STUDIES ON THE MICROFLORA OF MAKIN SAND, (NORMAL PHASE) IN THE COUNTY BUCKINGHAM IN THE UPPER SOUTH EAST OF SOUTH AUSTRALIA.

by

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I INTRODUCTION

In 1950 a project was commenced by members of the Botany School, Adelaide to study among other projects, the effect of nutrients such as nitrogen (N), phosphorus (P), copper (Cu) and zinc (Zn) upon the natural vegetation at Dark Island Swamp, 10 miles north east of Keith.

A study of the microflora and the relation of micro-organisms to nitrogen metabolism within the soil was instigated as a separate project and the methods, results and discussion of this project are reported here. Such a study was considered to be of general interest to the people engaged on studies concerning the natural vegetation.

The work is presented in three parts.

(1) Description of the Area

(2) Composition of the Microflora

(3) Relationship of micro-organisms to soil problems.

  (a) Decomposition of soil organic matter and the return of nitrogen from the organic matter to the soil

  (b) Nitrification and problems arising from nitrification

II DESCRIPTION OF THE AREA

Coaldrake (1951) described the soil studied as a Makin Sand normal phase and salient points regarding its properties were taken from his description.

1. Location

The area studied was a portion of the University experimental block at Dark Island Swamp which lies in virgin country 10 miles north east of Keith, in the Hundred of Makin, County of Buckingham. Maps 1 and 2 give the location.

2. Climate

The effective rain falls in winter with a virtual summer drought. Temperatures are in the warm range giving the area a 'mediterranean' type of climate. Summer temperatures may exceed 100°F while frosts occur in winter. The mean annual temperature for Keith taken over 3 years was 57.7°F and the average rainfall for Keith for 43 years was 17.99 inches. No rainfall figures were available for the Dark Island Swamp area due to no permanent settlement. The most favourable conditions for plant growth probably occur between September and October but it has been noted that the growing season for Banksia ornata extends at least until February. Apparently the plants derive their moisture and nutrients from the clay B horizon.

3. Geology and Physiography

Coaldrake (1951) gave the following description "The area under

discussion is underlain at varying depth by Tertiary limestone, which usually is of great thickness. This, in turn, rests finally on a suite of Palaeozoic igneous rocks. The deposits above the limestone vary but they consist chiefly of Pleistocene fluviatile or lacustrine sediments (Howchin). Finally these are capped with a mantle of aeolianitic siliceous sands of varying depth. The origin of these sands has been traced by Crocker in a series of papers (1941, 1946a; Crocker and Wood 1947)". The silt and clay fractions in the Makin sand is non-existent to a depth of 60". The depth of clay existing below the sand, Coaldrake suggests may be from 40-60 feet.

4. Soils

(1) Soil type

The Makin Sand - normal phase has the following characteristics

A_1	horizon 0-4"	sand + organic matter	pinkish grey colour	topography of sand hills and undulations
A_2	horizon 4-60+"	sand	yellow colour	
B_1	horizoh 60" -	clay	reddish	

The soil type has a clay of uniform origin overlain by an undulating mantle of sand of varying depth.

(2) Laboratory analysis

(a) The Soil reaction The pH of the soil (by glass electrode) varied considerably from point to point within the soil but lay within the range 5.7 to 7.8 with the majority of recordings in the 5.9 to 6.5 range. Naturally, root systems and plants generally, together

with leaching, affect the pH readings from point to point within the soil.

(b) The exchangeable cations Magnesium (Mg) was in excess of the Calcium (Ca) in the soil in common with all soils of low fertility throughout Australia. Exchangeable cations in the A_1 and A_2 horizons were 2 Me%.

(c) Mineral content The total nitrogen content for the top 6" was 0.013% and was progressively lower throughout the soil to the clay layer. Phosphorus was similar ly low, 10 - 11 ppm in the sand, while Zn and Cu amounted to only 4 - 7 ppm for Zn and 2 - 3 ppm for Cu in the top 3" of soil. The highest total nitrogen found was 0.043%.

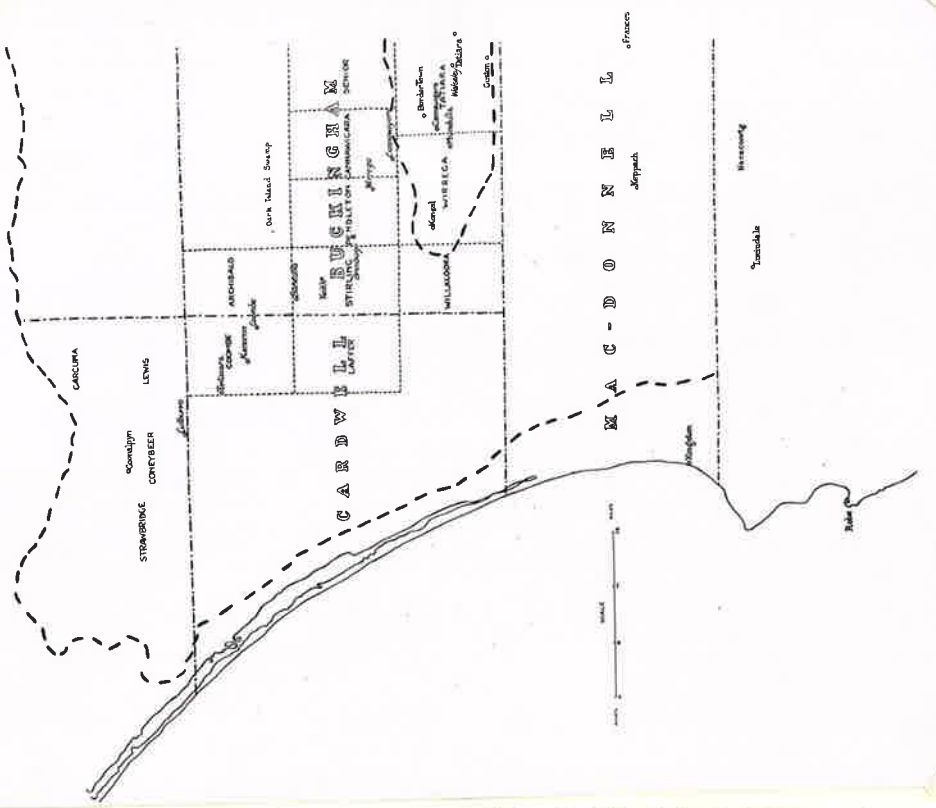
(d) Coaldrake gave the following figures for analysis of the Makin Sand. Results are expressed as percentages in the following tables.

Buckingham Suite - Makin Sand

