used in the present investigations because it appeared to be the only one suitable for a repetitious sampling programme.

RESULTS AND INTERPRETATION

The results of the periodic fungal enumerations are presented in Figs. 4.3 & 4.4. The results of the analyses are given in Tables 4.3 & 4.4.

Mitchell's Main

The significant treatment difference (Table 4.3) probably represented a difference between the shoddy and sewage-sludge plots as compared with the control and limestone ones. In this particular situation it is difficult to assess whether the difference was related to activity or was simply a reflection of the different number of spores present. Although both the organic amendments would be expected to provide utilizable energy sources for fungal growth, they were applied a number of years before the sampling programme was initiated. If decomposition had proceeded at all rapidly, these materials would have been completely decomposed before

the first fungal enumerations were performed. This being so, there would be no reason to suppose that fungal activity in the control plots should differ from that in the shoddy and sewage-sludge ones since all were very similar in terms of pH, spoil moisture and vegetation. The higher numbers could therefore be attributed to the presence of large numbers of spores, either introduced with the organic amendments or produced by the fungi that initially utilized the substrate provided. In later sections of the present chapter, however, it is shown that the shoddy at least, was not completely decomposed. The higher counts may, therefore, be indicative of higher fungal activity in these plots.

Upton

The analysis of the results (Table 4.4) indicates that the shoddy and sewage-sludge plots contained significantly more fungal propagules than did the control and limestone plots.

The large increase in the fungal count immediately after the application of the organic ameliorants indicates that these materials were carrying large numbers of fungal propagules. The change in numbers with time, after the ameliorant applications is very interesting. In the control and limestone plots, the numbers rose slowly, though somewhat eratically over the entire sampling period. In the shoddy plots, the numbers rose rapidly and reached a maximum in spring, 1971, some nine months after the additions were made, and then decreased to approximately the initial levels. The sewage-sludge plots showed

similar, though shorter initial increases, which were again followed by decreases to the initial levels. The gradual increase with time in these plots not receiving organic additions would be expected, because new propagules would be arriving in the spoil, and the conditions for fungal growth would be improving as the sown grasses developed. The more rapid increases in the numbers in the shoddy and sewage-sludge plots indicates that there was a burst of fungal activity during the first few months as a result of the increased availability of substrate. The decreases which occurred after the initial increase may possibly indicate the exhaustion of the bulk of easily decomposable material.

The numbers of propagules isolated from block III were generally higher than from the others, but the same general patterns emerged in all three field replicates.

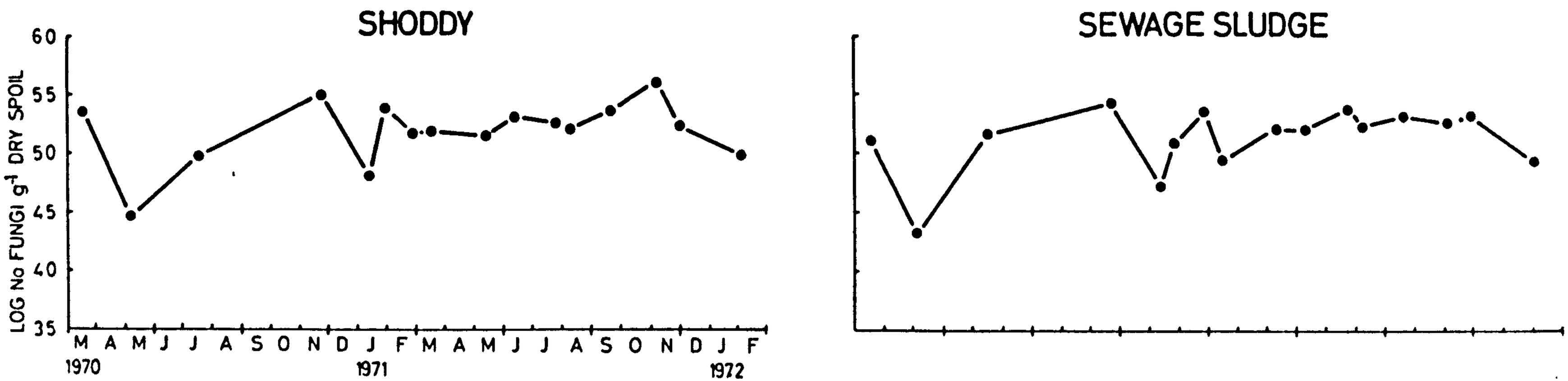
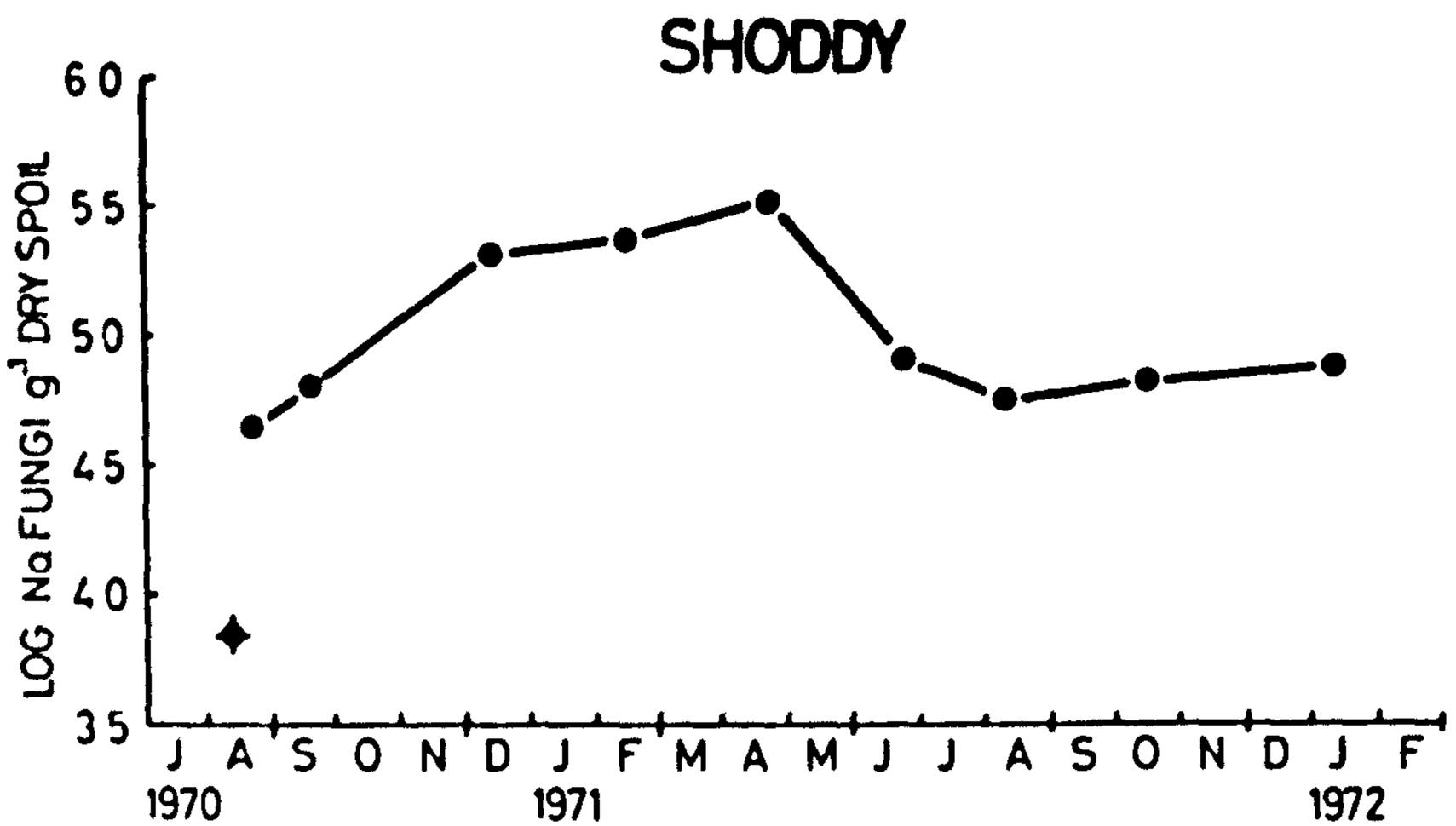
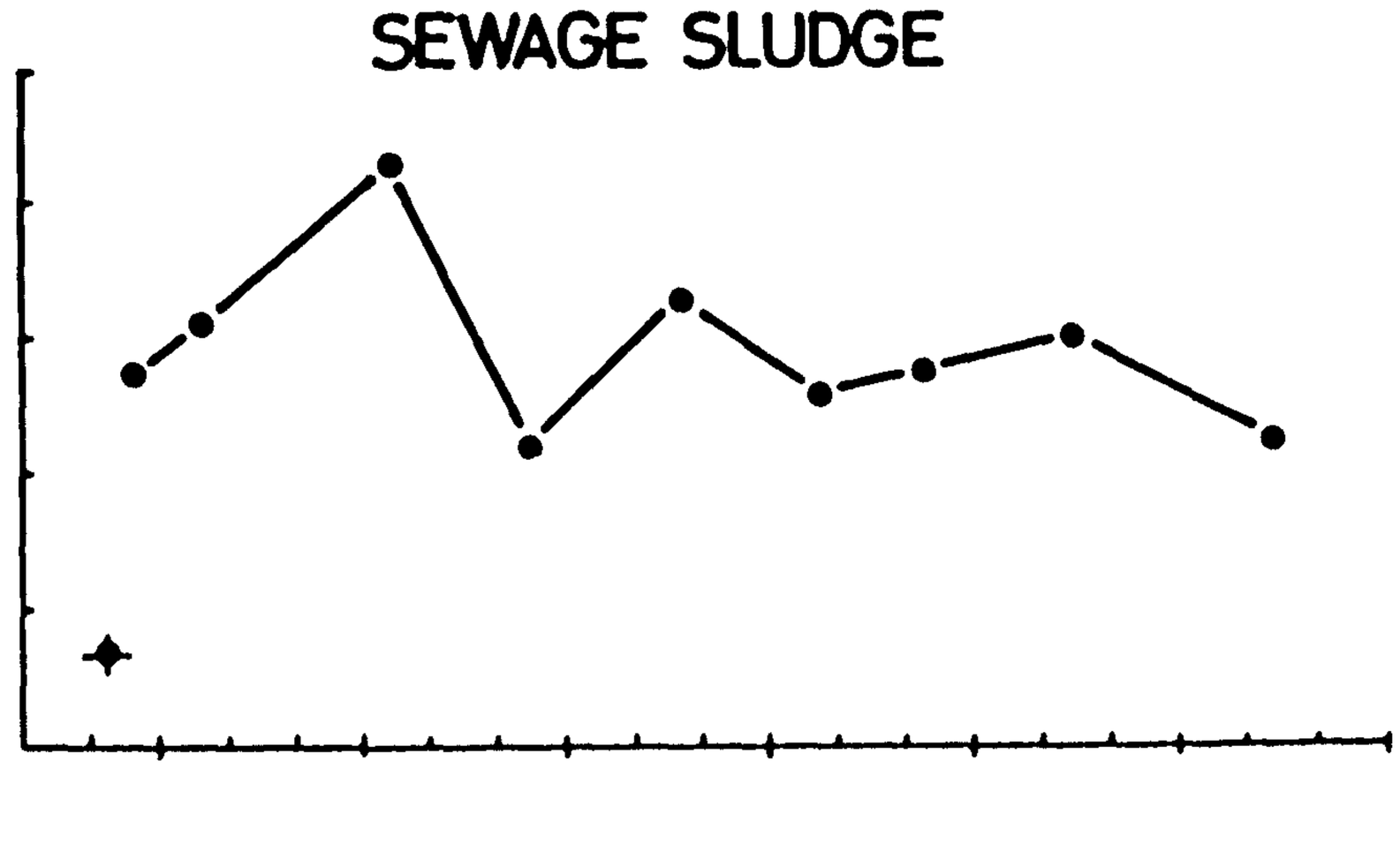
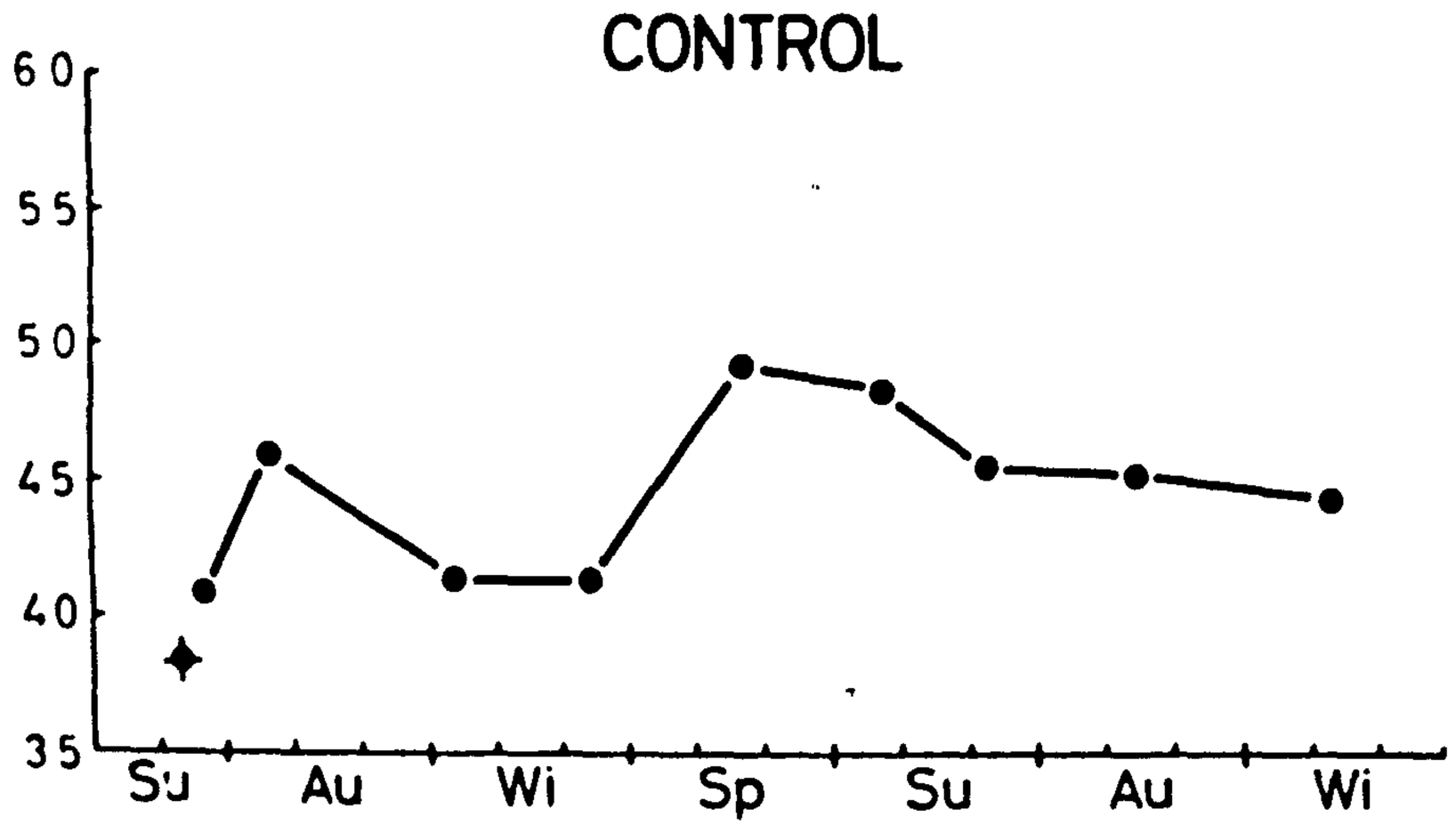


Fig. 4.3. Mitchell's Main. Seasonal variation of fungi.







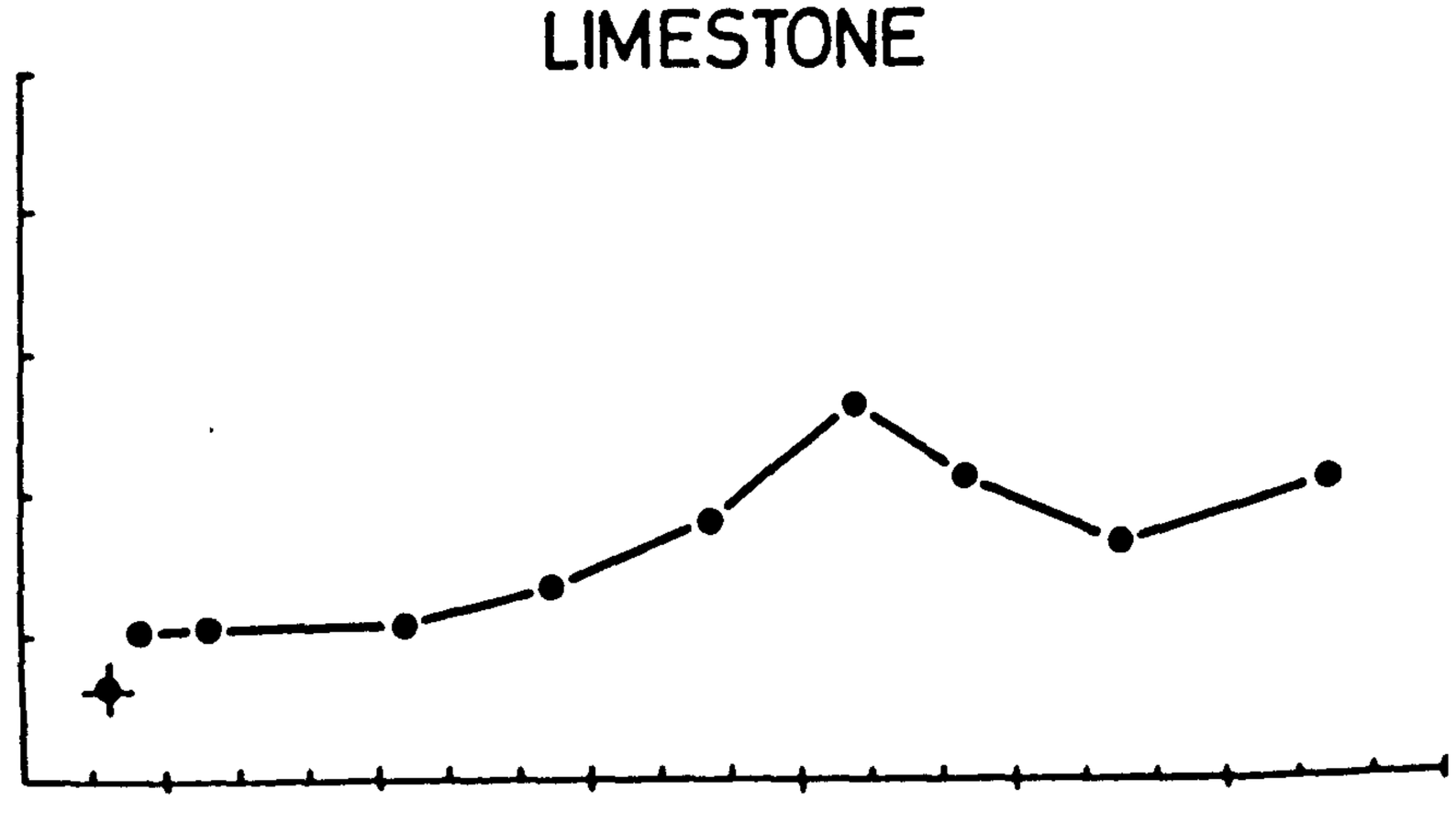


Fig. 4.4. Upton. Seasonal variation of fungi.

DISCUSSION

The fungal counts recorded at both sites fall within the range commonly recorded for soils, that is, between one thousand and one million per gramme (Russell 1961). Although the counts cannot be taken as a direct index of activity, the general spoil conditions, in terms of pH, soil moisture etc. did not appear to be so unfavourable that fungal activity would be seriously impaired. This does not mean, however, that on the acid plots at Mitchell's Main, decomposition would necessarily have proceeded rapidly. Indeed, it is often found that in acid soils, decomposition occurs only slowly because of the reduction in numbers of bacteria, and the size of the microfaunal populations that promote decomposition (Macfadyen 1964).

Although interpretation of numbers of fungi in terms of activity presents problems, the results of the fungal enumerations would suggest that fungal activity was greater in those plots receiving the organic amendments of shoddy and sewage-sludge.

2. NITROSOMONAS AND NITROBACTER

INTRODUCTION

Two genera of autrotophic bacteria are responsible for the great majority of the conversion of ammonium to nitrate in soils. These are Nitrosomonas and Nitrobacter. The former converts ammonium to nitrite and the latter, nitrite to nitrate. These two genera therefore, largely control the form in which mineral nitrogen occurs in a soil. Because the activity of the two groups is so intimately related, both will be considered together in the present section.

RESULTS AND INTERPRETATION

The results of the periodic enumerations of Nitrosomonas and Nitrobacter populations are shown in Figs. 4.5 & 4.6. The statistical analyses are shown in Tables 4.3 & 4.4.

Mitchell's Main

Nitrosomonas

Although the numbers were low in all treatments, the analysis indicated that a significant treatment effect occurred. The rank totals indicate that the four treatments fell into two distinct groups. These were the shoddy and limestone and the sewage-sludge and control treatments, the numbers of Nitrosomonas being higher in the former group. The significant block difference is probably attributable to the lower counts of block II. Seasonal fluctuations were not apparent.

Nitrobacter

The numbers of Nitrobacter were extremely low in all

treatments. In fact, in all but the shoddy plots, Nitrobacter were seldom isolated. Because the numbers were so very low statistical analysis has not been performed.

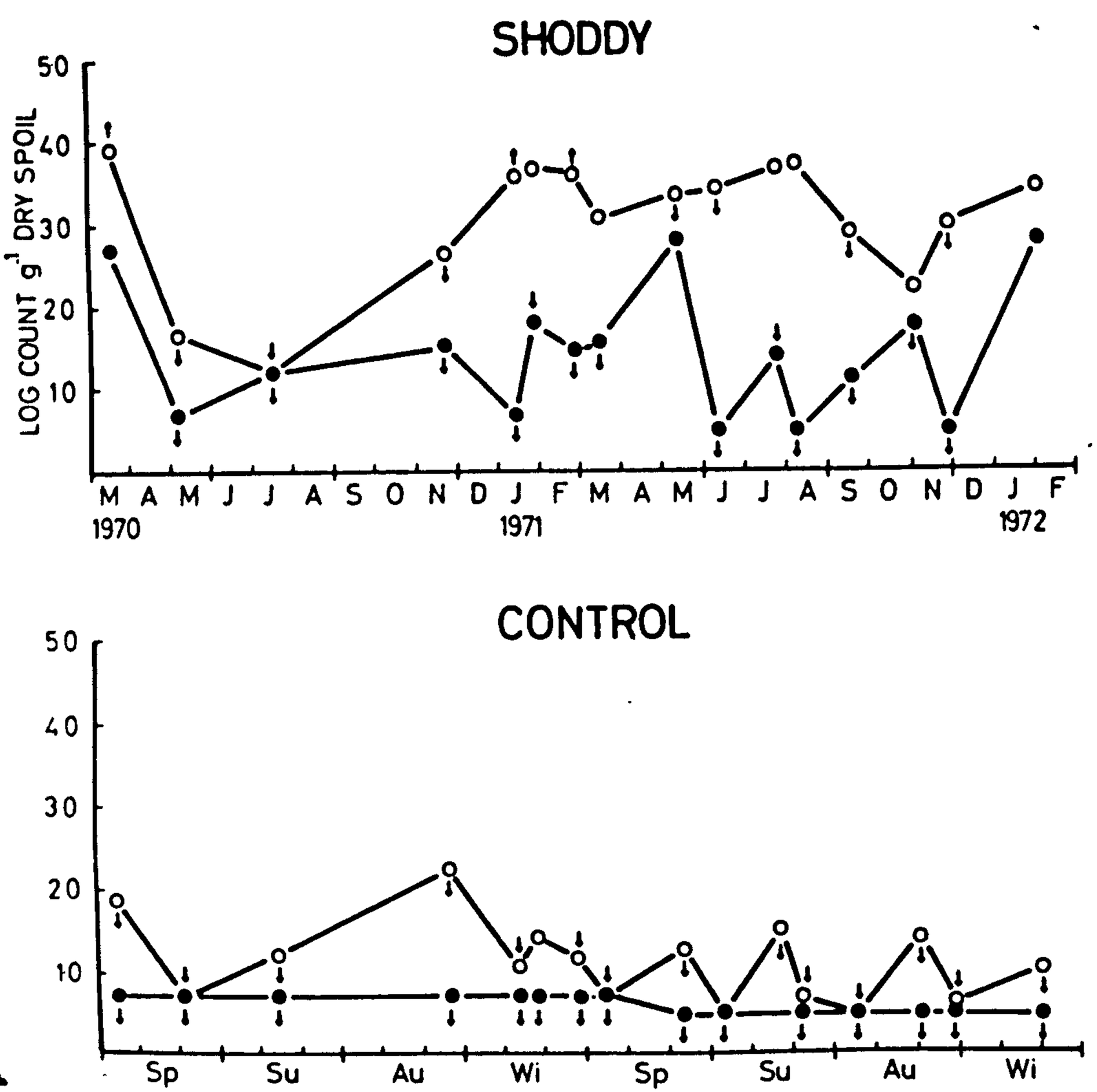
Upton

Nitrosomonas

The numbers of Nitrosomonas in all treatments at this site were very high indeed. No significant treatment differences were observed, although a significant block effect was apparent. From the ranked totals it can be suggested that this significant difference was attributable to the higher counts of block III. Seasonal fluctuations were not apparent.

Nitrobacter

Whilst the numbers of Nitrobacter were high in all treatments, the statistical analysis suggested that the greatest number occurred in the shoddy plots. The significant difference between blocks indicated that block I contained significantly more organisms than the other two.



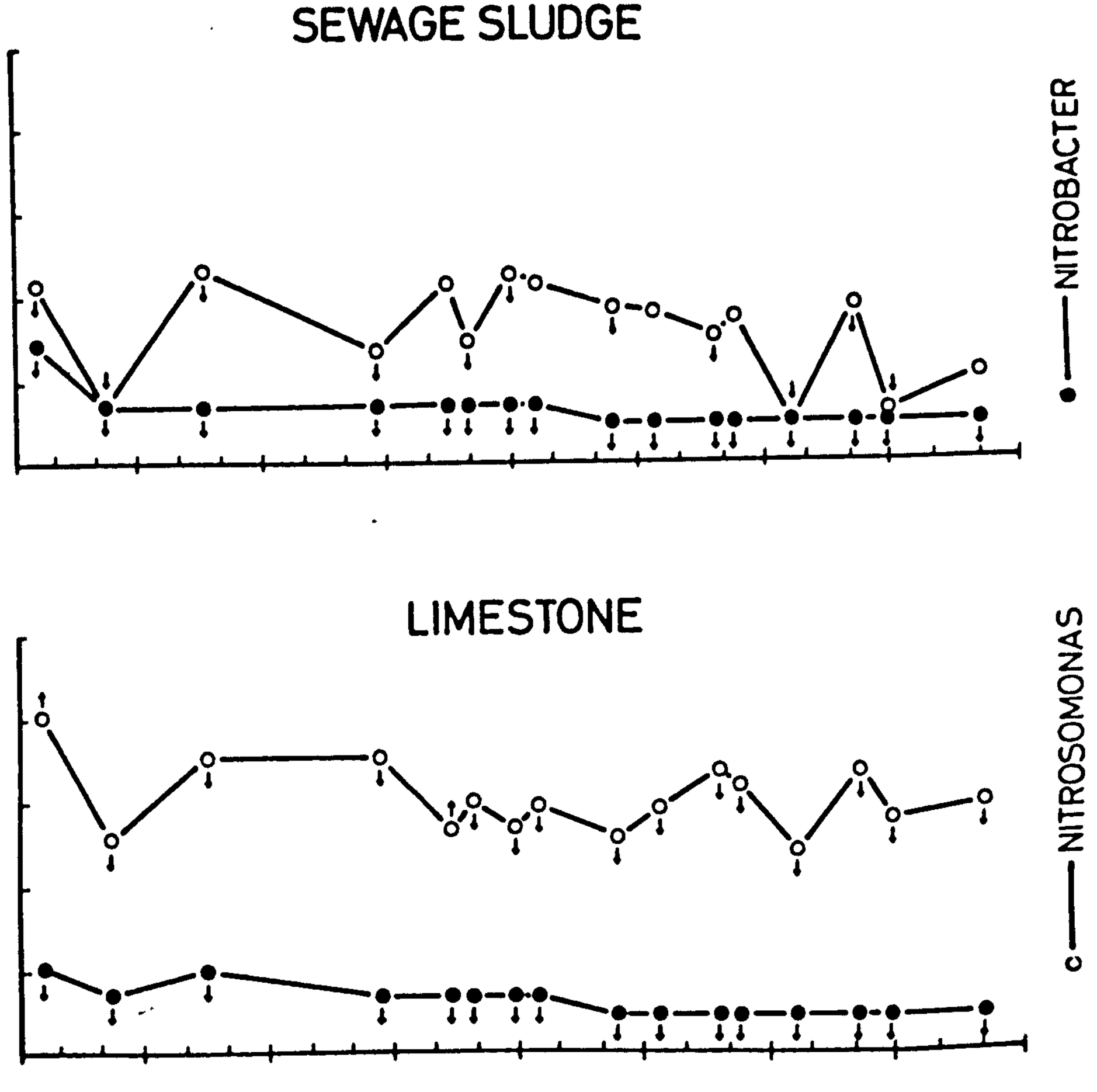


Fig. 4.5. Mitchell's Main. Seasonal variation of Nitrosomonas and Nitrobacter.

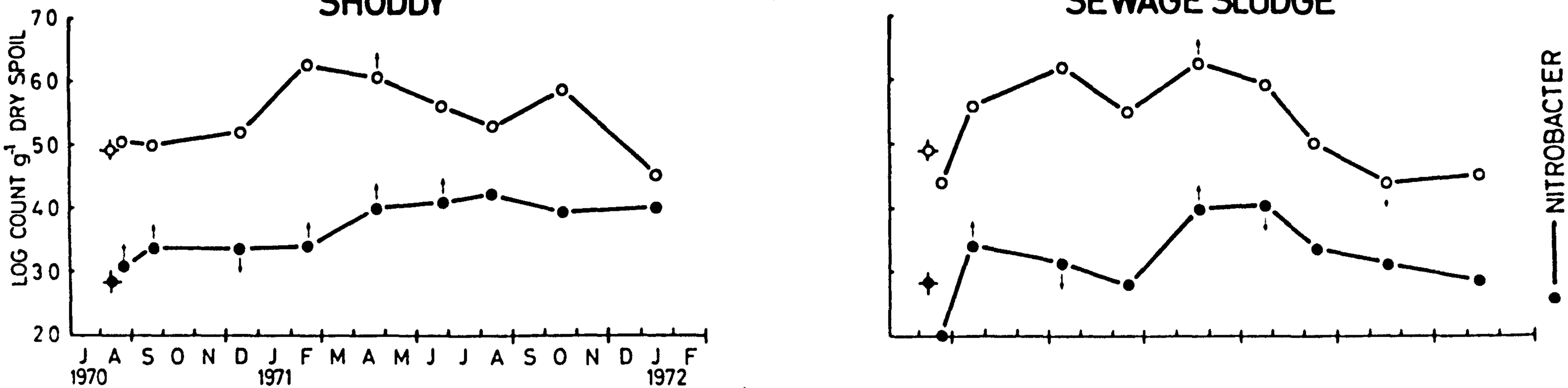


Fig. 4.6. Upton. Seasonal variation of Nitrosomonas and Nitrobacter.

DISCUSSION

The very low numbers of Nitrosomonas and Nitrobacter at Mitchell's Main was probably related to some extent to the low spoil pH at this site because it is well known that both groups are very intolerant of acid conditions. Since, however, the numbers of Nitrosomonas were as great in the shoddy plots (mean pH 3.6) as the limestone plots (mean pH 5.6), the situation was somewhat complicated. Further, the spoil from all the plots contained some nitrate and despite the low numbers of nitrifying autotrophs and the fact that some of this nitrate may have arrived in the rainfall, it seems likely that nitrification was occurring. Similar findings were reported by Weber & Gainey (1962) for acid soils where nitrate was produced at pH values as low as pH 4. Nitrification at such low pH values is limited to less acid or neutral microsites within the generally acid soil mass. The extreme variability of colliery spoil has already been discussed and it would seem probable that such less acid microsites would occur in colliery spoil. The higher number of Nitrosomonas in the shoddy plots would perhaps suggest that the presence of shoddy in the spoil increased the number of these sites and thus allowed the development of Nitrosomonas numbers to values as high as those found in the limestone plots.

The effect of substrate availability cannot, however, be ruled out because although it was previously shown that the control and sewage-sludge plots contained high levels of ammonium, the shoddy plots contained significantly more. There would, therefore, appear to have been an interaction between pH and substrate availability that

resulted in the shoddy and limestone plots showing approximately equal numbers of Nitrosomonas. This interaction is further indicated by the fact that Nitrobacter numbers, although very low, were higher in the lower pH shoddy plot, where ammonium (and hence nitrite) was more available than the higher pH limestone plots where ammonium was less available.

The very high numbers of nitrifying organisms found at Upton is in complete contrast to the Mitchell's Main situation. The remarkable fact about this site is that these very high numbers were found to occur in the spoil only a few weeks after the spoil heap had been regraded, and before the ameliorants were applied. The spoil surface upon which the trial was established was composed of freshly exposed spoil and the microorganisms must have recolonised naturally. Nitrosomonas and Nitrobacter are strict autotrophs that do not form resistant spores. Their numbers are often therefore taken as an index of their activity and since their activity depends upon the presence of substrate (i.e. initially ammonium), of nitrogen availability. The high numbers found so soon after regrading would therefore suggest that the freshly exposed soil was rich in ammonium. Since no fertilizer additions were made and the quantity of ammonium arriving in the spoil by natural agencies would not be expected to support this number of organisms, the source of this nitrogen is of interest. Whilst it is known that colliery spoil contains appreciable quantities of fossilized organic nitrogen, it is generally assumed that little or none of this becomes available to plants or microorganisms

(Doubleday 1972b). Cornwell & Stone (1968, 1973), however, stated that ammonium fixed in the lattice structures of clay minerals can be released under conditions of extreme acidity, and subsequently used by plants growing in the spoil. This explanation would not fit the present situation because of the neutral reaction of the Upton spoil. During the reshaping operations at Upton, the subsurface layers of spoil were found to be burning. During combustion, the nature of the spoil may undergo changes, (Doubleday 1972a) and Glover (personal communication) has suggested that some of the organic nitrogen may be converted to ammonium compounds including ammonium sulphate. Ammonium produced by this means could possibly account for the high initial numbers of nitrifying autotrophs at this site but no experimental evidence is available at present, to support this theory. When the reshaping operations were carried out, the new surface was heavily compacted, thus excluding oxygen and hence the possibility of further combustion. A continued supply of ammonium as a result of combustion would, therefore, seem unlikely.

The numbers of nitrifyers at Upton remained high during the whole of the sampling programme. This is interesting because very few nitrifyers are found to occur in grassed soils (the numbers found at Upton are as high as the highest levels found in unfertilized cultivated soils, Alexander 1965b). Various explanations have been put forward to explain the low numbers beneath grass swards. These include the inhibition of the nitrifyers by grass root exudates, (Soulides & Clark 1958; Theron 1951) and

the effects of adverse pH conditions and, or, substrate availability (Robinson 1963). Since the grass developed quite quickly at Upton, the numbers of nitrifying organisms might be expected to fall off rapidly as competition for the ammonium developed. This may not be so because despite the fact that the nitrifying organisms do not form resistant spores it seems likely that they may persist in soil in inactive forms for relatively long periods of time. Thus, it is well known that soil can be air dried but still retain its ability to nitrify when remoistened. Cooper (personal communication) has demonstrated that when soils containing few nitrifying autotrophs are incubated with an ammonium source, the numbers of nitrifyers rapidly increase to a high level at which they remain for several months after all of the added ammonium had been converted to nitrate. Thus, high counts of Nitrosomonas and Nitrobacter may only indicate the past availability of ammonium.

At both sites the numbers of Nitrosomonas were greater than Nitrobacter. This situation is commonly found in soils and results from the fact that more energy is released when ammonium is oxidised to nitrite, than when nitrite is oxidised to nitrate. In soils, populations of Nitrosomonas range between four and twenty times greater than Nitrobacter, but because Nitrobacter are potentially more active than Nitrosomonas (Alexander 1965b) nitrite is rapidly converted to nitrate. At Upton the population of Nitrosomonas was approximately one hundred times as great as Nitrobacter and this might explain the higher than usual levels of nitrite in the spoil.

Similarly at Mitchell's Main, whilst the numbers of Nitrosomonas were low, Nitrobacter were only infrequently isolated.

Since Nitrosomonas and Nitrobacter largely control the form in which mineral nitrogen appears in the spoil, it is interesting to compare the results of the enumeration study with the mineral nitrogen determinations presented in the section I. The two should be related because both sets of determinations were performed on the same bulked samples. At Mitchell's Main, ammonium was the predominant form of mineral nitrogen in the shoddy, sewage-sludge and control plots. The results of the microbial enumerations indicate that this was due to the inhibition of these microorganisms. In the limestone plots no predominance of ammonium over nitrate was observed. This is probably due to the fact that the pH of these plots was high enough to allow nitrification of any mineralized ammonium to proceed.

At Upton, the very high numbers of nitrifying microorganisms suggests that any ammonium released into the spoil would rapidly be converted to nitrate. No accumulation of ammonium was, in fact, observed at this site.

3. PSEUDOMONAS AND DENITROBACILLUS

INTRODUCTION

In order to evaluate the possibility of nitrogen losses from spoil as a result of denitrification, the populations of two prominent groups of denitrifying bacteria were estimated. These were Pseudomonas and Denitrobacillus. Whilst both groups have the ability to denitrify, that is, to use nitrate or nitrite as a terminal electron acceptor for respiratory purposes, oxygen is preferentially used when available. Both groups can utilize a wide variety of organic substrates and so long as oxygen is available they perform beneficial roles in the decomposition processes in soils. Their activity is somewhat limited to neutral soils because both have pH optima for growth near neutrality. Unlike Pseudomonas, Denitrobacillus species can form resistant spores and may, therefore, exist in soils and spoils in inactive forms. This feature presents interpretive difficulties similar to those discussed for the fungi.

RESULTS AND INTERPRETATION

The results for the enumeration of Pseudomonas and Denitrobacillus are shown diagramatically in Figs. 4.7 - 4.10, and the statistical analyses in Tables 4.3 & 4.4.

Mitchell's Main

Pseudomonas

Whilst the numbers in all treatments were low, a significant difference between the treatments was observed. The numbers were highest in the limestone and lowest in the control plots with the shoddy and sewage-sludge plots occupying intermediate positions.

The higher numbers in the limestone treatments would

be expected because of the known sensitivity of these organisms to acid conditions. The difference between the control, sewage-sludge and shoddy plots is interesting because the pH of all three treatments was previously shown to be insignificantly different. The finding that the numbers were highest in the shoddy plots, therefore, suggests that there was a greater availability of substrate or that more neutral microsites occurred in these plots.

No trends of counts with time were apparent and significant block differences were not found.

Denitrobacillus

The distribution pattern of Denitrobacillus between the treatments was very similar to that recorded for Pseudomonas. Thus, the highest counts were recorded in the limestone, and the lowest in the control plots, with the shoddy and sewage-sludge filling the intermediate positions. The interpretation is similar to that already given for Pseudomonas results.

Upton

Pseudomonas

The numbers of Pseudomonas present in the spoil prior to, and immediately after ameliorant additions were very small. There was a general tendancy for the counts to increase with time. This would be expected because the development of a surface cover of vegetation would improve the general conditions for microbial growth.

The statistical analysis indicated that a significant treatment difference occurred. Observation of the mean values and rank totals suggests that this was due to the higher numbers of organisms isolated from the shoddy plots.

Since the pH was uniformally high in all the treatment plots, this result suggests that there was a greater availability of substrate in the shoddy plots than the others.

A significant block difference was found to occur and this is attributable to the higher counts given by samples from block I.

Denitrobacillus

The numbers of Denitrobacillus isolated from the spoil before ameliorant application were very high, and were only increased slightly by the application of shoddy and sewage-sludge. Since the spoil surface was initially completely devoid of vegetation, and hence easily decomposable organic matter that could be used as substrate, it seems likely that the majority of the organisms isolated before the ameliorant additions were applied, were present in the spoil as spores.

The statistical analysis showed that a significant treatment effect occurred. The highest numbers were once again found in the shoddy plots and the lowest in the control plots.

The counts fluctuated greatly with time and no trends were apparent.

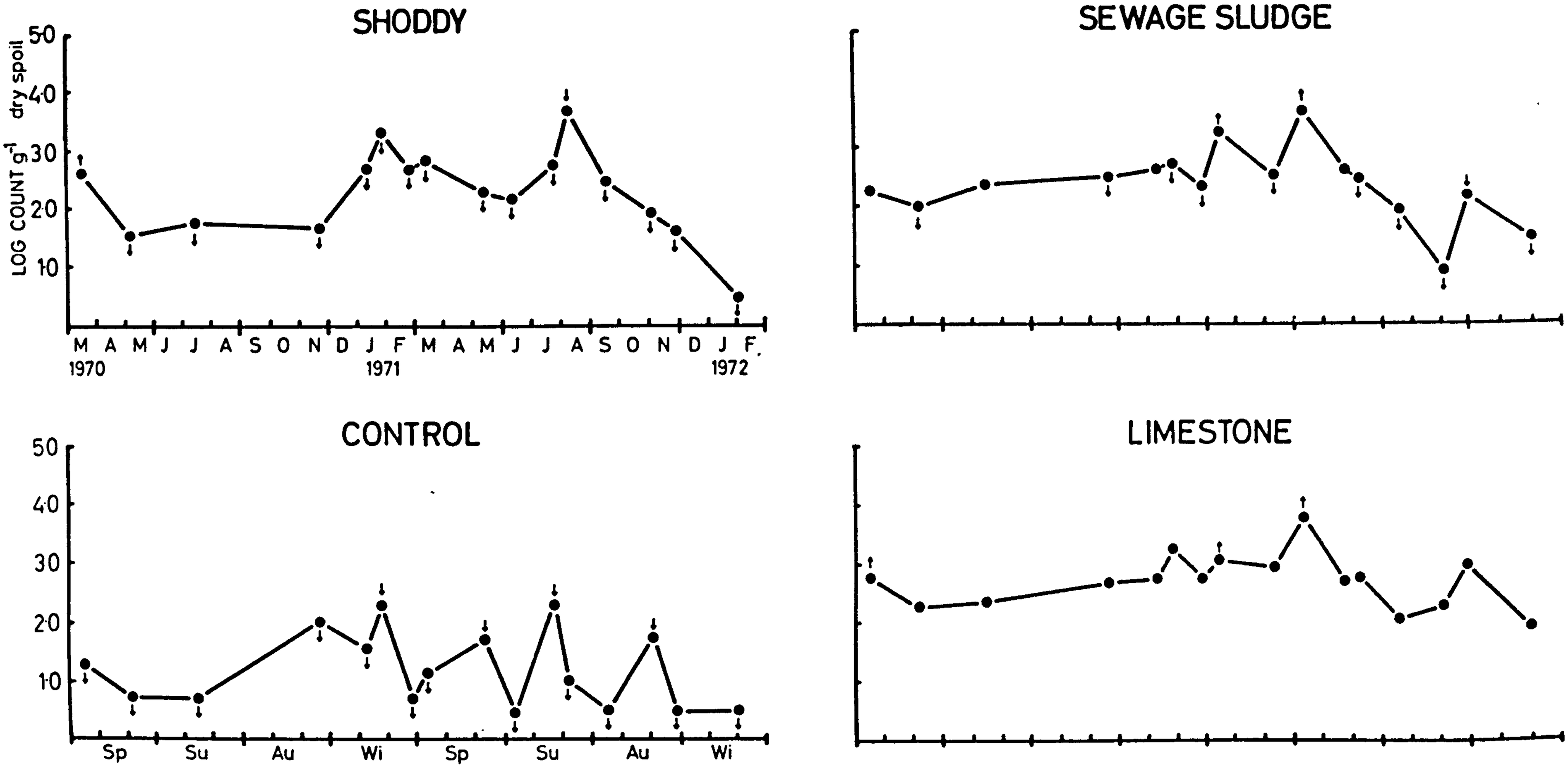


Fig. 4.7. Mitchell's Main. Seasonal variation of Pseudomonas.

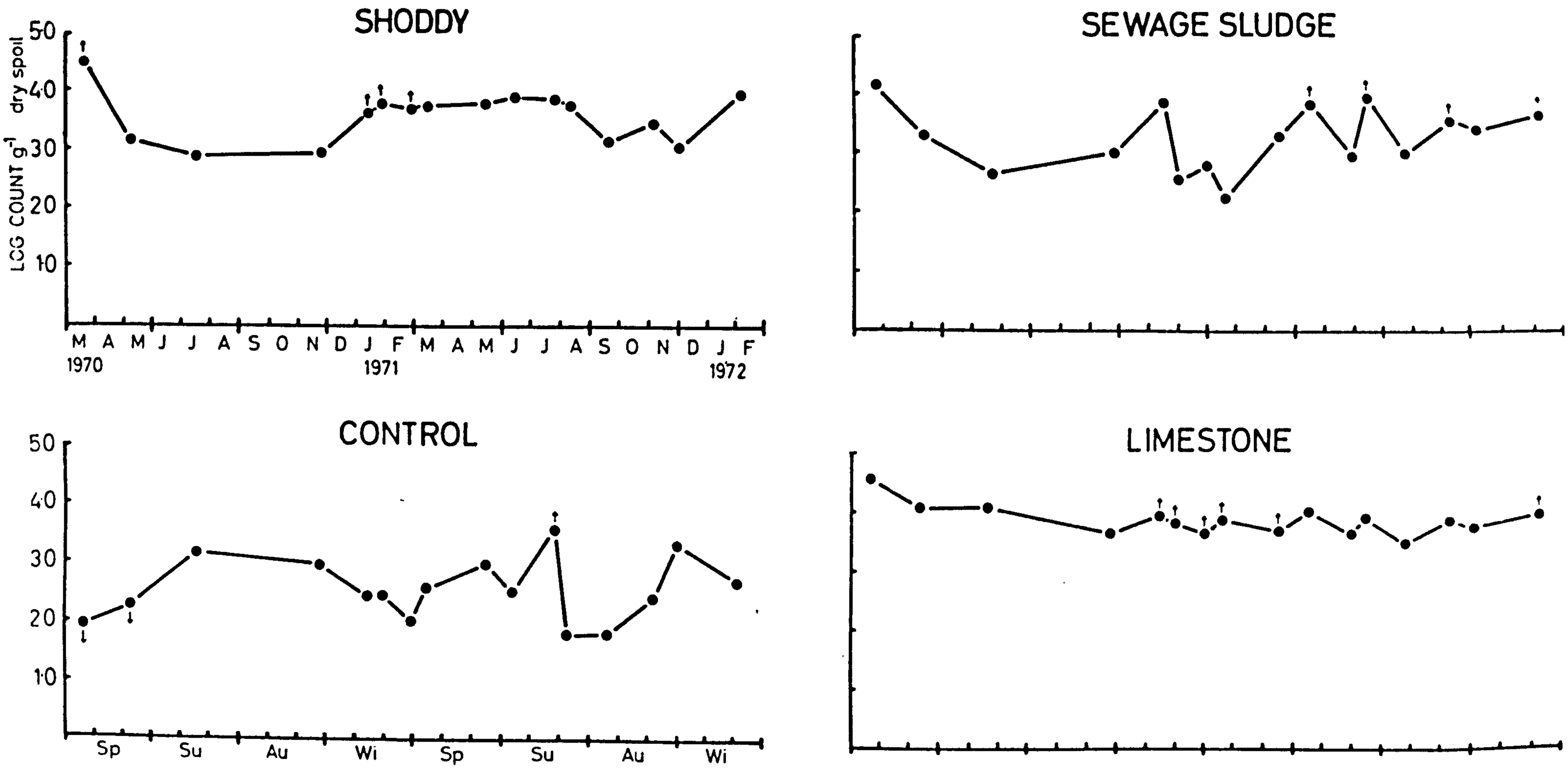
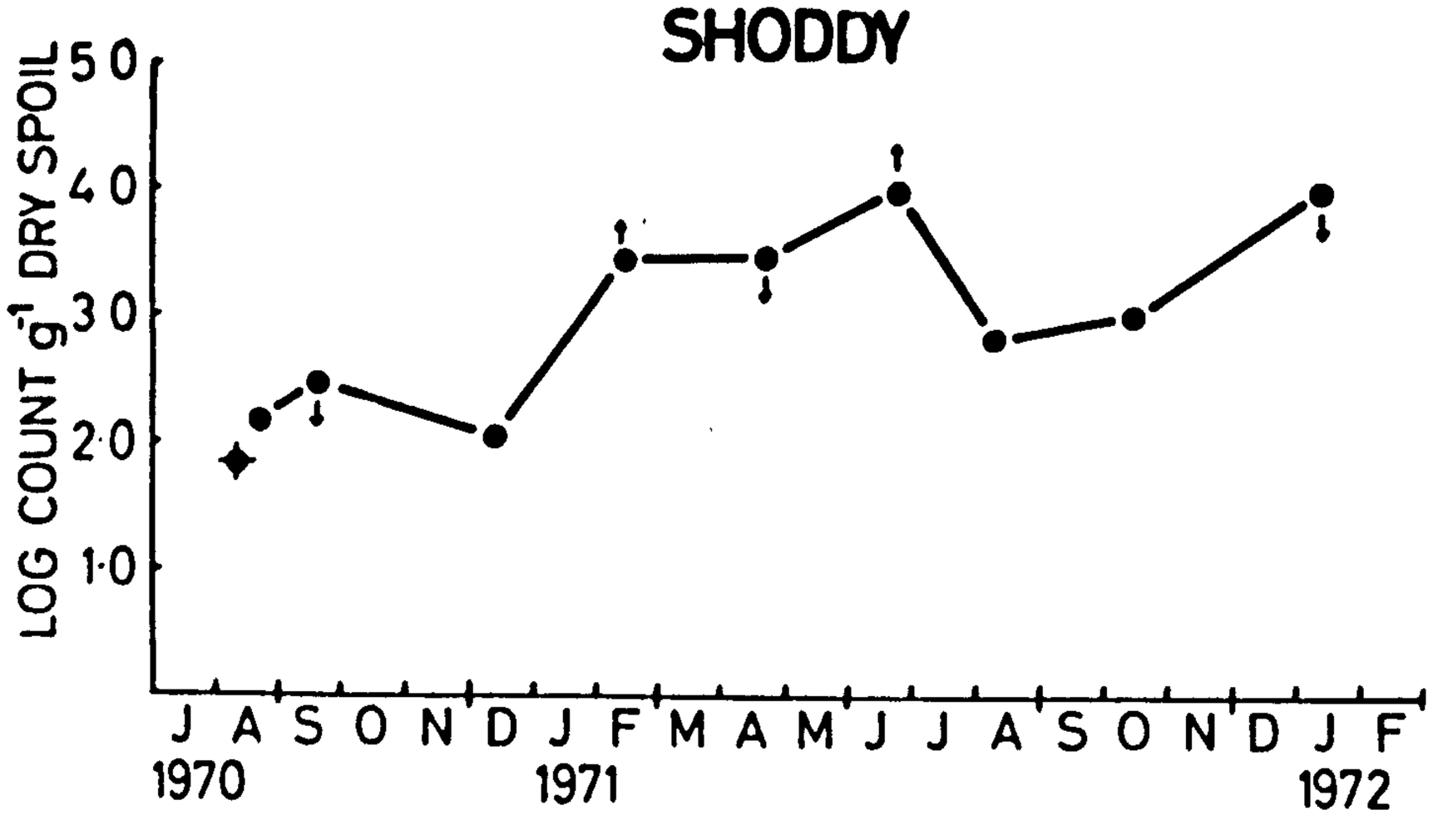
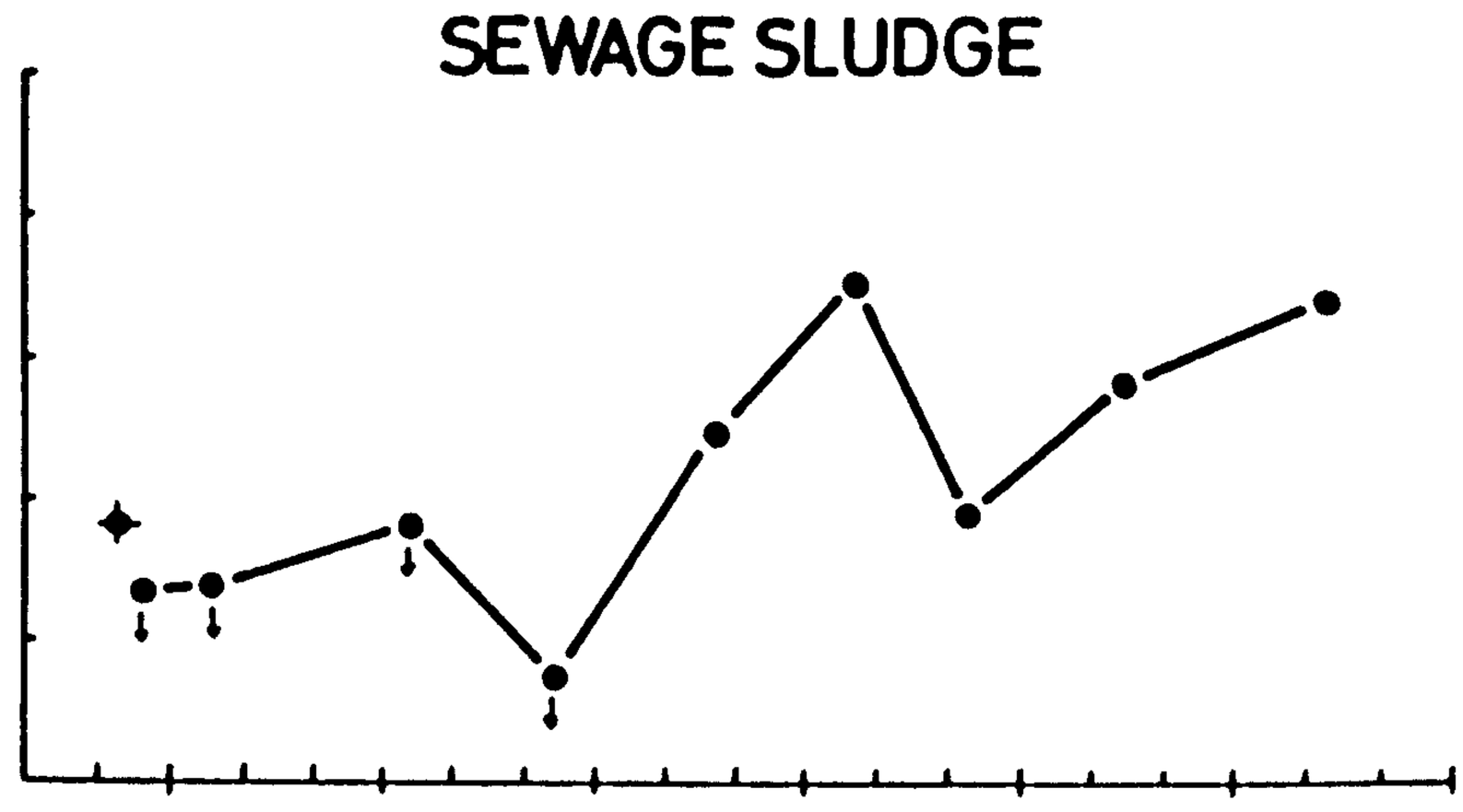
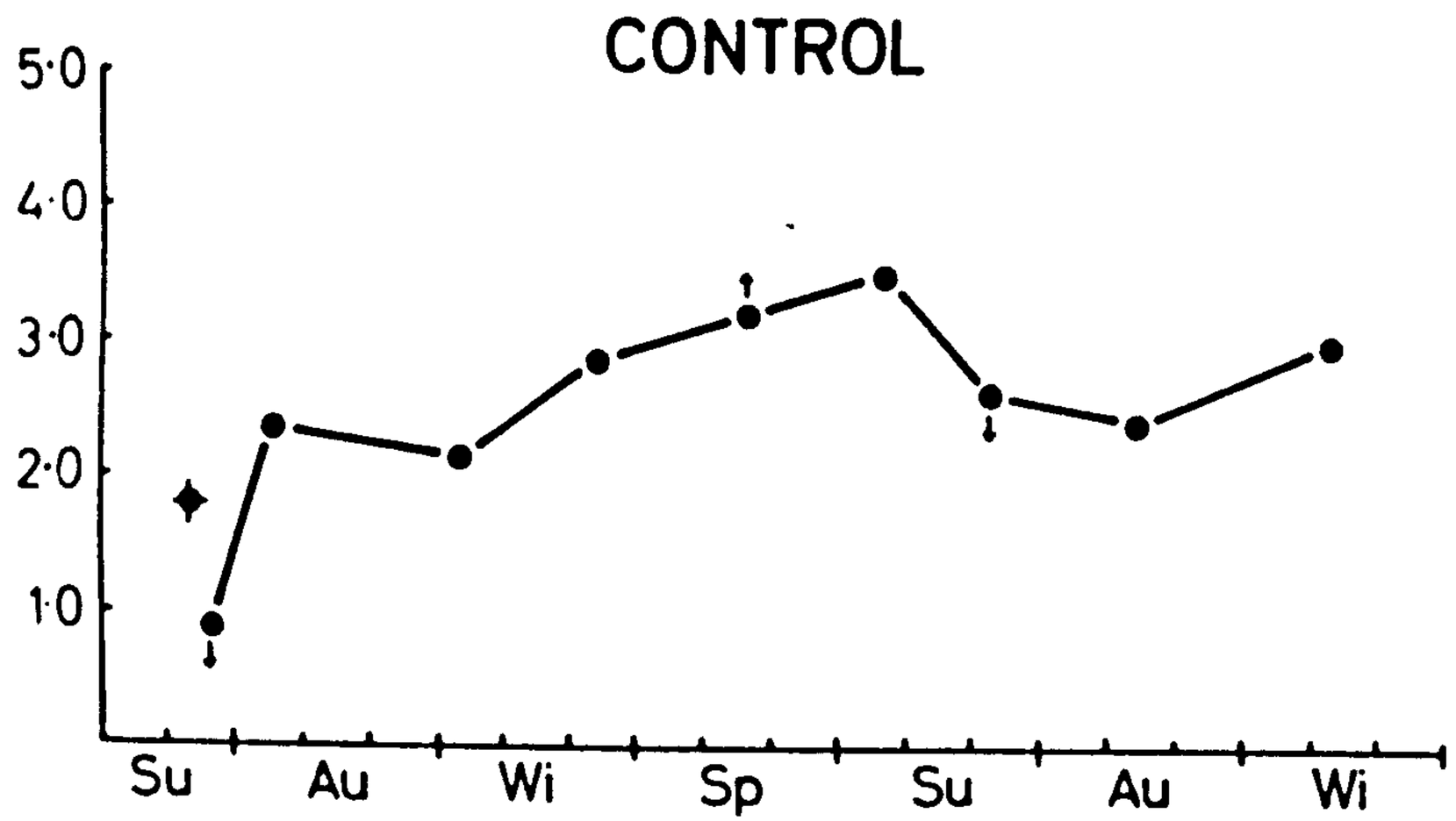


Fig. 4.8. Mitchell's Main. Seasonal variation of Denitrobacillus.







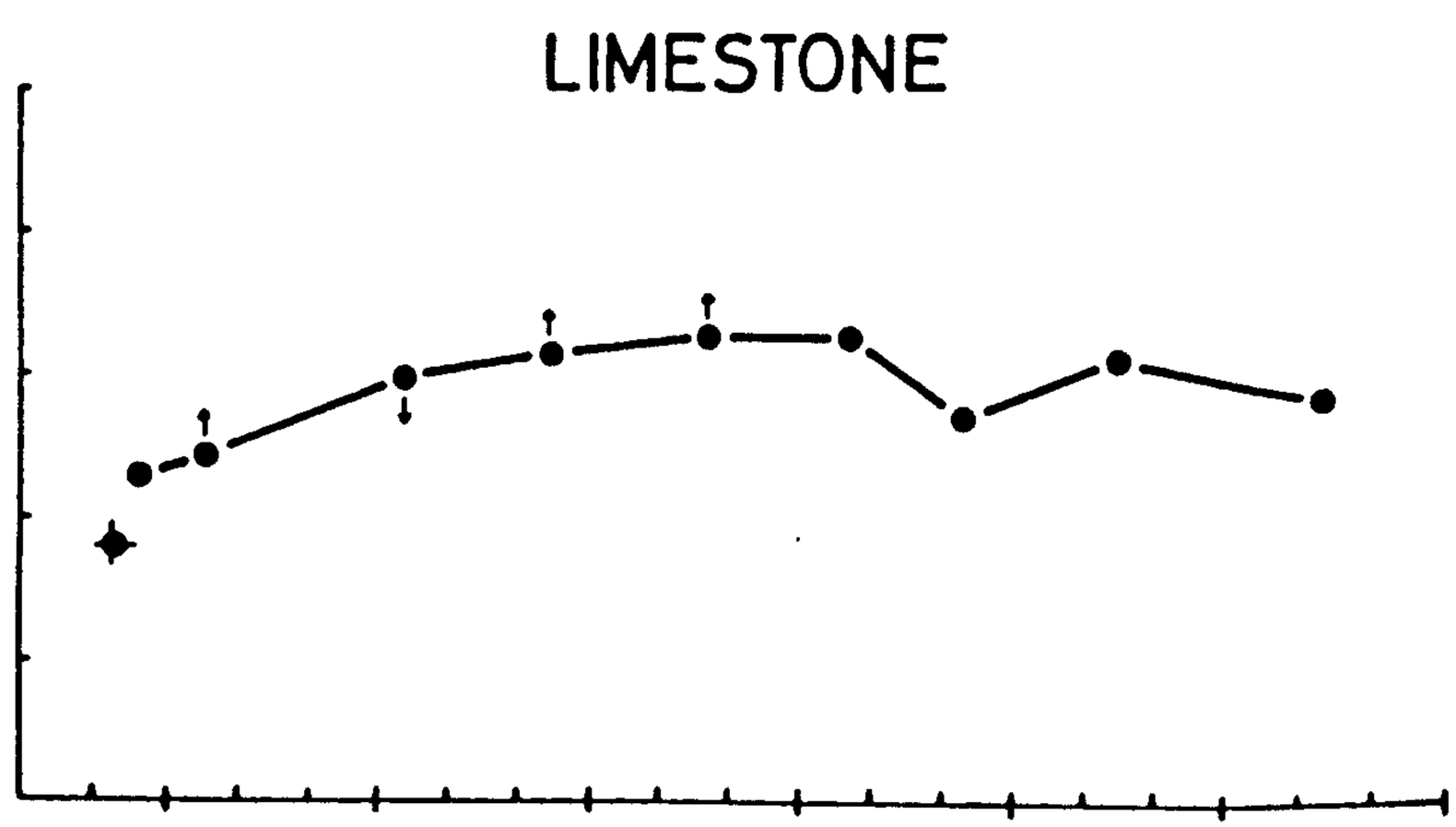


Fig. 4.9. Upton. Seasonal variation of Pseudomonas.

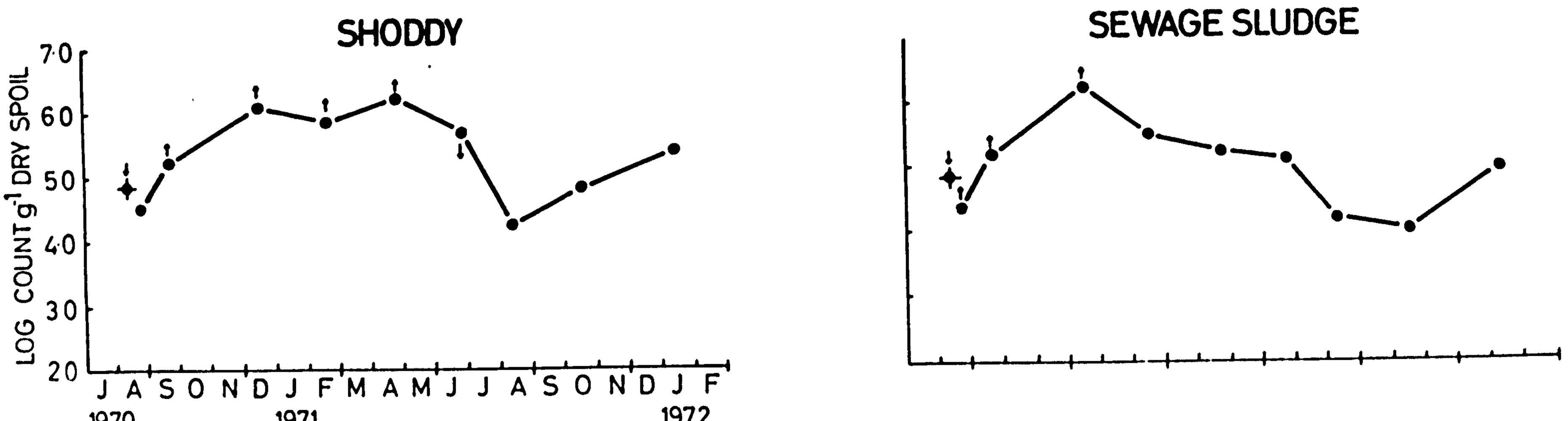


Fig. 4.10. Upton. Seasonal variation of Denitrobacillus.

DISCUSSION

Despite the fact that large populations of denitrifying organisms were found to occur in some of the spoil plots, it cannot be concluded that rapid denitrification was proceeding. This situation arises because some of the Denitrobacillus propagules isolated has probably existed in the spoil only as spores, and more importantly, because both groups are not obligatively anaerobic. In fact, oxygen is used in preference to nitrate or nitrite as the terminal electron acceptor until the oxygen tension falls to a low level. The high counts do, however, suggest that the spoils have a large potential for denitrification should conditions become suitable.

The conditions that favour denitrification are, low oxygen tension coupled with high substrate availability, and the presence of nitrate or nitrite (Harmsen & Kolenbrander 1965). Under field conditions a low oxygen tension is usually brought about by flooding or the addition of relatively large quantities of easily decomposable organic matter. Since the field trial plots at both Mitchell's Main and Upton were established on fairly steep slopes, the spoils never became submerged, and indeed, drained rapidly. It would, therefore, seem unlikely that anaerobic conditions would develop as a result of waterlogging at either site.

The shoddy and sewage sludge applications did represent additions of easily decomposable materials and rapid decomposition of these could have lead to the development anaerobic microsites. At Mitchell's Main, however, the

very low pH of the spoil to which these additions were made probably prevented their rapid decomposition and hence the production of anaerobic conditions and subsequent denitrification. Further, denitrification proceeds only very slowly under acid conditions (Bremner & Shaw 1958) and hence losses of nitrogen through denitrification are likely to have been very small at Mitchell's Main.

The situation at Upton was very different. The spoil was of neutral reaction and contained large numbers of denitrifying and nitrifying organisms even before the organic additions were made. Rapid decomposition would be expected at this site, and may have lead to the production of anaerobic microsites where denitrification could proceed rapidly.

The quantities of nitrogen lost through denitrification cannot be assessed from the results of the present investigations. Nevertheless, they do indicate that losses could occur in reclamation schemes where nitrogen was supplied in the forms of heavy dressing of organic materials, like sewage-sludge, to large poorly drained areas of neutral or limed acid spoils.

Table 4.3. Mitchell's Main. Non-parametric analysis of variance results for the microbial enumerations.

FUNGI

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	56	50.5	31.5	22
mean	173,000	151,000	69,000	54,000

$\chi r^2 = 28.59$ ***

between blocks

	I	II	III
rank total	44	20	32
mean	155,000	60,000	114,000

$\chi r^2 = 18.00$**

NITROSOMONAS

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	57.5	28	22.5	53
mean	2546	73	28	1883

$\chi r^2 = 37.71$ ***

between blocks

	I	II	III
rank total	40	19	37
mean	1875	288	1234

$\chi r^2 = 16.13$ **

Table 4.3. continued

DENITROBACILLUS

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	42	36	21	61
mean	5253	3368	662	8089

χr^2 = 30.83 ***

between blocks

	I	II	III
rank total	28	32	36
mean	3877	4004	5147

χr^2 = 2.00 n.s.

PSEUDOMONAS

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	42.5	41	18.5	58
mean	628	561	42	940

χr^2 = 29.76 ***

between blocks

	I	II	III
rank total	28	35	33
mean	660	482	489

χr^2 = 1.63 n.s.

All mean values are numbers per gram dry spoil

Table 4.4. Upton. Non-parametric analysis of variance results for the microbial enumerations.

FUNGI

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	31	30	16	13
mean	121,000	118,000	36,000	26,000

$\chi r^2 = 17.41$ ***

between blocks

	I	II	III
rank total	27	11	16
mean	112	51	63

$\chi r^2 = 14.90$ ***

NITROSOMONAS

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	22	26	28	14
mean	493,336	546,356	465,297	184,383

$\chi r^2 = 7.67$ n.s.

between blocks

	I	II	III
rank total	16	14	24
mean	369,722	225,004	672,304

$\chi r^2 = 6.23$ *

Table 4.4. continued

NITROBACTER

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	33.5	14.5	19.5	22.5
mean	6930	3239	5005	5268

χ_r^2 = 12.94 **

between blocks

	I	II	III
rank total	26	12.5	15.5
mean	9098	2752	3481

χ_r^2 = 11.18 **

DENITROBACILLUS

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	30	23	15	22
mean	464,567	297,489	60,922	183,862

χ_r^2 = 7.54 n.s.

between blocks

	I	II	III
rank total	17	19	18
mean	274,350	217,960	262,821

χ_r^2 = 0.23 n.s.

Table 4.4. continued

PSEUDOMONAS

between treatments

	shoddy	sewage-sludge	control	limestone
rank total	33	15	18	24
mean	2718	771	842	913

$\chi r^2 = 12.61$ ***

between blocks

	I	II	III
rank total	25	14	15
mean	1437	1073	1424

$\chi r^2 = 8.23$

All mean values are numbers per gram dry spoil

GENERAL DISCUSSION OF MICROBIAL ENUMERATIONS

The results of all the microbial enumerations taken together indicate two main points. Firstly, the low pH of the unlimed plots at Mithcell's Main was inhibitory to the bacterial groups studied. Secondly, the numbers of organisms were generally highest in those plots receiving organic amendments, especially the shoddy plots. This applied both to the neutral plots at Upton and to the acid ones at Mitchell's Main. Although for the numerous reasons already discussed it is difficult to relate microbial numbers to activity, the finding that the numbers of all groups or organisms studied were, almost without exception, highest in the shoddy plots, would strongly suggest that microbial activity was correspondingly higher in these plots. This indicates that there was a greater availability of decomposable substrate in these plots. Some evidence that this was in fact so is provided by the data for the levels of mineral nitrogen at Mitchell's Main. Thus, the levels of ammonium were higher in the shoddy plots than the others. This was not just due to a greater inhibition of nitrification in these plots because it was previously shown that the largest populations of nitrifying organisms were also found in these plots, and in fact, it has been suggested that the two factors were probably related. The same sort of evidence for the Upton site is not available because the large number of nitrifying organisms present in the spoils would rapidly oxidise any ammonium to nitrate, which, because of its high solubility might subsequently be lost through leaching. Further, mineral nitrogen determinations were unfortunately

not available for the period immediately after ameliorant application when the microbial numbers were generally at their highest levels.

The large number of organisms, both spore formers and non-spore formers, found at Upton in the enumeration performed before the ameliorant additions were made illustrates the very rapid rate at which the freshly exposed and presumably sterile spoil became naturally colonised.

This indicates that provided the condition for microbial growth in terms of substrate availability, pH and spoil moisture were suitable, there would be no reason why a normal soil-like microflora should not develop rapidly. Factors like the provision of substrate and soil moisture are intimately related to the occurrence of vegetation and hence the effects of nutrient deficiencies and toxicities on plant growth would be reflected by the microbial populations. Investigation into these interesting relationships was, unfortunately, beyond the scope of the present investigations.

SECTION III INCUBATION EXPERIMENT I

INTRODUCTION

Whilst the results of the periodic mineral nitrogen determinations and microbial population studies provided useful information on the form and factors affecting the interconversions of nitrogen in the spoils, no information was obtained on the quantity of, or rate at which, organic nitrogen reserves in the spoils became mineralized and hence made available to plants.

This information can be obtained from estimations of mineral nitrogen released when a spoil is incubated under conditions of temperature and moisture that promote mineralization of spoil organic matter. Since the fate of mineralized nitrogen can be studied, such techniques also provide information on the process of nitrification.

Incubation techniques have been widely used to investigate the availability of nitrogen in diverse soil types but very few investigations have been made for colliery spoils. Indeed, the only published work appears to be that of Wilson & Stewart (1955). This will be examined in some detail. These authors used incubation techniques to study the processes of mineralization and nitrification in West Virginian, acid, stripmine spoils. Comparisons were made between bare and vegetated spoils, and a local undisturbed (placeland) spoil. Mineralization studies were based on a technique whereby a number of organic nitrogen sources including egg albumen, urea and peptone were incubated both in the presence and absence of calcium hydroxide. The results indicated that all

the nitrogen sources could be mineralized but the rate was generally greater in the vegetated spoil and placeland soil than in the bare spoil.

Less ammonium accumulated when samples were incubated in the presence of calcium hydroxide than in its absence and the authors concluded, probably incorrectly, that this result was due to the utilization of the mineralized ammonium by microorganisms. Estimates of nitrate do not appear to have been made and it is possible that the lower ammonium levels were due to increased nitrification in the presence of the neutralizing reagent.

In the nitrification studies the spoil and soil samples were incubated with 500 ppm of nitrogen supplied as ammonium sulphate, both in the presence and absence of calcium hydroxide. The results showed that nitrification was inhibited in acid spoils. The results of this investigation were rather unusual because of the very slow rate of nitrification. This could have been due to the low pH at which the samples were incubated, even those samples receiving the theoretical amount of calcium hydroxide to neutralize the spoil acidity soon became quite acid. Further, no mention was made of the possibility of mineralization occurring during the incubation period. Certainly, for the placeland soil, some mineralization of organic matter initially present in the soil would be expected.

In summary, the work of Wilson & Stewart (1955), showed that organic nitrogen sources could be broken down in spoils and that nitrification was inhibited by the acid conditions. The way in which these workers

interpreted their results meant that no information was provided on the nitrogen supplying power of the spoils for plant growth. This fact, together with the unusual nature of their results and experimental designs reduces the usefulness of their investigations.

In the present investigations samples of the ameliorated and control spoils from both sites were incubated both in the presence and absence of ammonium sulphate and, or calcium carbonate. This approach provided information both on the availability of nitrogen in the spoils andon the factors that affect the processes of mineralization and nitrification.

For comparative purposes, two unfertilized soils, one of acid, the other of neutral reaction, were incubated under identical conditions. All samples were collected within a few days of each other in early spring, 1972.

RESULTS AND INTERPRETATION

The results are shown graphically in Figs 4.11, 4.12 & 4.13. Each plotted value represents the mean of two replicates. Nitrite was determined but the levels were always very low (<2ppm) and have therefore been added to the nitrate values. The initial pH at which the samples were incubated is indicated on each graph. The numbers of Nitrosomonas and Nitrobacter present in each sample immediately prior to incubation are shown in Table 4.5.

1. Mitchell's Main (Fig.4.11)

The shoddy, sewage-sludge and control spoil samples responded similarly to the laboratory additions of ammonium sulphate and, or calcium carbonate. These three spoil treatments will, therefore, be described

together.

On incubation with both ammonium sulphate and calcium carbonate, the ammonium level remained high during the nought to ten day period but thereafter rapidly fell to a low value, at which it remained for the duration of the experiment. The nitrate level was initially very low but increased with time showing that the ammonium was being converted to nitrate.

Incubation with ammonium sulphate alone gave rise to a different response pattern. The concentration of ammonium increased slowly whilst that of nitrate did not alter markedly and remained at the low initial level. The conversion of ammonium to nitrate did not occur to any great extent.

The results for the other pair of laboratory treatments i.e. calcium carbonate alone, and no addition, resembled the first pair that have been discussed. Thus in the presence of added calcium carbonate, nitrate accumulated, and in its absence, ammonium accumulated.

The limestone treatment responded differently on incubation. In the presence of ammonium sulphate, both with and without calcium carbonate, nitrate accumulated at the expense of the added ammonium. In the absence of ammonium sulphate, calcium carbonate addition had little effect since in both instances small quantities of nitrate accumulated.

The results demonstrate that for all treatments except the limestone one, the low pH of the spoil was inhibitive to nitrification. The pH of the limestone treatment was high enough to allow nitrification to proceed.

Nitrification is, of course, dependant upon the active presence of the autotrophic nitrifying bacteria. Counts of these organisms in the spoil prior to incubation (Table 4.5) showed that whilst the numbers were low in all treatments, more were found in the limestone treatment than the others. The low initial numbers of Nitrosomonas and Nitrobacter probably explains why the conversion of ammonium to nitrate in the neutralized spoils was greater between the 10-20 day period than before. A 'lag phase' occurred during which the organisms were presumably multiplying in response to the onset of more favourable conditions.

During the twenty to forty day period, the rate at which ammonium was oxidised decreased. This was probably due to substrate depletion. The experiment was discontinued after forty days but it seems likely that a further small increase in the levels of ammonium and nitrate would have occurred if the incubation period could have been extended.

It is apparent from Fig. 4.11 that mineralization of organic nitrogen reserves initially present in the spoil samples occurred during the incubation period. The quantity of mineral nitrogen present at the end of the incubation period minus that present initially represents the quantity of mineral nitrogen that had accumulated as a result of the mineralization of spoil organic nitrogen. The actual value varied both with the different treatments and the nature of the laboratory additions, and are shown in Table 4.6. These data have been analysed statistically and the results are shown in Table 4.7.

The statistical analysis shows that significant differences arose as a result of the spoil amelioration applied in the field some years before sampling and the laboratory additions. Further, a significant interaction between the two classes is observed.

Of the laboratory additions, calcium carbonate had the more profound effect. The quantity of mineralized nitrogen was significantly greater in the presence of calcium carbonate, regardless of whether or not, ammonium sulphate applications were made. The magnitude of the effect of calcium carbonate was related both to the initial pH at which the spoil was incubated and the spoil treatment. Thus on incubation without calcium carbonate, the higher pH of the sewage-sludge as compared with the shoddy treatment resulted in the greater accumulation of mineral nitrogen in the sewage-sludge treatment. When calcium carbonate was added to both, raising the pH to 7.2 in each case, the shoddy samples produced much more mineral nitrogen than the sewage-sludge ones. This result indicates that when the pH of the spoil was raised, many groups of heterotrophic microorganisms, previously inhibited by the acid conditions proliferated and promoted the mineralization of organic materials that previously remained unattacked, or were attacked only slowly.

The addition of ammonium sulphate tended to reduce the quantity of nitrogen mineralization. Of the two comparisons that can be made i.e. calcium carbonate + ammonium sulphate vs. calcium carbonate alone, and ammonium sulphate alone vs. no addition, the depressive effect is only significant for the first comparison.

The depressive effect of ammonium sulphate probably occurred because the oxidation of ammonium sulphate results in a drop in pH and hence a decrease in the activity of mineralizing microorganisms. Similar effects are known to occur under field conditions when fertilizer nitrogen is applied as ammonium sulphate (Whitehead 1971).

Assessment of the effects of the ameliorative treatments i.e. the original applications of shoddy, sewage-sludge and limestone, is complicated both by the effect of the laboratory additions and the fact that the original pH of the incubated samples were different. Comparison of the overall treatment means suggests that the shoddy and sewage-sludge treatments contained similar quantities of mineralizable nitrogen. These values were significantly higher than that produced by the control, which in turn, was significantly higher than for the limestone treatment. Comparison of treatment means at the same calcium carbonate regime demonstrates however, that the shoddy samples produced more mineral nitrogen than the sewage-sludge ones.

The difference between mineralizable nitrogen values produced on incubation of samples with and without calcium carbonate demonstrates that although the acid spoils contain appreciable quantities of potentially mineralizable nitrogen, the low spoil pH prevented its rapid mineralization. It is interesting to observe that the results suggest that limestone treatment contained the smallest quantity of mineralizable nitrogen. This indicates that the pH of this treatment was high enough to allow rapid

mineralization of easily decomposable organic matter.

The results show that the spoil treatments differed in their potential reserves of mineralizable nitrogen and the following order is suggested. Shoddy> sewage-sludge> control> limestone.

2. Upton (Fig.4.12)

All four treatments responded similarly to the laboratory additions of calcium carbonate and ammonium sulphate and will, therefore, be described together.

On incubation with ammonium sulphate and calcium carbonate, added ammonium largely disappeared within the first ten days. A quantity of nitrate, approximately equal to that of the added ammonium appeared. After the first ten days, no large changes in the levels of ammonium or nitrate occurred.

Similar results were obtained when samples were incubated with ammonium sulphate alone.

Both sets of incubations without ammonium sulphate responded similarly; the concentration of ammonium and nitrate remaining low throughout the incubation period. The concentration of nitrate did, however, rise slightly over the forty day period and was generally higher than that of ammonium.

The very rapid oxidation of applied ammonium would be expected because of the high numbers of nitrifying bacteria present in the spoils before incubation (Table 4.5).

The results show that the neutral reaction of the Upton spoil enabled large populations of nitrifying autotrophs to exist and to rapidly nitrify any ammonium added to, or mineralized in, the spoil.

The quantities of mineralized nitrogen produced after forty days incubation are shown in Table 4.6. These data have been analysed statistically and the results of this analysis are shown in Table 4.8. This analysis demonstrates that no significant difference was observed between the different spoil treatments. A significant difference did occur for the laboratory additions. Incubations with calcium carbonate gave significantly higher mineralizable nitrogen values than those which included only ammonium sulphate. Incubation with ammonium sulphate either alone, or in combination with calcium carbonate, resulted in a reduction of mineralizable nitrogen when compared to incubations made either with no addition or with calcium carbonate alone. These differences were not, however, large enough to be statistically significant (at the 5% level).

Calcium carbonate had a significant stimulatory effect on mineralization of organic matter, although the quantities of mineral nitrogen involved were not great.

3. Acid moorland soil (Fig.4.13)

The pattern of response for this soil to the laboratory additions of calcium carbonate and ammonium sulphate was very similar to that of the acid Mitchell's Main spoils. Thus, in the presence of calcium carbonate, added or mineralized ammonium was oxidised to nitrate, whilst in its absence, it remained largely unconverted. Some nitrate was, however, formed even in the incubation performed without laboratory additions, despite the low soil pH.

The low numbers of nitrifying bacteria in the soil

at the beginning of the incubation period (Table 4.5) probably accounted for the initial rise in ammonium concentration in samples incubated with added calcium carbonate.

The quantity of mineralized nitrogen produced after forty days incubation is shown in Table 4.6. Ammonium sulphate does not appear to have a depressive effect on mineralization. This may be due to the greater buffering capacity of this soil as compared with the spoils previously described. Calcium carbonate, however, had the same stimulatory effect on this soil as it had on the acid spoils.

4. Woodland soil (Fig. 4.13)

In all incubations, nitrate rather than ammonium was the predominant form of mineral nitrogen. This result could perhaps have been predicted from the high counts of nitrifying bacteria initially present in the soil (Table 4.5).

The laboratory additions of calcium carbonate and ammonium sulphate had little effect on the quantity of mineralized nitrogen (Table 4.6). This is probably due to the fact that the pH of the soil was initially high and remained so during the incubation period.

5. Comparison of the quantities of mineralizable nitrogen released by the spoils and soils

Table 4.9 gives the quantities of mineralized nitrogen produced after forty days incubation without laboratory additions. The results suggest that Mitchell's Main spoils could supply more nitrogen for

plant growth than the Upton spoils. Both soils could, however, supply very much more nitrogen than the Mitchell's Main spoils.

Table 4.9. Nitrogen mineralized in spoils and soils.

	Mitchell's Main	Upton
Shoddy	23.30	1.84
Sewage-sludge	47.12	0.00
Control	21.55	7.79
Limestone	11.35	3.89
	Moorland Soil	Woodland Soil
	97.35	71.37

All values ppm N dry spoil/soil

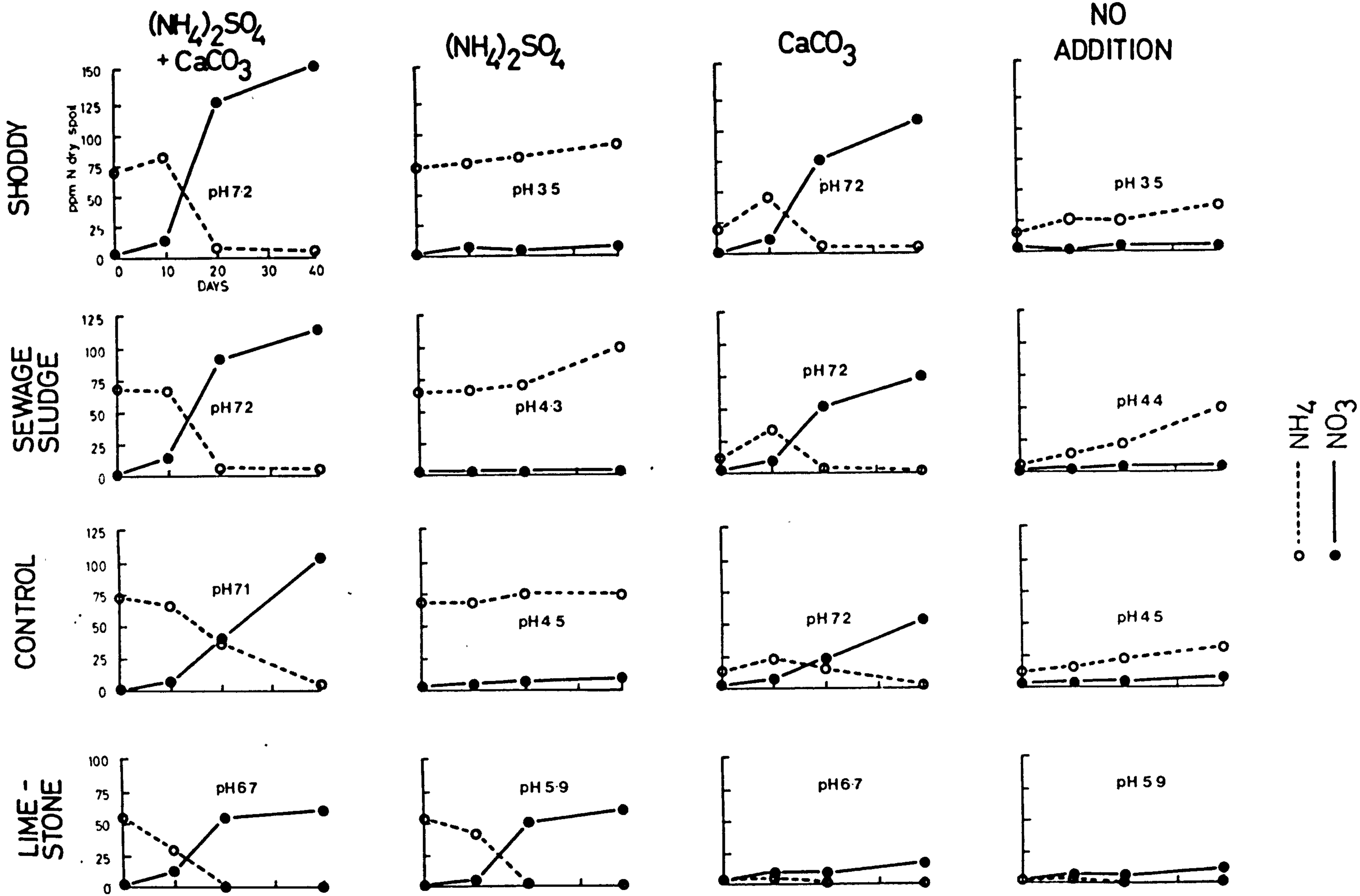


Fig. 4.11. Mitchell's Main. Incubation of spoil samples.

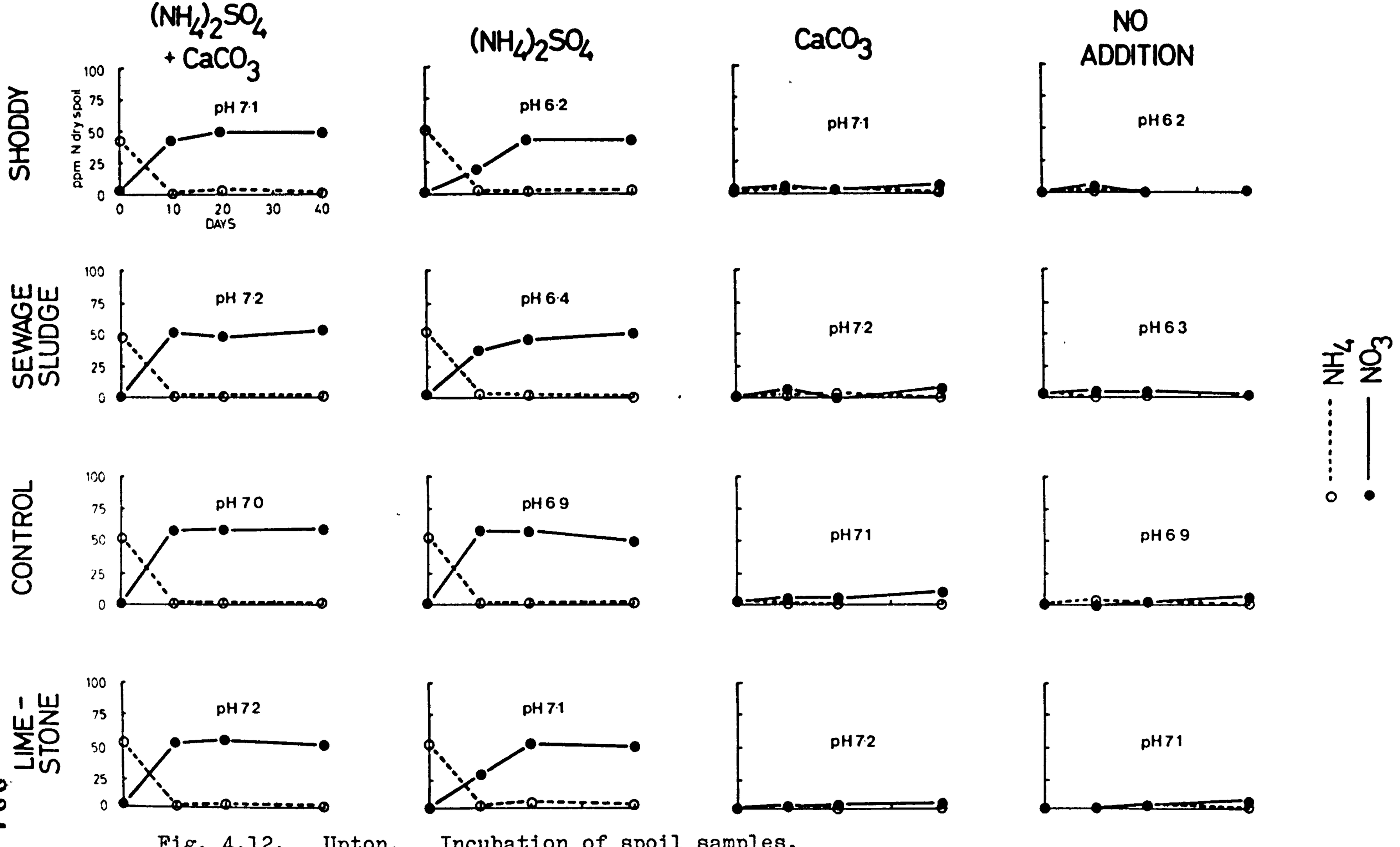


Fig. 4.12. Upton. Incubation of spoil samples.

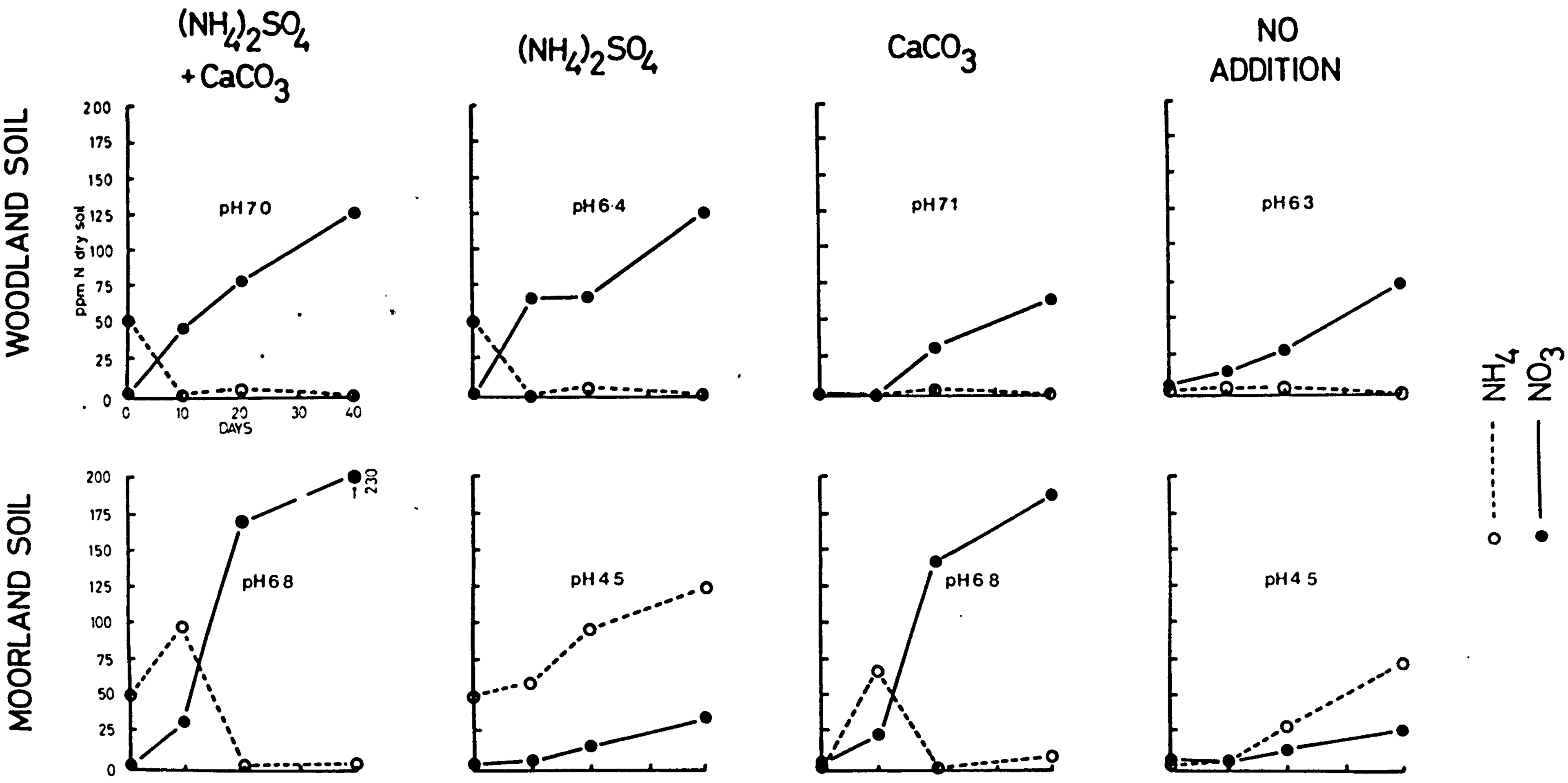


Fig. 4.13. Incubation of soil samples.

Table 4.5. Results of enumeration of nitrifying organisms, before incubation.

Site	Treatment	Count	
		Nitrosomonas	Nitrobacter
Mitchell's Main			
	Shoddy	7	less than 5
	Sewage-sludge	less than 5	" " 5
	Control	5	" " 5
	Limestone	293	" " 5
Upton			
	Shoddy	203034	75038
	Sewage-sludge	47755	4744
	Control	204784	14084
	Limestone	106676	3477
Moorland soil	-	1512	24
Woodland soil	-	16055	1086

Table 4.6. Net mineralized nitrogen (ppm N dry spoil/soil) after 40 days incubation.

Treatment	rep.	$(NH_4)_2 SO_4$ + $Ca CO_3$	$(NH_4)_2 SO_4$	$Ca CO_3$	No addition
MITCHELL'S MAIN					
Shoddy	1	82.38	19.96	79.69	23.87
	2	83.93	20.79	100.05	22.72
Sewage-sludge	1	59.51	34.75	58.31	57.90
	2	40.73	37.94	70.29	36.34
Control	1	36.09	15.91	39.98	25.63
	2	41.14	17.86	34.55	17.46
Limestone	1	5.99	5.99	15.59	13.19
	2	3.99	1.20	14.79	9.51
UPTON					
Shoddy	1	17.56	0.00	8.13	1.23
	2	1.63	0.00	5.29	2.45
Sewage-sludge	1	8.96	0.00	8.55	0.00
	2	2.44	0.00	9.35	0.00
Control	1	4.51	0.00	9.44	9.84
	2	7.80	0.00	7.80	5.74
Limestone	1	0.00	0.00	8.09	7.68
	2	0.00	0.00	7.69	0.00
MOORLAND SOIL					
	1	182.76	116.55	208.56	91.58
	2	175.83	99.26	177.78	103.12
'WOODLAND' SOIL					
	1	70.88	71.43	67.49	74.76
	2	66.02	71.85	59.22	67.97

Table 4.7. Mitchell's Main. Statistical analysis.

Net Mineralized nitrogen.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Ameliorants	10443.75	3	3481.25	71.55	***
Laboratory additions	5542.5	3	1847.50	37.97	***
Interaction	4576.43	9	508.49	10.45	***
Residual	778.41	16	48.65		
Total	21341.09	31			

MEANS TABLE

	Laboratory Addition				
Ameliorant	$(NH_4)_2SO_4$ $+CaCO_3$	$(NH_4)_2SO_4$	$CaCO_3$	No addition	Overall mean
Shoddy	83.16	20.38	89.87	23.30	54.17
Sewage-sludge	50.12	36.35	64.30	47.12	49.47
Control	38.62	16.89	37.27	21.55	28.58
Limestone	4.99	3.60	15.19	11.35	8.78
Overall mean	44.22	19.30	51.66	25.83	

LSD (p = 0.05)

Ameliorants = 7.39

Laboratory additions = 7.39

Interaction = 14.79

Table 4.8. Upton. Statistical analysis.

Net mineralized nitrogen.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Ameliorants	31.7241	3	10.5747	0.961	ns
Laboratory additions	268.8995	3	89.6331	8.147	**
Interaction	142.5539	9	15.8393	1.440	ns
Residual	187.2047	16	11.7002		
Total	630.3822				

MEANS TABLE

Ameliorant	Laboratory addition				
	$(NH_4)_2SO_4$ + $CaCO_3$	$(NH_4)_2SO_4$	$CaCO_3$	No addition	Overall mean
Shoddy	9.60	0.10	7.71	1.84	4.56
Sewage-sludge	5.70	0.10	8.95	0.10	3.71
Control	6.67	0.10	8.62	7.79	5.67
Limestone	0.10	0.10	7.89	3.89	3.00
Overall mean	5.39	0.10	8.04	3.41	

LSD (p = 0.05)

Laboratory additions 3.626

N.B. zero values are converted to 0.10

DISCUSSION

The results of this incubation experiment provide information on the processes of nitrification and mineralization. Nitrification will be discussed first.

The experimental results tend to confirm the conclusions drawn from the periodic study of spoil mineral nitrogen level and nitrifyer populations (sections I & II). Thus at Mitchell's Main nitrification of added or mineralized ammonium only occurred in the shoddy, sewage-sludge and control treatments when the pH was raised by the addition of calcium carbonate. This shows that low pH was the factor limiting the population size of the nitrifyers in these plots. The finding that the spoil from the limestone treatment at Mitchell's Main accumulated nitrate even in the absence of calcium carbonate indicates that the pH of this spoil was high enough for nitrifying activity and suggests that the low numbers usually found were due to a deficiency of substrate, i.e. ammonium.

At Upton, ammonium did not accumulate under any condition; applied ammonium being rapidly converted to nitrate. This again confirms the results presented in previous sections.

The similarity between the nitrification processes in the acid soil and spoils and neutral soil and spoils demonstrates that in respect of this process, there were no fundamental differences between soils and spoils.
The data produced on the process of mineralization, and hence availability of nitrogen in the spoils is very interesting. For Mitchell's Main spoils the results

suggest that the ameliorants applied in 1967, five years before the samples for this incubation study were taken, were not exhausted. From the previous studies of nutrients other than nitrogen it was known that the limestone applications were still effective, but no information on the shoddy or sewage-sludge treatments was available. The results suggest, however, that whilst the shoddy and sewage-sludge applications were not exhausted, neither were they of great consequence to plant growth. This situation arose because the spoils to which they were applied were so acid that the development of a large active microflora capable of mineralizing the organic nitrogen, was prevented. The fact that these organic materials were rapidly mineralized when the pH alone was raised showed that pH and not some other factor, like an unfavourably wide C:N ratio of the organic matter was, in fact, responsible for the slow rate of mineralization.

At Upton no ameliorant effects were apparent. At first sight this seems surprising because the ameliorants were applied a number of years after those at Mitchell's Main. The explanation is probably related to the fact that the spoil at this site was of near neutral reaction, thus allowing rapid decomposition of the applied ameliorants; the shoddy and sewage-sludge applications were therefore exhausted before the samples used for the present investigation were collected. It is interesting to note that when spoil samples were collected from the sites for the incubation experiment, remnants of shoddy were sometimes found in the Mitchell's Main, but not in the Upton spoils.

The incubations performed in the absence of laboratory additions are most likely to indicate the availability of nitrogen under field conditions (Table 4.9). Although the conditions of incubation are more favourable to mineralization than would occur in the field, it has often been shown that such laboratory studies do give a reasonable estimate of the availability of nitrogen to plants under field conditions (Allison & Sterling 1949; Black *et al*. 1947; Gasser & Williams 1963; Pritchett *et al*. 1959). If, as seems likely, this is also true for colliery spoils, the results presented here suggest that nitrogen deficiency was an important factor affecting plant growth at Mithcell's Main, and perhaps, more especially at Upton.

SECTION IV INCUBATION EXPERIMENT II

INTRODUCTION

The result of the first incubation experiment indicated that whilst Mitchell's Main spoils could provide some nitrogen for plant growth, Upton spoils could supply very little. The results for Mitchell's Main indicated that the potential availability of nitrogen in the ameliorated and control spoil plots followed the order:- shoddy > sewage-sludge > control > limestone. The significance to plant growth of this result could not be assessed because each spoil treatment had been represented by a single pooled sample that was prepared from a number of spoil cores collected without reference to vegetation. In order to examine the relationship between nitrogen availability and the occurrence, density and nitrogen status of the vegetation, and the possible interactions between nitrogen availability and deficiencies and toxicities of other elements, a second incubation experiment was performed at Mitchell's Main.

For this experiment three locations were chosen in each treatment main plot such that one was bare, the second supported a small quantity of vegetation, and the third was densely vegetated. Samples of spoil and vegetation, (where present) were taken from each location. Individual samples of spoil and vegetation were not pooled in this experiment.

For each spoil sample, the initial pH and the concentration of ammonium and nitrate (+ nitrite) was measured both before, and after, incubation for forty days in the presence and absence of calcium carbonate.

The dry weight and percentage nitrogen composition was measured for each vegetation sample. In addition to these determinations, the total nitrogen content of each spoil sample was estimated.

RESULTS AND INTERPRETATION

The results of all the determinations have been analysed by a split-plot analysis of variance technique. The spoil treatments i.e. shoddy, sewage-sludge, lime-stone and control are the main plots, and the vegetational status i.e. bare, poorly and densely vegetated, the sub-plots.

1. The pH of the spoil samples.

The results of the pH determinations are shown in Table 4.10. The analysis of these data is shown in Table 4.11. No significant difference is observed between the pH of the mainplots. This result is at first sight surprising because the periodic analysis of pH (Chapter 3) had shown that the limestone plots had a significantly higher pH than the others. Observation of the data in Table 4.10 shows that some uncharacteristically low pH values were recorded for certain of the limestone samples, and high values for some of the control samples. It must be remembered that the pH values quoted here refer to single cores of spoil and that the pH determinations in the periodic study of spoil pH were made on bulked samples, thus reducing the apparent variability of the substrate. The results serve to indicate once again, the extreme variability of colliery spoil and illustrates that neutral locations occurred in generally acid areas and vice-versa.

Table 4.10. Spoil pH.

Treatment	BLOCK I			BLOCK II			BLOCK III		
	0	1	2	0	1	2	0	1	2
Shoddy	3.20	3.20	4.95	3.20	3.85	3.35	3.10	3.40	3.50
Sewage-sludge	3.20	3.30	3.50	3.10	5.95	4.70	3.30	4.80	3.25
Control	3.15	3.35	3.60	3.30	3.50	3.40	3.05	6.85	4.70
Limestone	3.20	5.30	3.95	4.80	5.53	4.60	6.10	4.90	6.60

Each value is the mean of two determinations

0 = Bare

1 = Low vegetation

2 = High vegetation

Table 4.11. Statistical analysis. Spoil pH.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	10.9844	3	3.6615	2.870	ns
Blocks	3.8972	2	1.9486	1.528	ns
Error a	7.6530	6	1.2755		
Total	22.5346	11			
Sub-plots	5.4318	2	2.7159	3.598	ns
Interaction	2.1878	6	0.3646	0.483	ns
Error b	12.0747	16	0.7547		
Total	42.2289	35			

MEANS TABLE

Main plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Bare	3.167	3.200	3.167	4.700	3.558
Low veg.	3.483	4.683	4.567	5.243	4.494
High veg.	3.933	3.817	3.900	5.050	4.175
Mean	3.528	3.900	3.878	4.998	

The mean pH of the bare spoil samples was lower than that of both vegetated spoil samples. This difference was not quite large enough to be significant at the 5% level.

2. <u>The concentration of ammonium and nitrate in the spoils prior to incubation</u>

The results of these determinations are expressed graphically in Fig. 4.14.

The results of the ammonium and nitrate (+ nitrite) determinations were added to give an estimate of the total mineral nitrogen and analysed statistically. The results of this analysis are shown in Table 4.12. Significant main plot and sub-plot differences are observed. The calculated LSD values show that whilst the shoddy, sewage-sludge and control treatments were similar, both the shoddy and sewage-sludge treatments contained significantly more mineral nitrogen than the limestone treatment.

The significant sub-plot difference was shown to be a difference between the bare spoils and the two vegetated ones, the latter being insignificantly different.

The relationship between the initial concentration of mineral nitrogen and the occurrence of vegetation is shown in Fig. 4.14. This shows that the highest concentration of mineral nitrogen occurred where the vegetation was absent, and the lowest, usually nought, where it was densest. These results indicate that the vegetation had taken up most of the mineral nitrogen released into the spoil.

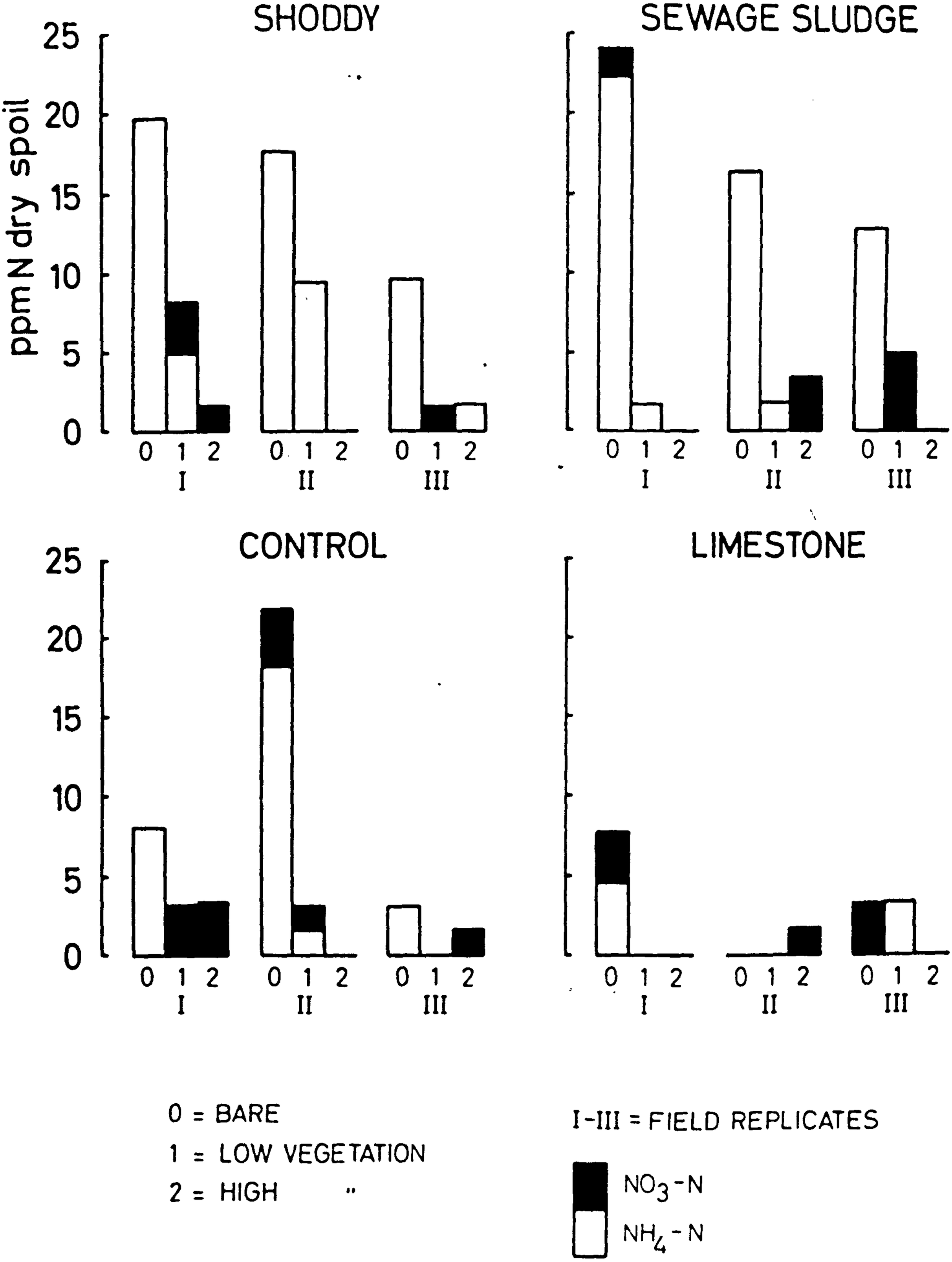


Fig. 4.14. Histogram. Levels of mineral nitrogen in spoil samples prior to incubation.

Table 4.12. Statistical analysis. Total mineral nitrogen before incubation.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	190.5903	3	63.5301	5.1847	*
Blocks	64.1644	2	32.5822	2.6600	ns
Error a	73.5197	6	12.2533		
Total	329.2744	11			
Sub-plots	796.2465	2	398.1233	23.617	***
Interaction	197.3279	6	32.8880	1.9509	ns
Error b	269.7207	16	16.8575		
Total	1592.5695	35			

MEANS TABLE

Main plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Bare	15.523	17.587	11.030	3.703	11.961
Low veg.	6.353	2.710	2.167	1.177	3.101
High veg.	1.140	1.163	1.707	0.607	1.154
Mean	7.672	7.153	4.968	1.829	

LSD (p = 0.05)

Main plots	4.038
Sub-plots	3.554

3. Mineralized nitrogen after forty days incubation

The results and statistical analyses of the incubation data are given in Fig. 4.15 and Tables 4.13 & 4.14. The values quoted are mineralized nitrogen i.e. the quantity of mineral nitrogen present at the end of the incubation period minus that present initially.

The only difference found to be significant when spoil samples were incubated without calcium carbonate, was that between the sub-plots. Thus the densely vegetated spoil samples produced significantly more mineralized nitrogen than the poorly vegetated but not than the bare samples (Table 4.13).

Although the main treatment means appear very different, suggesting perhaps a mainplot effect, the statistical analyses shows that the difference was not great enough to be significant at the 5% level.

When incubated with calcium carbonate, a significant main plot difference occurred (Table 4.14). This is shown to be a difference between the shoddy treatment and the others which were not significantly different one from another. A significant sub-plot difference was not observed.

The quantities of nitrogen mineralized were generally greater in the presence of calcium carbonate. This is shown diagramatically in Fig. 4.15, which also demonstrates the effect of calcium carbonate on the form of mineral nitrogen that accumulated i.e. in the presence of calcium carbonate nitrate was the predominant form, and in its absence, in acid spoils, ammonium was the predominant form. The explanation for these effects of calcium

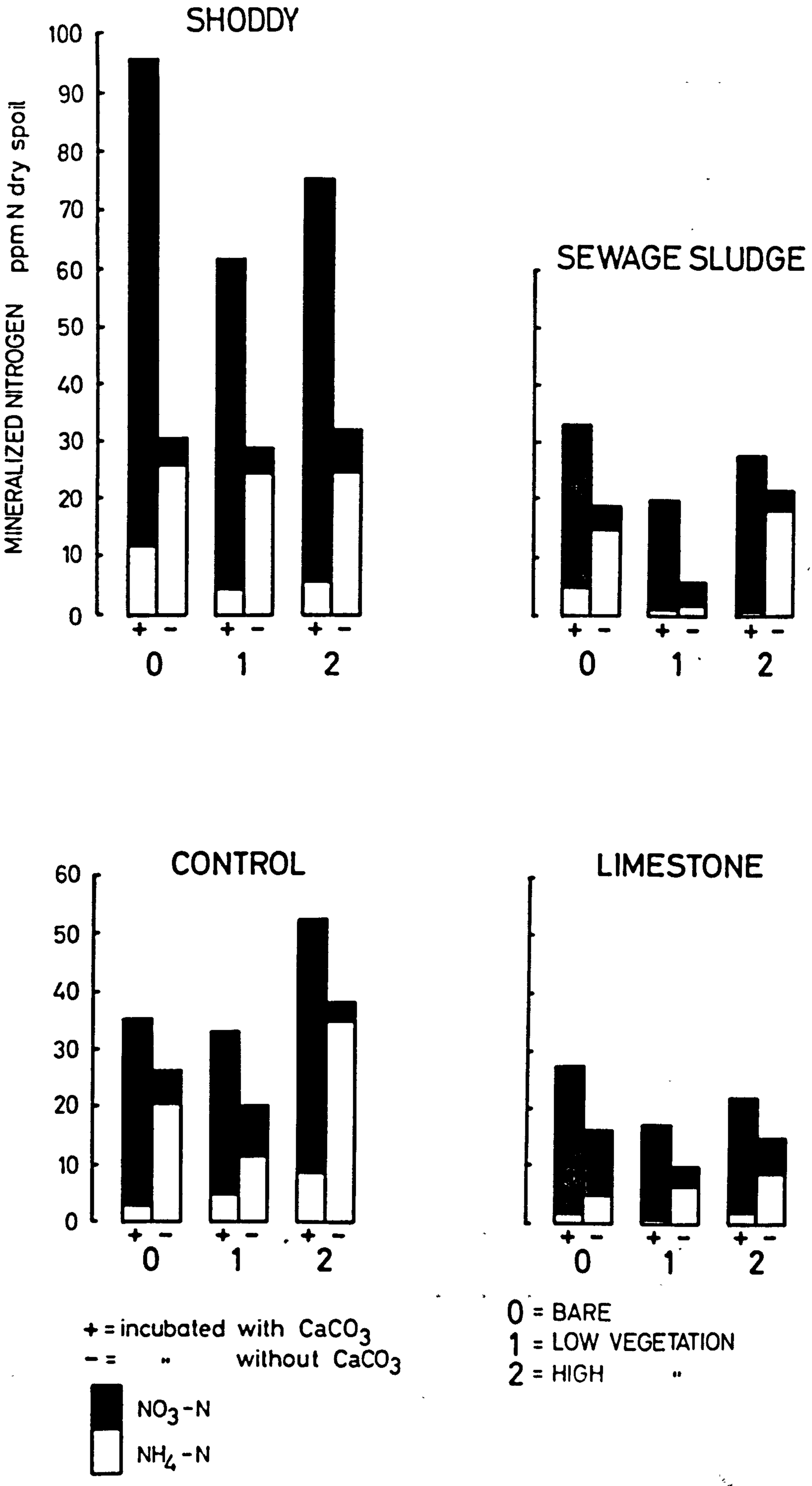


Fig. 4.15. Histogram. Results of incubating Mitchell's Main spoil samples for forty days.

Table 4.13. Statistical analysis. Mineralized nitrogen after forty days incubation without calcium carbonate.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	1962.6218	3	654.2073	1.827	ns
Blocks	1097.1756	2	548.5878	1.532	ns
Error a	2148.6360	6	358.1060		
Total	5208.4335	11			
Sub-plots	683.7515	2	341.8757	3.7567	*
Interaction	375.6304	6	62.6051	0.6879	ns
Error b	1456.0873	16	91.0054		
Total	7723.9027	35			

MEANS TABLE

Main plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Bare	30.290	18.870	24.673	15.657	22.373
Low veg.	28.657	5.557	20.497	10.007	16.179
High veg.	32.037	22.240	38.313	14.633	26.806
Mean	30.328	15.556	27.828	13.432	

LSD (p = 0.05)

Sub-plots = 8.257

Table 4.14. Statistical analysis. Mineralized nitrogen after forty days incubation with calcium carbonate.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	17768.9510	3	5922.9836	6.468	*
Blocks	1344.7994	2	672.3997	0.734	ns
Error a	5494.8099	6	915.8017		
Total	24608.5600	11			
Sub-plots	1527.9680	2	763.9840	2.127	ns
Interaction	1473.2215	6	245.5369	0.683	ns
Error b	5746.5792	16	359.1612		
Total	33356.329	35			

MEANS TABLE

Main plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Bare	98.190	33.417	35.210	27.260	48.519
Low veg.	64.093	19.497	32.700	16.943	33.308
High veg.	75.013	31.037	52.400	21.923	45.093
Mean	79.099	27.983	40.103	22.042	

LSD (p = 0.05)

Main plots 34.908

carbonate has already been given (p 216).

4. Percentage nitrogen content and dry weight of vegetation

The results and statistical analysis for the nitrogen determinations are shown in Tables 4.15 & 4.16.

A significant difference between the percentage nitrogen content of the vegetation from the different spoil treatments is observed (Table 4.16). Thus the vegetation from the shoddy plots contained a significantly higher proportion of nitrogen than that from the other treatments. Vegetation from the sewage-sludge and control, and the control and limestone plots did not differ in this respect. However, the vegetation from the sewage-sludge plots contained a significantly higher proportion of nitrogen than that from the limestone plots.

Care must be taken when making such comparisons because the nitrogen status of vegetation is not usually independent of vegetational density. A significant sub-plot difference is not however observed, thus indicating the independance of nitrogen content and abundance of vegetation in the present situation. Further, the dry weight data (Table 4.17) has been analysed statistically (Table 4.18) and shows that similar quantities of vegetation were removed from the corresponding sub-plots of each main treatment plot. Between treatment comparisons can justifiably be made and the observed difference in percentage nitrogen is probably a meaningful statistic.

5. Total nitrogen content of spoils

The results and statistical analysis are shown in Tables 4.19 & 4.20.

Table 4.15. Percentage nitrogen content of the vegetation.

Treatment	BLOCK I		BLOCK II		BLOCK III	
	1	2	1	2	1	2
Shoddy	1.439	0.989	0.917	1.272	0.989	1.142
Sewage-sludge	0.823	0.886	0.993	1.096	0.804	0.930
Control	0.868	0.899	0.765	0.785	0.738	0.794
Limestone	0.762	0.658	0.634	0.779	0.675	0.758

Each value is the mean of two determinations

1 = Low vegetation

2 = High vegetation

Table 4.16. Statistical analysis. Percentage nitrogen content of vegetation.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	0.56893	3	0.18964	16.331	**
Blocks	0.01749	2	0.00875	0.753	ns
Error a	0.06967	6	0.01161		
Total	0.65609	11			
Sub-plots	0.01407	1	0.01407	0.581	ns
Interaction	0.00518	3	0.00173	0.071	ns
Error b	0.19356	8	0.02420		
Total	0.86890	23			

MEANS TABLE

Main plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Low veg.	1.115	0.873	0.790	0.690	0.867
High veg.	1.134	0.971	0.826	0.732	0.916
Mean	1.125	0.922	0.808	0.711	

LSD (p = 0.05)

Main plots = 0.152

Table 4.17 Dry weight of vegetation ($g.m^{-2}$).

	BLOCK I		BLOCK II		BLOCK III	
	1	2	1	2	1	2
Shoddy	171.2	649.6	274.4	386.4	256.8	843.2
Sewage-sludge	100.0	184.0	414.4	696.8	316.8	620.8
Control	132.8	964.8	236.8	668.0	160.8	394.4
Limestone	159.7	240.0	200.8	246.4	221.6	488.8

1 = Low vegetation

2 = High vegetation

Table 4.18. Statistical analysis. Dry weight of vegetation ($g.m^{-2}$).

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	115179.8	3	38393.3	0.743	ns
Blocks	33170.1	2	16585.1	0.321	ns
Error a	310114.6	6	51685.8		
Total	458464.	11			
Sub-plots	581919.4	1	581919.4	25.342	***
Interaction	122955.3	3	40985.1	1.785	ns
Error b	183700.4	8	22962.6		
Total	1347039.6	23			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Low veg.	234.1	277.1	176.8	194.0	220.5
High veg.	626.4	500.5	675.7	325.1	531.9
Mean	430.3	388.8	426.3	259.5	

All the spoil samples contained very similar quantities of total nitrogen. Significant differences were not observed for any of the comparisons.

Table 4.19. Total nitrogen (%N dry spoil).

Treatment	BLOCK I			BLOCK II			BLOCK III		
	0	1	2	0	1	2	0	1	2
Shoddy	0.474	0.410	0.412	0.626	0.459	0.388	0.436	0.428	0.516
Sewage-sludge	0.422	0.421	0.474	0.414	0.344	0.417	0.413	0.366	0.446
Control	0.440	0.457	0.499	0.615	0.519	0.438	0.341	0.371	0.397
Limestone	0.493	0.368	0.536	0.441	0.332	0.453	0.345	0.401	0.403

Each value is the mean of two determinations

0 = Bare

1 = Low vegetation

2 = High vegetation

Table 4.20. Statistical analysis. Total nitrogen.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	0.01340	3	0.00447	0.772	ns
Blocks	0.01955	2	0.00977	1.689	ns
Error a	0.03472	6	0.00579		
Total	0.06766	11			
Sub-plots	0.01380	2	0.00690	1.933	ns
Interaction	0.01710	6	0.00285	0.798	ns
Error b	0.05713	16	0.00357		
Total	0.15569	35			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Bare	0.512	0.416	0.465	0.426	0.455
Low veg.	0.432	0.394	0.449	0.367	0.411
High veg.	0.439	0.446	0.445	0.464	0.448
Mean	0.461	0.419	0.453	0.419	

DISCUSSION

One of the aims of this second incubation experiment was to examine the relationship between mineralizable nitrogen and the distribution of vegetation. The results indicate that no very obvious relationship existed. In only one instance was a significant difference observed between the quantity of nitrogen mineralized by the spoils of different vegetational status (i.e. when samples were incubated without calcium carbonate where the densely vegetated samples mineralized more nitrogen than the poorly vegetated but not the bare samples). A logical explanation relating vegetational distribution and mineralizable nitrogen cannot be made from this result. Two conclusions can, however, be reached. Firstly, the absence of vegetation on the bare areas was not directly related to the availability of nitrogen, and secondly there was a source of mineralizable nitrogen even the bare spoil that did not receive organic nitrogen additions. Information was also provided on the quantity of mineralizable nitrogen in the different spoil treatments, and the relationship between this and the quantity of nitrogen in the vegetation. On incubation of samples without calcium carbonate, the shoddy treatment produced the most and the limestone treatment the least mineral nitrogen. The difference was not, however, large enough to be statistically significant at the 5% level. With added calcium carbonate, the quantity of mineralized nitrogen was increased, but the order in which the treatments could be arranged in respect of the quantities of nitrogen mineralized, remained the same. The order was shoddy> control>

sewage-sludge > limestone. In this case, however, the quantity of mineralized nitrogen produced by the shoddy treatment was significantly greater than the others. The results are therefore in general agreement with those obtained in the first incubation experiment where pooled, rather than individual samples were incubated.

The results for the percentage nitrogen content of the vegetation samples suggested that the availability of nitrogen in the spoils followed the order, shoddy > sewage-sludge > control > limestone. The whole order is not, however, statistically significant. Nevertheless, it is very interesting to observe that this order is similar to that suggested from the mineralizable nitrogen data produced in both incubation experiments. There would appear to be general agreement between the various data, that the shoddy plots could supply the greatest, and the limestone plots, the least nitrogen for plant growth. The control and sewage-sludge plots appear to be intermediate in this respect and the fact that the order of these two treatments is reversed in the two incubation experiments possibly suggests that their nitrogen supplying powers were similar.

Incubation studies indicate the future availability of nitrogen whilst the nitrogen content of vegetation indicates past availability. The apparent relationship between these two criteria in the present experiment therefore suggests that the past trends in nitrogen availability were being maintained.

The total nitrogen determinations showed that all spoils contained large quantities of total nitrogen, the mean value being 0.44%. The surface layer of most

cultivated soils contained between 0.06 - 0.50%N. (Bremner 1965b). The quantity of total nitrogen in the spoil therefore fell within the range of most soils. The proportion of this nitrogen that was potentially available to plants was likely, however, to be very small. Since there is no satisfactory method for distinguishing between fossil nitrogen and humus nitrogen, total nitrogen and nitrogen to carbon ratios, are meaningless determinations for colliery spoils and have largely been omitted from the present investigations.

SECTION V YIELD AND NITROGEN STATUS OF THE VEGETATION ON THE TRIAL PLOTS

INTRODUCTION

The results of the experiments already described in this chapter have indicated that the form and availability of nitrogen differed in response to ameliorant treatment and spoil type. Data for the average yield and nitrogen content of the vegetation on the trial plots has not been presented. The relationship between nitrogen supply, as influenced by ameliorant additions and spoil characteristics and yield of vegetation has not, therefore, been examined. In order to evaluate this relationship, sample cuts of the vegetation were taken in late July, 1972. This time was chosen because it allowed comparisons to be made between the availability of nitrogen at the beginning of the 1972 growing season, as indicated by the incubation experiments, and the quantity of nitrogen actually present in the vegetation some months later. In order to increase the precision of the comparison and also to provide information on the rate of breakdown of dead plant material, the vegetation was sorted into living and dead components and the dry weight and nitrogen status of each ascertained.

Dry weight determinations were made at the Upton site in September, 1971, approximately one year after establishment of the field trial. Similar data were provided by Cooper (1973) for the Mitchell's Main site. Using all these data, both the initial and longer term effects of ameliorant applications and spoil type on plant yield could be considered.

RESULTS AND INTERPRETATION

1. Dry weight yield of vegetation

The dry weight of the living and dead components of the vegetation in July, 1972 are shown in Table 4.21. The analyses of the results are given in Tables 4.22 & 4.23. In the statistical analyses, spoil treatments are taken as the main plots, and living and dead components as the sub-plots.

For Mitchell's Main, no significant main plot differences are observed. The significant sub-plot difference is due to the fact that samples contained very much more dead, than living material. This situation had obviously arisen because dead vegetation has not been rapidly decomposed.

At Upton, the analysis of the dry weight of the vegetation approximately one year after establishment (Table 4.24) shows that significantly greater yields occurred on the shoddy plots than the others. At this time, the vegetation was not divided into living and dead components but observation suggested that the great majority was living.

The results and analysis of the determinations made in July, 1972 (Tables 4.21 & 4.23) show that the yield of the vegetation on the shoddy treatment was still greater than that on the others but not significantly so (At the 5% level). A significant sub-plot difference is not found to occur, showing that the living and dead components occurred in approximately equal quantities. Whether the similarity in yield of living and dead material was attributable to the rapid breakdown of organic matter at

Table 4.21. Dry weight of vegetation ($g.m^{-2}$) at Mitchell's Main and Upton, July 1972.

MITCHELL'S MAIN

Treatment	Block I		Block II		Block III	
	Living	Dead	Living	Dead	Living	Dead
Shoddy	60.80	461.60	20.00	188.00	48.48	406.4
Sewage-sludge	37.60	201.12	31.68	224.48	41.60	176.32
Control	5.92	13.12	17.60	113.60	22.88	273.92
Limestone	76.32	166.88	17.28	242.88	36.48	660.80

UPTON

Treatment	Block I		Block II		Block III	
	Living	Dead	Living	Dead	Living	Dead
Shoddy	161.28	199.68	153.28	263.20	140.48	164.48
Sewage-sludge	87.20	88.48	38.40	30.08	96.48	69.60
Control	132.80	140.80	14.88	17.28	21.28	18.88
Limestone	186.08	170.08	51.68	14.88	43.68	28.00

Table 4.22. Mitchell's Main. Statistical analysis.

Dry weight of vegetation ($g.m^{-2}$) July, 1972.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	68541.67	3	22847.23	2.025	ns
Blocks	45858.05	2	22929.03	2.033	ns
Error a	67685.14	6	11280.86		
Total	182084.89	11			
Sub-plots	306564.49	1	306564.49	22.616	**
Interaction	45088.65	3	15029.55	1.109	ns
Error b	108443.03	8	13555.38		
Total	642181.06	23			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Living	43.09	36.96	15.47	43.36	34.72
Dead	352.00	200.64	133.55	356.85	260.76
Mean	197.55	118.80	74.51	200.11	
Total dry weight ($g.m^{-2}$)	395	238	149	400	

Table 4.23. Upton. Statistical analysis. Dry weight of vegetation (g. m^{-2}) July, 1972.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	57217.17	3	19072.39	4.657	ns
Blocks	28335.79	2	14167.89	3.460	ns
Error a	24570.83	6	4095.14		
Total	110123.79	11			
Sub-plots	252.98	1	252.98	0.811	ns
Interaction	5680.06	3	1893.35	6.069	*
Error b	2495.95	8	311.99		
Total	118552.78	23			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Living	151.68	74.03	56.32	93.81	93.96
Dead	209.12	62.72	58.99	70.99	100.45
Mean	180.40	68.37	57.65	82.40	
Total dry weight ($g.m^{-2}$)	361	137	115	165	

Table 4.24. Results and statistical analysis of dry weight determinations ($g.m^{-2}$) at Mitchell's Main, July, 1968 and Upton, September, 1971.

MITCHELL'S MAIN*

	Shoddy	Sewage-sludge	Control	Limestone
Mean values	61.8	32.0	41.1	47.0

Values not significantly different (p = 0.05)

*Data and interpretation of Chadwick (1973a)

UPTON +

	Block I	Block II	Block III
Shoddy	241.25	281.25	408.75
Sewage-sludge	84.38	76.87	112.50
Control	188.13	23.13	8.13
Limestone	255.0	26.25	37.50

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Ameliorant	221514.258	3	73838.006	29.143	***
Blocks	32775.912	2	16387.956	6.468	*
Interaction	105649.610	6	17608.269	6.95	**
Residual	30403.907	12	2533.659		
Total	390343.687	23			

+ Each value is the mean of two determinations, both of which were used in the computation of the above table.

MEANS TABLE

Ameliorants	Shoddy	Sewage-sludge	Control	Limestone
	310.40	91.25	73.13	106.30

LSD (p = 0.05) = 63.32

Blocks	I	II	III
	192.20	101.90	141.70

LSD (p = 0.05) = 58.84

this site as a result of the more favourable pH conditions for microbial growth, or to the fact that the field trial had only been established for two years, cannot be ascertained.

2. Nitrogen content of the vegetation

The results and statistical analysis of the nitrogen determinations are shown in Tables 4.25 4.26 & 4.27.

The results indicate that ameliorant addition had no effect on the percentage nitrogen content of the vegetation at either site. For the Upton data a significant difference is observed between the living and dead components, the living containing significantly higher percentages than the dead. This results from translocation of nitrogen from senescing to growing parts of the plant. At Mitchell's Main the same general pattern was observed but the difference was not quite large enough to be significant at the 5% level.

The actual percentages of nitrogen in the vegetation were higher at Mitchell's Main than at Upton for both the living and dead components. This perhaps suggests that the availability of nitrogen was greater at Mitchell's Main than Upton. Such comparisons can, however, be misleading because the percentage nitrogen content may be related to vegetational density and hence yield per unit area. This may have been so in present situation because the yield of living vegetation at Upton was higher than at Mitchell's Main. In order to overcome such difficulties, the yield and percentage nitrogen data have been combined to give values for the quantities of nitrogen per unit area. This is shown in Table 4.28, and the results of

the statistical analysis in Tables 4.29 & 4.30. The statistical analyses show that at Upton, the living vegetation contained significantly more nitrogen than the dead whilst at Mitchell's Main the reverse was true. Once again it is not possible to ascertain whether this was due to the different ages or spoil conditions of the two sites.

Table 4.25. Percentage nitrogen (dry weight) content of the vegetation at Mitchell's Main and Upton, July 1972.

MITCHELL'S MAIN

Treatment	Block I		Block II		Block III	
	Living	Dead	Living	Dead	Living	Dead
Shoddy	1.671	1.243	1.121	1.173	1.270	1.278
Sewage-sludge	1.277	1.347	1.646	0.993	1.226	0.939
Control	2.604	1.247	1.439	1.351	1.391	1.184
Limestone	1.039	0.865	1.141	1.226	1.199	1.312

UPTON

Treatment	Block I		Block II		Block III	
	Living	Dead	Living	Dead	Living	Dead
Shoddy	0.896	0.503	1.120	0.809	1.012	0.655
Sewage-sludge	0.993	0.454	0.973	0.548	1.065	0.616
Control	0.951	0.660	1.150	0.665	1.412	0.705
Limestone	1.800	0.891	1.076	0.725	1.048	0.640

Each value is the mean of two determinations

Table 4.26. Mitchell's Main. Statistical analysis. Percentage nitrogen (dry weight) content of the vegetation, July, 1972.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	0.5256	3	0.1752	1.831	ns
Blocks	0.1510	2	0.0755	0.789	ns
Error a	0.5741	6	0.0957		
Total	1.2507	11			
Sub-plots	0.3519	1	0.3519	3.922	ns
Interaction	0.2637	3	0.0879	0.979	ns
Error b	0.7177	8	0.0897		
Total	2.5839	23			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Living	1.354	1.396	1.811	1.126	1.422
Dead	1.231	1.093	1.261	1.134	1.180
Mean	1.293	1.245	1.536	1.130	

Table 4.27. Upton. Statistical analysis. Percentage nitrogen (dry weight) content of the vegetation, July, 1972.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	0.2239	3	0.0746	0.9938	ns
Blocks	0.0006	2	0.0003	0.0040	ns
Error a	0.4506	6	0.0751		
Total	0.6750	11			
Sub-plots	1.3184	1	1.3184	73.8062	***
Interaction	0.0323	3	0.0108	0.6023	ns
Error b	0.1429	8	0.0179		
Total	2.1686	23			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Living	1.009	1.010	1.171	1.308	1.125
Dead	0.6557	0.539	0.677	0.752	0.656
Mean	0.833	0.775	0.924	1.030	

Table 4.28. Nitrogen content of vegetation ($g.Nm^{-2}$) at Mitchell's Main and Upton, July 1972.

MITCHELL'S MAIN

Treatment	Block I		Block II		Block III	
	Living	Dead	Living	Dead	Living	Dead
Shoddy	1.016	5.738	0.224	2.205	0.616	5.194
Sewage-sludge	0.480	2.708	0.522	2.230	0.526	1.655
Control	0.152	0.163	0.253	1.535	0.318	3.242
Limestone	0.792	1.429	0.198	2.978	0.437	8.670

UPTON

Treatment	Block I		Block II		Block III	
	Living	Dead	Living	Dead	Living	Dead
Shoddy	1.444	1.004	1.716	2.129	1.421	1.077
Sewage-sludge	0.866	0.401	0.374	0.164	1.027	0.429
Control	1.263	0.929	0.170	0.114	0.299	0.133
Limestone	3.350	1.518	0.555	0.107	0.457	0.179

Table 4.29. Mitchell's Main. Statistical analysis. Nitrogen content of vegetation ($g.Nm^{-2}$), July, 1972.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	10.8108	3	3.6036	1.505	ns
Blocks	7.6199	2	3.8100	1.592	ns
Error a	14.3640	6	2.3940		
Total	32.7948	11			
Sub-plots	43.2366	1	43.2366	17.164	**
Interaction	7.8336	3	2.6111	1.037	ns
Error b	20.1526	8	2.5191		
Total	104.0175	23			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Living	0.619	0.509	0.241	0.476	0.461
Dead	4.379	2.198	1.647	4.359	3.146
Mean	2.499	1.354	0.944	2.417	

Table 4.30. Upton. Statistical analysis. Nitrogen content of vegetation ($g.Nm^{-2}$), July, 1972.

VARIANCE TABLE (untransformed data)

Source	ss	df	ms	vr	sig.
Main plots	3.8025	3	1.2675	1.434	ns
Blocks	2.6188	2	1.3093	1.481	ns
Error a	5.3045	6	0.8841		
Total	11.7257	11			
Sub-plots	0.9433	1	0.9433	7.521	*
Interaction	0.4918	3	0.1639	1.307	ns
Error b	1.0033	8	0.1254		
Total	14.1641	23			

MEANS TABLE

Main Plots

Sub-plots	Shoddy	Sewage-sludge	Control	Limestone	Mean
Living	1.527	0.756	0.577	1.454	1.079
Dead	1.403	0.331	0.392	0.601	0.682
Mean	1.465	0.544	0.485	1.028	

DISCUSSION

The results for Mitchell's Main indicate that the ameliorative applications of shoddy, sewage-sludge and limestone did not increase the yields of vegetation. Despite the fact that the ameliorants were applied five years before the sampling in July, 1972, the periodic determinations of pH and the incubation studies indicated that ameliorant exhaustion had not occurred. Further, Cooper (1973) (see page 286) examined the dry weight yields of vegetation on this trial in July, 1968, sixteen months after establishment, and even at this time, significant ameliorant effects were not observed. The ameliorant treatments were therefore ineffective in increasing the dry weight yield of vegetation.

At Upton, significantly more vegetation was present on the shoddy plots than the others, after one, but not two years. The sewage-sludge application did not have any significant effect on vegetation yield despite the fact that the quantity of nitrogen supplied in this ameliorant was similar to that in the shoddy application. The effect of the ameliorant additions on the yield of vegetation will be discussed in the next section (page).

The finding that the dead component of the vegetation was between five and ten times greater than the living at Mitchell's Main indicates that the rate of decomposition was slow. It is interesting to observe that the relative proportions of the living and dead components was approximately the same for all treatments. Since the pH of the limestone plots was significantly higher than

that of the others, this observation suggests that low pH was not the only cause of the slow rate of decomposition.

It is well known that the C:N ratio of organic matter affects the rate of decomposition. When the ratio is narrow, i.e. less than 6:1, breakdown occurs rapidly (provided the materials are not particularly resistant) and mineral nitrogen is released. This nitrogen represents the proportion of nitrogen in the substrate that was in excess of the requirements of the microorganisms utilising the carbonaceous energy source. When the ratio is wide, the breakdown rate is slow unless additional nitrogen is available to enable the microorganisms to utilise all of the carbon present. Addition of organic matter with a wide C:N ratio to soil initially decreases the availability of nitrogen, because any mineral nitrogen present in the soil becomes temporarily incorporated into microbial protoplasm. Mineral nitrogen fixed in this way becomes available later, when the microbial tissues are themselves decomposed.

The C:N ratio of plant remains is of the order of 30:1. Breakdown does not therefore occur rapidly unless mineral nitrogen is available. In a well established soil, the humus fraction represents a large reserve of nitrogen that is slowly mineralized. Mineral, or easily mineralized nitrogen sources are also added in the rainfall and animal excrement, and all forms can be utilized by the microflora to aid decomposition. Since the availability of nitrogen at Mitchell's Main was much smaller than normal soils, the accumulation of dead but

undecomposed vegetation may be at least partially due to the unavailability of nitrogen.

Other factors were probably also of importance and of these the failure of the incorporation of dead vegetation into the surface layers of the spoil was possibly the most important. In soils, this activity is performed by earthworms and other small animals. The complete absence of earthworms from colliery spoil heaps may be one of the reasons for the distinct interface that occurs between the spoil surface and the dead vegetation.

Whatever the cause, the failure of the decomposition processes can result in the formation of a dense mat of vegetation that physically inhibits new growth. Whilst this situation had not been reached at Mitchell's Main it has often occurred during reclamation attempts on colliery spoil heaps, especially where high yielding agricultural Rye grass seed mixtures have been sown, heavily fertilized, and left ungrazed or uncropped.

Macfadyen (1964) stated that the productivity of a grassland ecosystem is limited by the rate of decomposition of organic matter. This must be especially true for a grassland community existing on a basically nutrient deficient substrate such as colliery spoil. In this situation it can be envisaged that a slow rate of decomposition would result in the situation where a significant proportion of the potentially available nitrogen in the ecosystem occurred in an unavailable form, thus accentuating the deficiency.

The incubation studies indicated that at Mitchell's

Main, the availability of nitrogen at the beginning of the growing season in 1972 followed the order, shoddy > sewage-sludge ≏ control > limestone. The results of the nitrogen analysis of vegetation in section IV tended to confirm this view. The results of the determinations described in the present section, however, showed that no significant differences were observed either in the percentage, or the quantity of nitrogen present in the vegetation from the different spoil treatments. The discrepancy may result from the fact that the previous nitrogen determinations were made on vegetation samples not selected randomly, and hence, not being truly representative of the whole treatment plot from which they were removed. The generally poor relationship that was found between mineralizable nitrogen and plant nitrogen probably indicates that factors other than, or more likely, in addition to, the supply of nitrogen were important in controlling the growth of plants at Mitchell's Main.

For the Upton site, the incubation experiment indicated that all treatments could supply very little nitrogen for plant growth at the beginning of the 1972 growing season. The yield, and percentage nitrogen content of the vegetation was similar on all plots towards the end of this growing season. There is, therefore, good agreement between the various data. The fact that the significantly higher yield on the shoddy plots after one year's growth was not maintained after two years, indicates that the ameliorant had been depleted during the first year. The yield on all plots

in the second year was approximately half those of the first year and all the results taken together indicate that nitrogen availability was limiting the yield of vegetation at this site.

SECTION VI NITROGEN BUDGET OF COLLIERY SPOIL

INTRODUCTION

Using the data previously presented, a nitrogen budget for the two sites can be drawn up. Because this involves making a number of assumptions and estimations, the values presented must only be approximate. General patterns do, however, emerge and despite the approximate nature of much of the derived data, meaningful information can be obtained.

Using this approach a number of lines of investigation have been followed. These are: the assessment of mineralization studies as an indicator of nitrogen availability; the fate and effectiveness of the nitrogen applied in the sewage-sludge and shoddy; the quantity and possible sources of nitrogen to vegetation growing on unfertilized plots.

Throughout this section, yields of vegetation and quantities of nitrogen are all expressed in $g.m^{-2}$.

1. Comparison of the quantities of available nitrogen at the beginning of the 1972 growing season with the nitrogen content of the living vegetation towards the end of the season

Although a comparison of this nature has already been made in general terms, the following account represents an attempt to quantify this comparison.

The available nitrogen is taken as the total quantity of nitrogen present after incubation for forty days without laboratory additions. This value represents the nitrogen mineralized plus the mineral nitrogen present

initially. For Mitchell's Main the mean values of the two incubation experiments will be used. Both were performed early in the growing season and the combined data should give a better estimate than either experiment on its own. The assumption is made that 1 hectare of spoil to a depth of 15 cms (samples were always taken to this depth) has an air dry weight of 2.24×10^6 kg.

The values for the nitrogen content of the living vegetation (Table 4.28) only included that present in the above ground parts. It is generally assumed that the roots contain approximately the same quantity of nitrogen as the tops, and hence the value for the vegetation tops has been doubled to take account of this.

Table 4.31. Relationship between available nitrogen in spoil and that in living vegetation ($g.N\ m^{-2}$).

Mitchell's Main

Treatment	Available Nitrogen	Nitrogen in living vegetation
Shoddy	8.81	1.24
Sewage-sludge	8.86	1.02
Control	7.93	0.48
Limestone	3.31	0.95

Upton

Treatment	Available Nitrogen	Nitrogen in living vegetation
Shoddy	0.96	3.05
Sewage-sludge	0.59	1.51
Control	2.11	1.15
Limestone	1.58	2.91

The data indicates that at Mitchell's Main, considerably more nitrogen was available at the beginning of the growing season than appeared in the vegetation at the end

of July. This is another pointer to the fact that factors other than nitrogen availability were important at this site.

For the field trial at Upton, the date indicates that the plots receiving shoddy, sewage-sludge and lime-stone could not provide enough nitrogen to account for the quantities found in the vegetation at the end of the growing season. This discrepancy probably arose because the plants are perennial and there was a carry over of nitrogen from year to year. The effect of nitrogen supply in the past was still evident as a result of trans-location within the sward. The data for the control plots suggests that the spoil could provide slightly more nitrogen than is accounted for in the vegetation. The magnitude of the difference is much smaller than occurred for Mitchell's Main samples and suggests that plant growth may have been directly limited by the unavailability of nitrogen at this site.

2. <u>The fate and effectiveness of the shoddy and sewage-sludge ameliorants</u>

The shoddy applied as an ameliorant contained 6.5% nitrogen and the sewage-sludge 3.25%. The moisture contents were 17.5% and 65% respectively (Chadwick 1973a). The application of shoddy at 4 tons per acre represented the addition of 54 g.N m^{-2}, and sewage-sludge at 20 tons per acre, of 57 g.N m^{-2}.

In order to obtain information on the proportion of the added nitrogen remaining in the plant-spoil system, Table 4.32 has been prepared. The values for the nitrogen

reserves in the spoils are based on the incubations made under the conditions most favourable to mineralization, i.e. with added calcium carbonate alone. The total quantity of nitrogen present after forty days incubation is taken as the estimate of nitrogen in spoil. This represents the mineralized plus the initially present mineral nitrogen. For Mitchell's Main the mean values for both incubation experiments are used.

Values for the nitrogen content of the vegetation are based on the values used in Table 4.31 above. To each of these values, the quantity of nitrogen in the dead vegetation is added. This latter value is not multiplied by two to account for nitrogen in the roots because the roots will be dead, and their breakdown may be responsible for a proportion of the mineralized nitrogen already estimated.

Table 4.32. Total potentially available nitrogen in spoil and nitrogen in vegetation ($g.N\ m^{-2}$).

Mitchell's Main

Treatment	Nitrogen in Spoil	Nitrogen in Vegetation	Total
Shoddy	22.1	5.6	27.7
Sewage-sludge	12.8	3.2	16.0
Control	11.3	2.1	13.4
Limestone	4.6	5.3	9.9

Upton

Treatment	Nitrogen in Spoil	Nitrogen in Vegetation	Total
Shoddy	1.5	4.5	6.0
Sewage-sludge	2.4	1.8	4.2
Control	2.6	1.5	4.1
Limestone	2.3	3.5	5.8

The control and limestone treatments did not receive fertilizer additions of nitrogen, but still supported vegetation and produced mineral nitrogen on incubation. Nitrogen in addition to that added in the ameliorant treatments was, therefore, available. In order to take this extra nitrogen into account, corrections have to be made to the shoddy and sewage-sludge treatment values. For Mitchell's Main data, the values for spoil and vegetation nitrogen present in the control treatment will be subtracted from the corresponding shoddy and sewage-sludge treatment values. The control values are used in preference to the limestone ones because of the similarity in several respects between the control, shoddy and sewage-sludge plots. For the Upton data, the correction is applied by subtracting the mean of the control and limestone treatments. At this site all four spoil treatments were similar in most respects. These operations result in the following data.

Table 4.33. Total available nitrogen in the spoil, and nitrogen in the vegetation attributable to shoddy and sewage-sludge ameliorants (g.N m^{-2}).

Site and treatment	Nitrogen in spoil	Nitrogen in vegetation	Total	Nitrogen supplied by ameliorant.
Mitchell's Main				
Shoddy	10.8	3.5	14.3	54
Sewage-sludge	1.5	1.1	2.6	57
Upton				
Shoddy	-1.0	2.0	1.0	54
Sewage-sludge	-0.1	-0.7	-0.8	57

Before the data expressed in Table 4.33 can be discussed and evaluated some mention of the sources of error involved must be made. The available nitrogen values quoted for the Mitchell's Main spoil treatments are likely to be under estimates because the mineral nitrogen levels were still rising slowly when the study was terminated after forty days incubation. Further, the appearance of shoddy particles during sampling suggests that all the shoddy had not been broken down and hence would not be included in the nitrogen estimates. A small amount of mineral nitrogen would also become fixed in the microbial protoplasm of the organisms that accomplished the decomposition.

The incubation study at Upton showed that only very small changes were occurring when incubation was discontinued thus suggesting that these values were more accurate.

It could be argued that the quantities of nitrogen represented by the vegetation at both sites is underestimated because of the effect of grazing. In the situation that existed, however, grazing was limited to insect and occasional small mammals and was probably unimportant because the similar yields on all treatments would suggest that all would be subjected to approximately the same level of grazing. By applying the corrections mentioned above, grazing is removed as a factor of importance when the fate of the nitrogen applied in the ameliorants is being discussed.

The data in Table 4.33 suggests that very little of the nitrogen supplied by the shoddy, and more particularly the sewage-sludge applications could be found in the spoil

or vegetation. It was previously shown that the sewage-sludge ameliorative treatment had no significant beneficial effect on the yield of vegetation at either site. The data expressed in Table 4.33 suggests that this was due to the fact that very little of the nitrogen in the sewage-sludge became available to the vegetation. There are two possible explanations for this. The first would be that some adverse factor of either the spoil, or the material prevented its decomposition. At Mitchell's Main the low pH of the spoil could possibly have been responsible for such a failure. This could not have been the case at Upton because the spoil was of neutral reaction. The C:N ratio of the sewage-sludge may have been too wide to promote rapid breakdown. This seems unlikely however, because the mineralization study for Upton spoils showed that when 50 ppm of ammonium nitrogen were added to spoil samples from the sewage-sludge plots, 50^{+}ppm of nitrate were recovered. If breakdown had been prevented by the wide C:N ratio, much of the added mineral nitrogen would have been initially fixed in microbial protoplasm. Similar reasoning can be applied to the sewage-sludge incubations for Mitchell's Main samples. Thus if the sewage-sludge were still present, fixation of applied ammonium would have been expected in the incubations made with calcium carbonate additions. No marked fixation was observed for the sewage-sludge samples for either site and it can be concluded that the nitrogen had been mineralized. This being so, the lack of response of the vegetation at both sites must have been due to other

factors. The most likely explanation is that the nitrogen was mineralized and subsequently lost from the spoil as a result of leaching or denitrification before the sown grass species had germinated or reached a stage where they could utilize the mineral nitrogen. At Upton, the large populations of nitrifying bacteria would be able to rapidly convert ammonium to nitrate. The mobility of nitrate is well known, and it seems very probable that large quantities of nitrate were lost through leaching. At this site leaching of sodium was found to occur very rapidly in the first few months of weathering, and nitrate could have been lost simultaneously. Further, it was previously suggested that the very high numbers of denitrifying bacteria at this site could also have lead to losses through denitrification.

At Mitchell's Main the acid conditions would have prevented the rapid conversion of ammonium to nitrate. Although ammonium is not nearly as mobile as nitrate, Dennington (personal communication) found that large quantities of ammonium appeared in the run off water from fertilized areas of Mitchell's Main spoil heap. For the reasons given in section II, denitrification losses were likely to be very small at this site and leaching would, therefore, appear to have been the main way in which nitrogen could have been lost.

The significant effect of the shoddy on plant yield after one year at Upton suggests that some of its nitrogen had become available to the vegetation. The incubation study indicated that there was no residual effect of the shoddy in the Upton spoils at the beginning of 1972 and

this showed that all of the applied shoddy had been decomposed. The fact that the quantity of nitrogen in the vegetation represented only a very small proportion of that applied in the ameliorant suggests that much of the mineralized nitrogen was lost. This would have occurred in the manner already described for the sewage-sludge. The fact that some of the mineralized nitrogen appeared in the vegetation indicates that the breakdown of shoddy occurred more slowly than that of sewage-sludge. At Mitchell's Main particles of shoddy were still visible in March, 1972, some years after the application was made. This shows that the low pH had an adverse effect on the breakdown of this material. This view is confirmed by the fact that a very large increase in mineralized nitrogen was observed when the pH of the incubated samples was increased, and in fact most of the value presented in Table 4.32 represented potentially rather, than actually available nitrogen. Some of the organic nitrogen in shoddy would obviously have been mineralized, and the results for the percentage nitrogen content of vegetation growing on the different treatments, (section IV) would tend to confirm this, i.e. shoddy was decomposed locally. The usefulness of such mineral nitrogen to the general plant community on the shoddy plot is perhaps questionable because it has already been shown that factors other than nitrogen were probably important in limiting plant growth on the acid treatments, (e.g. high aluminium and manganese, and low potassium levels). The discrepancy between the total nitrogen content is the spoil-plant system and that applied in the ameliorative treatment at

Mitchell's Main was probably due both to the fact that some of the shoddy was still undecomposed and some had been lost, probably largely through leaching.

The conclusions that can be reached regarding the fate and effectiveness of the shoddy and sewage-sludge applications are these. At Upton both materials were rapidly decomposed and mineral nitrogen released. This was largely lost from the trial plots before the grass had become established. At Mitchell's Main, decomposition occurred more slowly because of the acid nature of the spoil, but despite the fact that the grass probably had become established before the majority of mineralized nitrogen was lost from the plots, the increased availability of nitrogen was only locally beneficial. In other areas the presence of toxicities or deficiencies so limited plant growth that the mineralized nitrogen could not be utilized.

If the ameliorants had been added after the grass had become established at Upton, the very great capacity of grasses to take up mineral nitrogen (Walker 1956) may have resulted in a great reduction in the quantity of mineral nitrogen lost at this site. At Mitchell's Main liming the spoil would not only have increased the rate at which the ameliorants were broken down but also reduced the toxicities that were apparently responsible for the failure of the vegetation to utilize available nitrogen.

3. <u>The source and extent of naturally occurring nitrogen</u>

The nitrogen contribution that the unameliorated spoils could make when the field trials were set up is

unknown because initial nitrogen determinations were not performed. It is generally assumed, however, that freshly exposed i.e. unweathered, colliery spoil can supply little or not nitrogen for plant growth (Doubleday 1972b). Observation of the data presented in Table 4.32 and reproduced below, for the control and limestone treatments, at both sites indicates that some nitrogen was available to the developing vegetation. What proportion of the total came from the unameliorated spoil is unknown.

Table 4.34. Part of Table 4.32 (g.N m^{-2}).

	Nitrogen in Spoil	Nitrogen in Vegetation	Total
Mitchell's Main			
Control	11.3	2.1	13.4
Limestone	4.6	5.3	9.9
Upton			
Control	2.6	1.5	4.1
Limestone	2.3	3.5	5.8

Nitrogen is added to the spoil from rainfall, animal excrement and wind and animal-borne organic debris. Rainfall is likely to be the single largest source of nitrogen. Dennington (personal communication) measured the nitrogen content of the rainfall at three spoil heaps, including Mitchell's Main, in the West Riding of Yorkshire. The mean value was equivalent to 0.9 g.m^{-2} yr^{-1} of plant available nitrogen. Dennington (personal communication) considered that this quantity was great enough to account for all the nitrogen present in birch trees growing on the Mitchell's Main site.

If it is assumed that at Upton, the nitrogen content

of the rainfall was also 0.9 g.N m^{-2} yr^{-1}, since regrading operations, the freshly exposed spoil surface would have received 1.8 g of nitrogen during the two year period up to July, 1972. Observation of the data in Table 4.32 shows that if all the nitrogen were retained in the spoil surface layers, which is obviously unlikely, nitrogen in rainfall could account for about half of the total quantity. The remainder must have been added by other agencies or occurred initially in the spoil. It was previously suggested (section II of this chapter) that some ammonium was initially present in the spoil at this site, and may have resulted from the combustion of the spoil.

At Mitchell's Main the situation was complicated by the fact that the spoil heap was regraded a number of years before the field trial was established and fertilizer additions made during the original revegetation schemes. In the five years from 1967 when the trial was layed down, to 1972 very approximately, about 4.5 g of nitrogen would have arrived in the rainfall. This again represents about half the quantity of nitrogen in the vegetation and spoil estimated in 1972. The remainder may have been largely due to the fertilizer additions made in the initial reclamation scheme.

If nitrogen was a growth limiting factor, the annual increase in yield should be related to the input of this element. In order to assess whether this was the case, the accumulation of organic matter has been estimated. The yield approximately one year after establishment should reflect the past availability of nitrogen, i.e. the

contribution made by the spoil, provided that other factors were not limiting. Yield data for the Mitchell's Main trial sixteen months after establishment was obtained by Cooper (1973). Similar data for the Upton trial was obtained in the present investigations. By comparing these 'initial' yields with those obtained in July, 1972 a yearly increase has been estimated, and using the average nitrogen value obtained from the analysis of the vegetation sampled in 1972, the yearly input of nitrogen has been calculated. These data are shown in Table 4.35. No correction for below ground material has been included.

Table 4.35. Yearly increase in nitrogen content of the vegetation at Mitchell's Main and Upton.

Mitchell's Main.

	Yield * July, 1968	Yield July, 1972	Increase per year	Increase in nitrogen per yr.
Shoddy	61.8	395.1	83.3	1.04
Sewage-sludge	32.0	237.6	51.4	0.59
Control	41.1	149.0	27.0	0.36
Limestone	47.0	400.3	88.3	1.00

Upton

	Yield Sept.1971			
Shoddy	310.5	360.7	50.2	0.40
Sewage-sludge	91.3	136.7	45.4	0.36
Control	73.1	115.2	42.1	0.39
Limestone	106.3	164.8	58.5	0.63

* Data of Cooper (1973) discussed by Chadwick (1973a)

The data indicates that the yearly increase in total dry weight and nitrogen is very small when compared with a similar community growing on a soil. Thus, MacFarlan (1939) reported that an old permanent pasture consisting

mainly of Agrostis and fescue produced in each of two years approximately 390 $g.m^{-2}$ dry matter which contained 8.4 $g.m^{-2}$ of nitrogen.

The yearly input of nitrogen from the rainfall estimated by Dennington (personal communication) to be 0.9 $g.m^{-2}$ yr^{-1} would appear to be great enough to account for the yearly yield increase, for those treatments not receiving nitrogen applications. At Mitchell's Main the difference between the yield of the control and limestone plots is interesting because it shows the interaction between nitrogen and other factors. Thus at the low pH of the control plots, the added nitrogen may not be useful because the plants are limited by toxicities or deficiencies. On the limestone treatment where toxicities are absent, the vegetation can utilize the added nitrogen. The increase in nitrogen in the shoddy treatment is similar to that shown on the limestone treatment despite the dissimilar pH status of the two treatments. This probably resulted from the local mineralization and subsequent utilization of the organic nitrogen in the shoddy. It was previously indicated that the shoddy had not been exhausted at this site. At Upton, the dry matter increase between September 1971 and July 1972 was smaller than the annual increase at Mitchell's Main. All treatments showed similar yield increases confirming the conclusion reached previously that the organic ameliorants were exhausted. The reason for the smaller dry matter increase at Upton than at Mithcell's Main is not obvious. Although the nutrient content of the precipitation was not measured

at Upton the value is probably very similar to that at Mitchell's Main because Dennington (personal communication) found very little variation between sites. The vegetation at Upton was well established during this period and loss of mineral nitrogen would therefore be expected to be reduced. The largest proportion of nitrogen from the rainfall occurs, however, during the winter months, when growth is very slow. Thus some nitrogen may still have been lost in run off. The lower pH of the treatments at Mitchell's Main have meant that less of the nitrogen added in the rainfall was lost in this way because the ammonium fraction, which accounted for 50% of the total nitrogen input in rainfall would not be rapidly converted to nitrate.

SECTION VII SUMMARY

Mitchell's Main

On all but the limestone plots ammonium was the predominant form of mineral nitrogen available to the vegetation. This was shown to be due to the low numbers of nitrifying organisms, which in turn was related to the low pH of the spoils.

The highest numbers of organisms were generally found to occur in the shoddy and limestone plots. The reason would appear to be the greater availability of substrates in the former and the higher pH of the latter plots.

The first incubation experiment suggested that the shoddy plots could supply the most, and the limestone plots, the least mineral nitrogen. The great response to calcium carbonate showed that the low pH of the spoils prevented rapid mineralization of organic nitrogen reserves.

The second incubation experiment demonstrated that the distribution of vegetation was not directly related to the availability of nitrogen and that other factors were of importance. Both incubation experiments did, however, show that the spoils contained much less mineralizable nitrogen than unfertilized soils.

The results of the dry weight determinations showed that the ameliorants were ineffective in increasing the dry weight of vegetation. It was concluded that this situation arose because the nitrogen released from the two organic ameliorants could not generally be utilized by the vegetation because other factors were limiting plant growth.

Upton

The mineral nitrogen levels remained low throughout the year and ammonium did not accumulate. This was shown to be due to the large numbers of nitrifying organisms present. Since these organisms were present in large numbers before ameliorant additions were made it was suggested that ammonium was present in the freshly exposed spoil and postulated that this may have resulted from the combustion of coaly materials.

The microbial enumerations suggested that activity was greatest in those plots receiving organic amendments, especially shoddy. The large numbers of denitrifying bacteria were taken as indicative of the fact that mineral nitrogen may have been lost as a result of denitrification. Leaching losses were also considered to be important in this respect.

The incubation experiment showed that all spoil plots were very deficient in nitrogen, even those that had received heavy applications of shoddy and sewage-sludge.

The yield data showed that a response to shoddy was apparent only after one years growth. The quantity of nitrogen that this extra yield represented was, however, only a small proportion of the nitrogen added in the ameliorant. It was concluded that much of the nitrogen originally present in the ameliorants had been lost through denitrification and leaching before the sown species had germinated and reached a stage where they could utilize the nitrogen.

The yearly increase in yield could be accounted for

by the nitrogen input in the rainfall. Nitrogen deficiency was considered to be of prime importance to the low yields of vegetation at this site.

CHAPTER FIVE

CHAPTER FIVE

AN OVERALL ASSESSMENT OF THE SPOILS AT MITCHELL'S MAIN AND UPTON IN RELATION TO PLANT GROWTH

Introduction

In Chapters Three and Four, the significance to plant growth of the recorded levels of plant nutrients and physical features of the spoils were considered. An overall assessment of the spoils as plant growth media has not, however, been made. This will be presented in the present chapter.

Table 5.1 shows the mean, standard deviation and range of concentrations for each element in the control plots at both sites. Comments based on the literature referred to previously about the suitability for plant growth of the levels recorded are given. Five categories are included. These are the two extremes of toxicity and deficiency and three intermediate values. Normal, indicates that the concentration range was similar to that found in fertile soils, high and low indicates that the ranges departed somewhat from the norm, but not to an extent that could be considered definitely toxic or deficient.

Mitchell's Main

From Table 5.1 it can be seen that plants naturally colonising or artificially sown into unameliorated spoil would have been subjected to toxic levels of aluminium, locally toxic levels of manganese, low or deficient

Table 5.1. The suitability of the concentrations of various nutrients in the spoils at Mitchell's Main and Upton.

	MITCHELL'S MAIN				UPTON			
Element	Mean	S.D.	Range	Comment	Mean	S.D.	Range	Comment
Aluminium	52.3	57.0	0.0-220.0	Toxic	Not detectable			Normal
Manganese	12.87	17.15	0.50-67.00	Locally Toxic	2.12	3.51	0.01-15.00	Normal
Copper	0.47	0.45	0.00-1.40	Normal	0.07	0.11	0.00-0.35	Low
Zinc	1.36	1.12	0.05-4.36	High	0.15	0.19	0.02-0.69	Normal
Iron	3.15	4.51	0.00-15.47	High	0.44	1.01	0.05-5.38	Normal
Sodium	9.68	4.47	1.00-28.00	Normal	241.6	594.6	17.0 -28.05	Initially toxic then normal
Potassium	6.07	3.99	1.70-26.00	Deficient	26.81	12.72	14.00-54.00	Deficient
Calcium	215.3	154.9	16.0-700.0	High	500.2	171.8	93.0-724.0	High
Magnesium	117.0	92.1	6.0-340.0	High	120.3	82.2	13.0-383.0	High

All values are ppm in saturated paste extracts

levels of potassium and higher than usual levels of iron, zinc, calcium and magnesium. In addition it was shown that the spoil could provide such less nitrogen for plant growth than normal soils and surface layers became very hot in the summer months and could have dried out rapidly. Interaction of the various elements would make an already bad situation even worse. Thus aluminium toxicity was probably accentuated by the fact that ammonium was the predominant mineral form of nitrogen available to the vegetation. This, in turn, would have its effect on the uptake of essential nutrients like potassium that were present in deficient quantities, and water, because aluminium toxicity results in reduced root systems. The seasonally induced increases in levels of many potentially toxic cations when spoil temperatures were high and water availability low, would also appear to have been important. The situation obviously represented a very unfavourable environment for plant growth.

The ameliorants applied in the field trial did little to improve the situation because the organic additions were made without pH correction and the limestone additions merely corrected the pH (and thus reduced toxicities) but obviously did nothing to correct nutrient deficiencies.

Despite the unfavourable nature of the spoil at Mitchell's Main some natural colonisation had occurred. The vegetation was, however, patchily distributed and the question therefore arose as to whether the colonised

sites (or those retaining vegetation originally introduced in the unsuccessful reclamation programme) were in some way more favourable for plant growth than the general spoil surface, or whether the successful recolonisers were tolerant of the adverse conditions. The latter situation has been shown to exist on heavy metal-ore spoil heaps where distinct ecotypes of grass species were found to be resistant to high levels of copper, zinc and lead (Bradshaw, McNielly & Gregory 1965). In an attempt to resolve this problem Harding (1970) investigated the naturally recolonising awards of Agrostis tenuis on a number of spoil heaps in the West Riding of Yorkshire including Mitchell's Main. His results demonstrated that distinct ecotypes did not occur. Varnam (1970) used a paired sampling technique at Mitchell's Main (that involved analysis of saturated paste extracts of spoil samples taken from beneath vegetated swards and from the nearest bare area) and showed that compared to bare sites, vegetated ones were characterized by higher pH and potassium and lower, aluminium, manganese copper, iron and zinc levels (nitrogen and phosphorus were not included in this investigation). Similar findings were reported by Oldershaw (1969) for another acid spoil heap (Bullcroft) in the West Riding of Yorkshire. The results of all three workers would suggest that the patchy distribution of vegetation on the colliery spoil heaps studied resulted from the extreme variability of the spoil (Harding 1970; Oldershaw 1969; Varnam 1970).

Upton

The situation at Upton appeared to be somewhat better than that at Mitchell's Main because of the general absence of elements in phytotoxic concentrations. Only the initial sodium levels were potentially phytotoxic and these rapidly fell as a result of leaching.

On this site nutrient deficiences were important and whilst the levels of potassium were low, nitrogen deficiency was probably more important.

The application of shoddy significantly increased the yield of vegetation in the first year but this was not maintained for a second year. The increase in yield on the shoddy plots has been solely attributed to the effect of the added nitrogen. It was, however, shown (Chapter Three) that the levels of potassium were significantly higher in the spoil on the shoddy plots than on the others and it could be argued that the response was due not to nitrogen, but to potassium. There are a number of reasons why this was probably not the case. Firstly, the yields on all plots were similar in the second year despite the fact that the levels of potassium remained highest in the shoddy plots. Secondly, the increase in yield in the second year at Upton was not as great as the yearly increase in yield on the limestone plots at Mitchell's Main despite the fact that the levels of potassium were between three and four times higher at Upton than that at Mitchell's Main. Thirdly, the Upton spoils were shown to be extremely deficient in nitrogen at the beginning of the 1972

growing season, thus indicating the importance of nitrogen.

Whilst the shoddy applications did produce a significant yield increase in the first year, it was demonstrated that a large proportion of the added nitrogen did not remain in the application area for long enough to be beneficial. The situation was even more extreme in the case of the sewage-sludge applications where none of the applied nitrogen could be accounted for in the vegetation.

Phosphorus determinations were not performed at either site but the findings of other workers that colliery spoils are frequently deficient in phosphorus (Davison & Jefferies 1966; Doubleday 1972b; Fitter 1972) would suggest that the available phosphorus levels may have been low at both sites. The situation is likely to have been less favourable at Mitchell's Main than Upton in respect of phosphorus availability because of the lower pH of the Mitchell's Main spoil (see Doubleday 1972a).

Conclusions

The results of the investigations presented in this thesis indicate that the spoils at both sites studied represented poor growth media for plants and the likely causes can be summarized as follows:

Mitchell's Main Toxicities attributable to aluminium and manganese and the combined effects of high spoil temperatures and higher than usual levels of iron, zinc, calcium and magnesium together with deficiencies of potassium, nitrogen and possibly phosphorus.

Upton Deficiencies of nitrogen, potassium and possibly phosphorus.

CHAPTER SIX

CHAPTER SIX

AMELIORATION OF COLLIERY SPOIL

Introduction

The results of the present investigations have indicated the likely causes of poor plant growth on colliery spoil and the effectiveness of a number of ameliorant additions. Using this information, suggestions of a qualitative nature can be made on general aspects of the amelioration of colliery spoils on which grass or grass/legume mixtures are to be established.

1. Time of seeding

The seed should be sown either early (February or March) or rather late in the year (November). This should allow the seedlings to develop a good root system before they become subjected not only to the desiccating effect of high spoil temperatures, but also to the seasonal increases in levels of potentially phytotoxic cations. Early and late sowing increases the risk of frost damage but this would appear to be less important than the other factors.

2. Saline spoils

Unweathered spoils that are exposed during recontouring operations can be very saline (e.g. Upton). In this situation it would be advisable to delay sowing until the heap has weathered over winter and the salinity decreased by leaching. This may be especially important where inorganic fertilizers are to be used to remedy

deficiencies because these additions would increase the total salinity of the spoil, possibly to a critical level.

3. pH and toxicity correction

The results for Mitchell's Main indicated that the application of eight tons per acre of limestone maintained the pH at approximately pH 5.5 for at least five years and thus suppressed the solution of phytotoxic levels of a number of cations, notably aluminium and manganese. This single application of limestone on this moderately acid spoil therefore had a fairly long term effect. Other sites have been investigated where quantities of limestone in excess of this quantity have become exhausted after only one or two years. It is, therefore, necessary to monitor the spoil reaction at least annually in order to prevent the reoccurrence of toxicities on limed, initially acid spoils. Occasionally particular areas of a spoil heap have been found to produce sulphuric acid at a rate much greater than that of the surface as a whole. These areas may need up to twenty tons per acre of limestone annually to maintain a favourable pH for plant growth and the addition of a thick soil layer is often the only solution in this situation.

Problems of toxicity are not usually encountered so long as the pH remains above about pH 4.5. It is not, therefore, necessary and probably undesirable to lime a site to pH 7 or above.

4. Nitrogen fertilization

The results of the present investigations show that

whilst the provision of nitrogen for plant growth is essential for colliery spoils, economical application is problematical. The form of mineral nitrogen applied, or to which nitrogen compounds become converted is of great importance in this context. Whilst nitrate can be utilized by plants it is susceptable to leaching and denitrification losses. pH values higher than about pH 5.8 encourage the oxidation of ammonium to nitrate. Liming acid spoils to values higher than pH 5.8 or the application of nitrogen in the nitrate form are therefore undesirable. Ammonium on the other hand is far less mobile and hence less prone to leaching losses. It is a utilizable form of nitrogen for grass species but its uptake could initiate or accentuate aluminium toxicity. pH values below about pH 5.5 favour the presence of ammonium in spoils by limiting the activity of the microorganisms responsible for its oxidation. However, very low pH values favour the release of cations like aluminium and manganese in phytotoxic concentrations and reduce the activity of the microflora and microfauna responsible for nutrient recycling. Clearly a compromise is desirable. This would be achieved in the situation where mineral nitrogen is kept predominantly in the ammonium form but in which cation toxicities are absent and nutrient recycling proceeds normally. On neutral sites the maintenance of ammonium as the predominant mineral form presents problems which are discussed below. On initially acid sites this compromise situation can be achieved by

liming to pH 5.5 (using the lime determination of Kamprath 1970) and sowing species of grass that are tolerant of mildly acid conditions.

Neutral spoils present difficulties with regard to nitrogen utilization because mineral or mineralized nitrogen will rapidly be converted to nitrate. This process had resulted at Upton in the loss of a very large proportion of the applied nitrogen before the vegetation had become established. This also serves to indicate that the time of nitrogen application is important. Application made before the seeds have germinated serve no useful purpose since germinating seeds do not require additional nitrogen, and may be very wasteful (as in the Upton situation) because leaching and denitrification losses may be substantial. A similar fate awaits autumn applications. The maximum effectiveness would be obtained in the first year by applying fertilizer after the seeds had germinated, and in subsequent years at the onset of the growing season in late April or May. Losses should be minimised at this time because grasses have a very great capacity to take up mineral nitrogen when they are actively growing (Walker 1956).

Other ways can be envisaged to reduce nitrogen losses from neutral spoils. These include the use of nitrification inhibitors (such as N-Serve) or the use of slow release fertilizers (e.g. sulphur coated urea). These methods may be of use but have not been assessed in comparative experiments on colliery spoils. Shoddy and sewage-sludge are sometimes considered to be slow

release fertilizers but the experimental design of the trial described in this thesis were not such that their effectiveness could be fairly assessed.

For non-specific land use, grass/legumes mixtures may be sown. The nitrogen fixing properties of legumes make their use a very attractive proposition since additions of fertilizer nitrogen could be reduced or dispensed with. Encouraging results have been reported by Chadwick (1973a) and Cooper (1973) with Trifolium repens, T. hybridum and Lotus corniculatus in field trials at a moderately acid site (Roundwood) in the West Riding of Yorkshire. Very little work has, however, been done on the nitrogen fixing properties of legumes in colliery spoils and this topic deserves a full investigation.

5. Potassium fertilization

Whilst some workers have commented on the deficiency of potassium in spoils (Beyer and Hutnik 1969; Coates 1964; Knabe 1965) Doubleday (1972b) has stated that spoils are not usually deficient in this nutrient. The disagreement probably arises because although the levels of potassium are generally low in unameliorated spoils, plant growth is more likely to be limited by deficiencies of nitrogen and phosphorus and/or toxicities of aluminium and manganese, than by potassium deficiency. If, however, potassium additions are not made when the other deficiencies and toxicities are corrected by ameliorant applications, the low availability of potassium will soon limit plant growth. Potassium additions must,

therefore, be included in fertilization programmes.

6. Phosphorus fertilization

Although many of the methods used for the determination of available phosphorus levels in colliery spoil appear to be rather unsatisfactory, the general conclusion reached by many workers that spoil can supply little phosphorus for plant growth is probably correct.

Spoil has the ability to adsorb applied phosphorus, thus making it unavailable to plants. This adsorbtion must be taken into account when the rates of application are being considered.

It is particularly important to maintain a relatively high level of phosphorus in spoil when grass/legume mixtures are sown because of the high phosphorus requirements of legumes.

7. Initial fertilization in reclamation schemes.

The three essential plant macronutrients are likely to be deficient in a newly reshaped spoil heap and fertilizer additions must be made to ensure the success of a revegetation programme. The application of a general purpose inorganic fertilizer after seed germination would appear to be the most appropriate technique to adopt.

The composition and rates of fertilizer application have, however, received very little attention and remains very much a matter of speculation. Work carried out in the Colliery Spoil Research and Advisory Group at the University of York lead to the suggestion that a 10:15:10 fertilizer should be applied at the rate of 6 cwt per acre.

8. 'After care' of revegetated sites

The regimes of fertilization and cropping practices that must be adopted to ensure the continued success of an initially well established cover of vegetation are not known. This area of reclamation practice, usually referred to as 'After care' has received very little attention despite its obvious importance. Future research is urgently needed in this field and should be directed at finding practical ways of promoting the development of revegetated spoil areas into "self-supporting" or "low maintenance", soil-like ecosystems that can become incorporated into normal land use patterns.

This thesis has reported investigations into the basic problems of plant growth on colliery spoil and the results have given some indication of the difficulties that are encountered, and hopefully some of the ways in which these may be overcome. Much more research is needed into the whole problem of the revegetation of colliery spoil heaps and other forms of derelict land and it is hoped that this thesis may be of some use to those undertaking this work.

APPENDIX

APPENDIX

COMPUTER PROGRAM FOR MPN DETERMINATIONS

1. Method

The procedure finds the MPN of organisms and the 95% confidence limits from a set of data by the iterative method suggested by Finney (1951).

2. Language

Algol 60.

3. Procedure title

```
PROCEDURE   MPN (NL, N, DF, P, EPS, MAX, MPNO,
                 UPLIM, LOWLIM, FAIL);
VALUE       NL, N, DF, P, EPS, MAX;
INTEGER     NL, N, DF, FAIL, MAX;
REAL        MPNO, UPLIM, LOWLIM, EPS;   ARRAY P;
```

4. Parameters

NL - An integer scaler called by value, set on entry to be the number of levels in the dilution series.

N - An integer scaler, called by value, set on entry to be the number of replicates at each level.

DF - An integer scaler called by value, set on entry to be the dilution factor.

P - A real array of dimensions 1:NL , called by value, set on entry to be the proportion of sterile responses at each dilution level.

EPS - A real scaler, called by value, the accuracy parameter. The routine will be left when two successive values of the expected log log of

the MPN differ by less than the EPS.

MAX - An integer scaler, called by value that is set to the maximum number of iterations that are allowed.

MPNO - A real scaler, the most probable number. On exit it will contain the most probable number of organism if the procedure has been successful.

UPLIM - A real scaler, the upper 95% confidence limit for the most probable number. On exit it will contain the upper 95% confidence limit if the procedure has been successful.

LOWLIM - A real scaler, the lower 95% confidence limit for the most probable number. On exit it will contain the lower 95% confidence limit if the procedure has been successful.

5. Procedure and Test Program using an ICL 4130 computer

The procedure has been incorporated into a test program and is shown below. "COMMENT" statements have been included to indicate the beginning and end of the procedure and to demonstrate particular parts of the program. The program includes instructions to convert the MPN and confidence limits to values per gram of dry spoil. This is achieved by multiplication by a dry weight correction factor (DW) and multiplication fact (MULT) in lines 160-165.

As an example, the program has been used to calculate the MPN and confidence limit for the following set of data.

Organism						<u>Nitrosomonas</u>	
Site						Mitchell's Main	
Treatment						Shoddy	
Field replicate						I	
Date						3rd November, 1971	
Dry weight correction factor (DW)						1.13	
Multiplication factor- reciprocal of the lowest dilution level (MULT)						10	
Number of replicates at each dilution level						3	
Dilution factor						4	
Data - Dilution level		1/10	1/40	1/160	1/640	1/2560	1/10240
No. of fertile tubes		3	3	3	1	0	0
<u>MPN</u>	392	Upper 95% Confidence limit		1108	Lower 95% Confidence limit		139

```
1.
2.      "BEGIN"
3.      "COMMENT" START OF THE PROCEDURE;
4.
5.
6.      "PROCEDURE" MPN(NL,N,DF,P,EPS,MAX,MPN,UPLIM,LOWLIM,FAIL);
7.      "VALUE"NL,N,DF,P,EPS,MAX;
8.      "INTEGER"NL,N,DF,FAIL,MAX; "REAL"MPN,UPLIM,LOWLIM,EPS;
9.      "ARRAY"P;
10.     "BEGIN"
11.         "ARRAY" Y[1:NL];
12.         "INTEGER"I,II,BB,L,K,M,J,NJSJL;
13.         "REAL"JY,SW,SWX,TRY,W,JU,XJ,NX,NY,SNW,VARSNW,SDSNW,
14.         X,DIMBJ;
15.         BB:=II:=0; TRY:=10.-20;
16.         "FOR"I:=1"STEP"1"UNTIL"NL"DO"
17.         "BEGIN"
18.             "IF"P[I]=0"THEN"
19.             "BEGIN"
20.                 BB:=BB+1; "GOTO"NEXT
21.             "END";
22.             "IF"P[I]=1"THEN"
23.             "BEGIN"
24.                 II:=II+1; "GOTO"NEXT
25.             "END";
26.             Y[I]:=LN(-LN(P[I]));
27.         NEXT:"END";
28.         "IF"BB=NL"THEN"
29.         "BEGIN"
30.             FAIL:=4; "GOTO"EXIT
31.         "END";
32.         "IF"II=NL"THEN"
33.         "BEGIN"
34.             FAIL:=3; "GOTO"EXIT
35.         "END";
36.         M:=NL"DIV"2;
37.         "IF"BB+II=NL"THEN"BB:=-10;
38.         II:=NJSJL:=10;
39.         BACK:"IF"NJSJL"LE"0"THEN"
40.         "BEGIN"
41.             DIMBJ:=DIMBJ+0.1; JY:=DIMBJ;
42.             "IF"DIMBJ"GE"5.0"THEN"
43.             "BEGIN"
44.                 FAIL:=2; "GOTO"EXIT
45.             "END";
46.             "GOTO"AGAIN
47.         "END";
48.         "IF"II"GE"0"THEN"M:=M-1"ELSE"M:=M+1;
49.         "IF"M"LE"0"THEN"
50.         "BEGIN"
51.             II:=-10; M:=NL"DIV"2;
52.         "END";
53.         "IF"M"GE"NL+1"THEN"
54.         "BEGIN"
55.             NJSJL:=-10; M:=NL"DIV"2; DIMBJ:=-3.0;
56.             "GOTO"AGAIN
57.         "END";
58.         "IF"BB=-10"THEN""GOTO"START;
59.         "IF"P[M]=0"JR"P[M]=1"THEN""GOTO"BACK;
60.         START:L:=0;
61.         "IF"P[M]=0"THEN"
62.         "BEGIN"
```

```
63.         OY:=LN(-LN(TRY)); "GOTO"AGAIN
64.       "END";
65.       "IF"P[M]=1"THEN"
66.       "BEGIN"
67.           OY:=LN(TRY); "GOTO"AGAIN
68.       "END";
69.       OY:=LN(-LN(P[M]));
70.       AGAIN:K:=1;
71.       "FOR"I:=M-1"STEP"-1"UNTIL"1"DO"
72.       "BEGIN"
73.           Y[I]:=LN(DF)*K+OY; K:=K+1;
74.       "END";
75.       K:=1;
76.       "FOR"I:=M+1"STEP"1"UNTIL"NL"DO"
77.       "BEGIN"
78.           Y[I]:=OY-K*LN(DF); K:=K+1;
79.       "END";
80.       SW:=SWX:=0.0;
81.       "FOR"I:=1"STEP"1"UNTIL"NL"DO"
82.       "BEGIN"
83.           "IF"Y[I]"LE"5"THEN"
84.           "BEGIN"
85.               "IF"Y[I]"GE"-9.0"THEN"
86.               "BEGIN"
87.                   W:=EXP(2*Y[I])/(EXP(EXP(Y[I]))-1);
88.                   JU:=EXP(EXP(Y[I])-Y[I]);
89.                   XO:=-EXP(-Y[I]); X:=XO+P[I]*JU;
90.                   SW:=SW+W; SWX:=SWX+W*X;
91.               "END";
92.           "END";
93.       "END";
94.       "IF"ABS(SW)"LE"TRY"THEN""GOTO"BACK;
95.       L:=L+1; NX:=SWX/SW; NY:=OY-NX;
96.       "IF"ABS(NY-Y[M])"LE"EPS"THEN"
97.       "BEGIN"
98.           SNW:=N*SW; VARSNW:=1/SNW; SDSNW:=SQRT(VARSNW);
99.           J:=M-1;
100.          LOWLIM:=EXP(OY-1.96*SDSNW)*DF↑J;
101.          UPLIM:=EXP(OY+1.96*SDSNW)*DF↑J;
102.          MPNO:=EXP(OY)*DF↑J; FAIL:=1; "GOTO"EXIT
103.      "END";
104.      Y[M]:=NY;
105.      "IF"NOSOL"LE"0"THEN"OY:=(NY+OY)/2"ELSE"OY:=NY;
106.      "IF"L"LE"MAX"THEN""GOTO"AGAIN"ELSE""GOTO"BACK;
107. EXIT:"END"MPN;
108. "COMMENT" END OF THE PROCEDURE;
109.
110.
111. "INTEGER""ARRAY"A[1:200];
112. "INTEGER"NL,N,NE,DF,I,J,MAX,FAIL,K,M,MULT;
113. "REAL"MPNO,UPLIM,LOWLIM,EPS,DW;
114. "PRINT"``F'';
115.
116. "COMMENT" READ IN THE NUMBER OF SERIES TO BE ANALYSED;
117. "READ"NE;
118. "FOR"K:=1"STEP"1"UNTIL"NE"DO"
119. "BEGIN"
120.     "COMMENT" READ IN THE TITLE OF THE SERIES;
121.     M:=1; INSTRING(A,M);
122.
123.     "COMMENT" PRINT OUT THE TITLE OF THE SERIES;
124.     "PRINT"``L2'';
     .      :=1; INSTRING(A,M);
```

```
126.
127.     "COMMENT" READ IN NL  THE NUMBER OF DILUTION LEVELS
128.                           DW THE DRY WEIGHT
129.                           MULT THE MULTIPLICATION FACTOR
130.                           N NUMBER OF REPLICATES AT EACH LEVEL
131.                          DF DILUTION FACTOR;
132. "READ"NL,N,DF,MULT,DW;
133.     "BEGIN"
134.         "INTEGER""ARRAY" PR[1:NL];
135.         "ARRAY" P[1:NL];
136.         "SWITCH"WRIT:=F1,F2,F3,F4;
137.
138.         "COMMENT" READ IN THE NUMBER FERTILE AT EACH LEVEL;
139.         "FOR"I:=1"STEP"1"UNTIL"NL"DO"
140.         "BEGIN"
141.             "READ"PR[I]; P[I]:=1-PR[I]/N;
142.         "END";
143.         SAMELINE; DIGITS(5);
144.         "PRINT"''L2`;
145.         "PRINT"'NUMBER OF LEVELS =`,NL,
146.         ''S10`DILUTION FACTOR =`,DF;
147.         "PRINT"''L`DRY WEIGHT =`,DW,
148.         ''S5`MULTIPLICATION FACTOR =`,MULT;
149.
150.         "COMMENT" PRINT OUT THE DILUTION SERIES;
151.         "PRINT"''L`DILUTION SERIES`;
152.         "FOR"I:=1"STEP"1"UNTIL"NL"DO" "PRINT"PR[I];
153.
154.         "COMMENT" SET MAXIMUM NUMBER OF ITERATIONS;
155.         MAX:=100;
156.
157.         "COMMENT" SET THE CONVERGENCE CRITERIA;
158.         EPS:=0.001;
159.         MPN(NL,N,DF,P,EPS,MAX,MPNO,UPLIM,LOWLIM,FAIL);
160.
161.         "COMMENT" MULTIPLY THE MOST PROBABLE NUMBER AND
162.         THE CONFIDENCE LIMITS BY DW AND MULT;
163.         MPNO:=MPNO*DW*MULT;
164.         UPLIM:=UPLIM*DW*MULT;
165.         LOWLIM:=LOWLIM*DW*MULT;
166.
167.         "COMMENT" PRINT OUT THE RESULTS;
168.         "GOTO"WRIT[FAIL];
169.         F1:"PRINT"''L`MPN = `,MPNO,
170.                  ''L`LOWER LIMIT = `,LOWLIM,
171.                   ''L`UPPER LIMIT =`,UPLIM;
172.         "GOTO"FIN;
173.         F2:"PRINT"''L2`NO SOLUTION OBTAINED`;
174.         "GOTO"FIN;
175.         F3:"PRINT"''L2`ALL OBSERVATIONS STERILE MIN. VALUE =`,
176.             MULT;
177.             "GOTO"FIN;
178.             F4:MPNO:=DW*MULT*DF↑(NL-1);
179.             "PRINT"''L2`ALL OBSERVATIONS FERTILE MAX. VALUE =`,
180.             MPNO;
181.     FIN:"END";
182. "END";
183. "END";
```

REFERENCES

Alexander, M. (1965a). Most-probable-number method for microbial populations. Methods of Soil Analysis, Part 2. (Ed. by C.A. Black, D.D. Evans, J.L. White, L.E.Ensminger & F.E. Clark), Agronomy no. 9. pp. 1467-1472. Amer. Soc. Agron.

Alexander, M. (1965b). Nitrification. Soil Nitrogen (Ed. by W.V. Bartholemew & F.E. Clark), Agronomy no. 10. pp. 307-343. Amer. Soc. Agron.

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

Allison, F.E. & Sterling, L.D. (1949). Nitrate formation from soil organic matter in relation to total nitrogen and cropping practices. Soil Sci. 67, 239-252.

Arnon, D.I. & Johnson, C.M. (1942). Influence of hydrogen ion concentration on the growth of higher plants under controlled conditions. Plant Physiol. 17, 525-539.

Ashmead, D. (1956). The influence of bacteria in the formation of acid mine waters. Colliery Guardian 192, 483-487.

Atkinson, J.R., Laurie, I.D., & Clay, A.J.M. (1957). The afforestation of pit heaps in Co. Durham. Pl. Outl. IV no. 3.

Baker, R.A. & Wilshire, A.G. (1970). Microbiological Factor in Acid Mine Drainage Formation. U.S.D.I. Federal Water Quality Admin. Water Pollution Control Research Series.

Berg, W.A. & Vogel, W.G. (1968). Manganese Toxicity of Legumes Seeded in Kentucky Strip-Mine Spoils. Res. Pop. Forest Serv. U.S. N.E. Forest Exp. Stn. 119.

Beyer, L.E. & Hutnik, R.J. (1969). Acid and Aluminium Toxicity as Related to Strip-Mine Spoil Banks in Western Pennsylvania. Coal Research Branch of the Commonwealth of Pennsylvania, Res. Rep. SR - 72.

Black, C.A., Nelson, L.B. & Pritchett, W.L. (1947). Nitrogen utilization by wheat as affected by rate of fertilization. Soil Sci. Soc. Amer. Proc. 11, 393-396.

Bould, C. (1963). Mineral nutrition of plants in soils. Plant Physiology (Ed. by F.C. Steward), 3 pp. 16-133.

Bower, C.A. & Wilcox, L.V. (1965). Soluble salts. Methods of Soil Analysis, Part 2 (Ed. by C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), Agronomy no. 9. pp. 933-951. Amer. Soc. Agron.

Bradshaw, A.D., McNeilly, T.S. & Gregory, R.P.G. (1965). Industrialisation, evolution and the development of heavy metal tolerance in plants. Ecology and the Industrial Society (Ed. by G.T. Goodman, R.W. Edwards & J.M. Lambert), pp 327-343. Blackwell Scientific Publications, Oxford.

Brandt, R.A. & Moulton, E.Q. (1960). Acid Mine Drainage Manual. Bull. Ohio Engng. Exp. Sta. 29.

Breeman, van N. (1973). Soil forming processes in acid sulphate soils. Acid Sulphate Soils (Ed. by H. Dost). International Institute for Land Reclamation and Improvement, Wageningen. Publication 18, 1, pp. 66-128.

Bremner, J.M. (1965a). Inorganic forms of nitrogen. Methods of Soil Analysis, Part 2 (Ed. by C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), Agronomy No. 9. pp. 1179-1237. Amer. Soc. Agron.

Bremner, J.M. (1965b). Total nitrogen. Methods of Soil Analysis, Part 2 (Ed. by C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), Agronomy no. 9. pp 1149-1178. Amer. Soc. Agron.

Bremner, J.M. & Shaw, K. (1958). Denitrification in soil II. Factors affecting denitrification. J. Agr. Sci. 51, 40-52.

Brierley, J.K. (1956). Some preliminary observations on the ecology of pit heaps. J. Ecol. 44, 383-390.

Brierley, W.B., Jewson, S.T. & Brierley, M. (1927). The quantitative study of soil fungi. Proc. First Int. Congr. Soil Sci. 3, 48-71.

Caruccio, F.T. (1974). Estimating acid potential of coal mine refuse with implications for reforestation attempts. In Press: The Ecology of Resource Degradation and Renewal (Ed. by M.J. Chadwick & G.T. Goodman). Blackwell Scientific Publications, Oxford.

Chadwick, M.J. (1973a). Amendment trials of coal spoil in the North of England. Ecology and Reclamation of Devastated Land (Ed. by R.J. Hutnik & G. Davis), pp. 105-119. Gordon & Breach, London.

Chadwick, M.J. (1973b). Methods of assessment of acid colliery spoil as a medium for plant growth. Ecology and Reclamation of Devastated Land (Ed. by R.J. Hutnik & G. Davis), pp. 81-91. Gordon & Breach, London.

Chadwick, M.J., Cornwell, S.M. & Palmer, M.E. (1969). Exchangeable acidity in unburnt colliery spoil. Nature, Lond. 222, 161.

Chadwick, M.J. & Salt, J.K. (1969). Population differentiation within Agrostis tenuis L. in response to colliery spoil substrate factors. Nature, Lond. 224, 186.

Chapman, H.D. & Liebig, G.F. (1952). Field and laboratory studies of nitrite accumulation in soils. Soil Sci. Soc. Amer. Proc. 16, 276-292.

Chapman, H.D. & Pratt, P.F. (1961). Methods of Analysis for Soils, Plants, and Waters. Univ. Calif. Div. Agric. Sci. California.

Clark, F.E. (1965). Aerobic spore forming bacteria. Methods of Soil Analysis, Part 2 (Ed. by C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), Agronomy no. 9. pp. 1473-1476. Amer. Soc. Agron.

Clarkson, D.T. (1966). Aluminium tolerance in species within the genus Agrostis. J. Ecol. 54, 167-178.

Coates, V.A. (1964). Experiment in Grassland Establishment on Colliery Shale. Bickershaw Reservoir Site. Abram 1954-1960. Lancashire County Council Planning Department Report.

Cochran, W.G. & Cox, G.M. (1957). Experimental Designs. 2nd Edn. John Wiley & Sons, Inc. London.

Cooke, G.W. & Cunningham, R.K. (1958). Soil nitrogen. IV. Mineralizable nitrogen determined by an incubation technique. J. Sci. Food Agric. 9, 324-330.

Cooper, J.E. (1973). A Study of the Microbiology and Plant Yield of Colliery Spoils Receiving Fertilizer Amendment. D. Phil. thesis, Univ. of York.

Cornwell, S.M. (1971). Anthracite mining spoils in Pennsylvania. I. Spoil classification and plant cover studies. J. Appl. Ecol., 8, 401-409.

Cornwell, S.M. & Stone, E.L. (1968). Availability of nitrogen to plants in acid coal mine spoils. Nature, Lond. 217, 768-769.

Cornwell, S.M. & Stone, E.L. (1973). Spoil type lithology and folia composition of Betula populifolia. Ecology and Reclamation of Devastated Land (Ed. by R.J. Hutnik & G. Davis), pp. 105-119. Gordon & Breach, London.

Cunningham, R.K. & Cooke, G.W. (1958). Changes in levels of inorganic nitrogen in a clay-loam soil caused by fertilizer additions, by leaching and uptake by grass. J. Sci. Food Agric. 9, 317-324.

Davison, A. & Jefferies, B.J. (1966). Some experiments on the nutrition of plants growing on coal mine waste heaps. Nature, Lond. 210, 649-650.

Doubleday, G.P. (1972a). Development and management of soils on pit heaps. Landscape Reclamation , Newcastle Univ. 2, pp. 25-35. I.P.C. Science and Technology, Guildford.

Doubleday, G.P. (1972b). Soil forming materials: Their nature and assessment. Landscape Reclamation , Newcastle Univ. 1, pp. 70-83. I.P.C. Science and Technology, Guildford.

Ekpete, D.M. & Cornfield, A.H. (1965). Effect of pH and addition of organic materials on denitrification losses from soil. Nature, Lond. 208, 1200.

Fenton, G.W., Adams, H.F. & Rumsby, P.L. (1962). The mapping and appraisal of the characteristics of British coal seams. Min. Engr. 121, 454-467.

Finney, D.J. (1951). The estimation of bacterial densities from dilution series. J. Hyg. 49, 26-35.

Finney, D.J. (1952). Statistical Methods in Biological Assay. Griffin, London.

Fisher, R.A. & Yates, F. (1963). Statistical Tables for Biological Agricultural and Medical Research. Oliver & Boyd, London.

Fitter, A.H. (1972). Mineral Nutrition of Grasses and the Reclamation of Colliery Shale. Ph. D. thesis, Univ. of Liverpool.

Friedman, M. (1937). The use of ranks to avoid the assumption of normality implicit in the analysis of variance. J. Amer. Statist. Ass. 32, 675-701.

Gasser, J.K.R. (1961). Effects of air-drying and air-dry storage on the mineralisable nitrogen of soils. J. Sci. Food Agric. 12, 778-784.

Gasser, J.K.R. & Williams, R.J.B. (1963). Soil nitrogen. VII. Correlation between measurements of nitrogen status of soils and nitrogen percentage and nitrogen content of crops. J. Sci. Food Agric. 14, 269-277.

Glover, H.G. (1974). The disposal of coal mine spoil in the United Kingdom. In Press; Waste Disposal and the Renewal and Management of Degraded Environments (Ed.by G.T. Goodman & M.J. Chadwick). Nordhoff, Holland.

Halvorson, H.O. & Ziegler, N.R. (1933). Application of statistics to problems in bacteriology. III. A consideration of the accuracy of dilution data obtained by using several dilutions. J. Bact. 26, 559-567.

Harding, C.P. (1970). Plant Available Nutrients in Colliery Spoil and their Relation to Ecotypic differentiation within Populations of Agrostis tenuis L. D. phil. thesis, Univ. of York.

Harmsen, G.W. & Kolenbrander, G.J. (1965). Soil inorganic nitrogen. Soil Nitrogen (Ed. by W.A. Bartholomew & F.E. Clark), Agronomy no. 10. pp. 43-92. Amer. Soc. Agron.

Hewitt, E.J. (1963). The essential nutrient elements: requirements and interactions in plants. Plant Physiology (Ed. by F.C. Steward), 3, pp. 137-360.

Jackson, M.L. (1962). Soil Chemical Analysis. Constable, London.

Jackson, W.A. (1967). Physiological effects of soil acidity. Soil Acidity and Liming (Ed. by R.W. Pearson & F. Adams), Agronomy no. 12. pp. 43-124. Amer. Soc. Agron.

Kamprath, E.J. (1970). Exchangeable aluminium as a criterion for liming leached mineral soils. Soil Sci. Soc. Amer. Proc. 34, 252-254.

Kirkby, E.A. (1969). Ion uptake and ionic balance in plants in relation to the form of nitrogen nutrition. Ecological Aspects of the Mineral Nutrition of Plants. (Ed. by I.H. Rorison), pp. 215-235. Blackwell Scientific Publications, Oxford.

Knabe, W. (1965). Observations on world-wide efforts to reclaim industrial land. Ecology and the Industrial Society (Ed. by G.T. Goodman, R.W. Edwards & J.M. Lambert), pp. 263-296. Blackwell Scientific Publications, Oxford.

Lau, C.M., Shumate, K.S. & Smith, E.E. (1970). The role of bacteria in pyrite oxidation kinetics. Proc. Third Sym. on Coal Mine Drainage Res. Mellon Inst. Pennsylvania. pp. 114-119.

Lipman, C.B. (1938). Importance of silicon, aluminium, and chlorine for higher plants. Soil Sci. 45, 189-198.

Lorio, P.L. & Gatherum, G.E. (1965). Relationship of tree survival and yield to coal spoil characteristics. Iowa St. Univ. agric. Home Econ. Exp. Sta. Res. Bull. 535, 394-403.

Martin, M.H. (1968). Conditions affecting the distribution of Mercurialis perennis L. in certain Cambridgeshire woodlands. J. Ecol. 56, 777-793.

Menzies, J.D. (1965). Fungi. Methods of Soil Analysis, Part 2 (Ed. by C. A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), Agronomy no. 9. pp. 1502-1505. Amer. Soc. Agron.

Macfadyen, A. (1964). Energy flow in ecosystems and its exploitation by grazing. Grazing in Terrestrial and Marine Environments (Ed. by D.J. Crisp), pp. 3-20.

MacFarlan, J. (1939). Time of application of nitrogen as a factor influencing the yield and herbage on permanent pasture. Exp. Agric. 7, 155-161.

Mulder, E.G. (1956). Nitrogen-magnesium relationships in crop plants. Plant and Soil 7, 341-376.

National Agricultural Advisory Service. (1963). Soil Microbiology Techniques, Bull. S.M. 3. Nitrogen cycle bacteria.

Oldershaw, J.H. (1969). Substrate Factors and the Distribution of Vegetation on Coal Spoil. B.A. thesis, Univ. of York.

Olsen, C. (1958). Iron uptake in different plant species as a function of the pH value of the nutrient solution. Physiologica Pl. 11, 889-905.

Palmer, M.E. (1972). Salinity in Colliery Spoil. Report of the Colliery Spoil Research and Advisory Group, Univ. of York.

Possingham, J.V., Vesk, M. & Mercer, F.V. (1964). The fine structure of leaf cells of manganese deficient spinach. J. Ultrastruct. Res. 11, 68-73.

Pratt, P.F. (1966). Aluminium. Diagnostic Criteria for Plants and Soils (Ed. by H.D. Chapman), pp.3-12. Univ. California.

Pritchett, W.L., Eno, C.F. & Malik, M.W. (1959). The nitrogen status of the mineral soils of Florida. Soil Sci. Soc. Amer. Proc. 23, 127-130.

Rains, D.W., Schmid, W.E. & Epstein, E. (1964). Absorption of cations by root. Effect of hydrogen ions and essential role of calcium. Plant Physiol. 39, 274-278.

Richards, S.J. (1965). Soil suction measurements with tensiometers. Methods of Soil Analysis, Part 1 (Ed. by C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger & F.E. Clark), Agronomy no. 9. pp. 153-163. Amer. Soc. Agron.

Richardson, H.L. (1938). The nitrogen cycle in grassland with especial reference to the Rothamstead Park grass experiment. J. Agric Sci., Camb. 28, 73-121.

Richardson, J.A. (1958). The effect of temperature on the growth of plants on pit heaps. J. Ecol. 46, 537-546.

Rickard, D.T. (1973). Sedimentary iron sulphide formation. Acid Sulphate Soil (Ed. by H. Dost). International Institute for Land Reclamation and Improvement, Wageningen. Publication 18, 1, pp 28-65.

Robinson, J.B. (1963). Nitrification in a New Zealand grassland soil. Plant and Soil 19, 173-183.

Rorison, I.H. (1971). The use of nutrients in the control of the floristic composition of grassland. The Scientific Management of Animal and Plant Communities for Conservation (Ed. by E. Duffey & A. S. Watt), pp 65-77. Blackwell Scientific Publications, Oxford.

Rorison, I.H. (1973). The effects of extreme soil acidity on the nutrient uptake and physiology of plants. Acid Sulphate Soils (Ed. by H. Dost), International Institute for Land Reclamation and Improvement, Wageningen. Publication 18, 1, pp 223-251.

Russell, E.W. (1961). Soil Conditions and Plant Growth. 9th Edn. Longmans.

Schramm, J.R. (1966). Plant Colonization Studies of Black Wastes from Anthracite Mining in Pennsylvania. Trans. Amer. Phil. Soc. 56, pp. 1-194.

Schmitt-Henner, A.P. & Williams, L.E. (1958). Methods for the Analysis of Soil-Borne Plant Pathogens and Associated Soil Fungi. Ohio Agric. Exp. Sta. Mimeo Serv. no. 29.

Seatz, L.F. & Peterson, H.B. (1964). Acid, alkaline, saline and sodic soils. Chemistry of the Soil (Ed. by F.E. Bear), pp. 292-319. Reinhold Publishing Corporation.

Smith, R.A.H. & Bradshaw, A.D. (1972). Stabilization of toxic mine wastes by the use of tolerant plant populations. Trans. Instn. Min. Metall. (Sect. A: Min-Industry), 81, 230-237.

Snedecor, G.W. (1956). Statistical Methods. The Iowa State Univ. Press.

Sommer, A.L. (1926). Studies concerning the essential nature of aluminium and silicon for plant growth. Univ. California Pub. Agr. 51, 57-81.

Soulides, D.A. & Clark, F.E. (1958). Nitrification in grassland soils. Soil Sci. Soc. Amer. Proc. 22, 308-311.

Street, H.E. & Goodman, G.T. (1967). Revegetation techniques in the Lower Swansea Valley. The Lower Swansea Valley Project (Ed. by K.J. Hilton), pp. 71-110.

Sutton, C.D. & Hallsworth, E.G. (1958). Studies on the nutrition of forage legumes. I. The toxicity of low pH and high manganese supply to lucerne as affected by climatic factors and calcium supply. Plant and Soil 9, 305-317.

Taylor, J. (1962). The estimation of numbers of bacteria by tenfold dilution series. J. appl. Bact. 25, 54.

Theron, J.J. (1951). The influence of plants on the mineralization of nitrogen and the maintenance of organic matter in the soil. J. Agric. Sci. 41, 289-296.

Varnam, D.J. (1970). The Micro-Distribution of Vegetation in Relation to Variation in the Chemical Factors of Colliery Spoil. B.A. thesis, Univ. of York.

Warwick, P.J. (1958). Plant Nutrition on Colliery Waste. Ph. D. thesis, Univ. of Birmingham.

Walker, T.W. (1956). The nitrogen cycle in grassland soils. J. Sci. Food Agric. 7, 66-72.

Weber, D.F. & Gainey, P.L. (1962). Relative sensitivity of nitrifying organisms to hydrogen ions in soils and in solutions. Soil Sci. 94, 138-145.

Weston, R.L., Gadgill, P.D., Salter, B.R. & Goodman, G.T. (1965). Problems of revegitation in the lower Swansea Valley, an area of extensive industrial dereliction. Ecology and the Industrial Society (Ed. by G.T. Goodman, R.W. Edwards & J. M. Lambert), pp. 297-325. Blackwell Scientific Publications, Oxford.

Whitehead, D.C. (1971). The Role of Nitrogen in Grassland Productivity. Bull. 48, Commonwealth Agri. Bureaux.

Wilson, H.A. (1965). The Microbiology of Strip-Mine Spoil. W. Va. Univ. Agric. Exp. Sta. Bull. 506T.

Wilson, H.A. & Stewart, G. (1956). Ammonification and Nitrification in a Strip-Mine Spoil. W. Va. Univ. Agric. Exp. Sta. Bull. 379T.

Withell, E.R. (1942). The significance of the variation in shape of time-survivor curves. J. Hyg. 42, 124.

Wood, R.F. & Thirgood, J.V. (1955). Tree Planting on Colliery Spoil Heaps. Forestry Comm. Res. Branch Paper no. 17.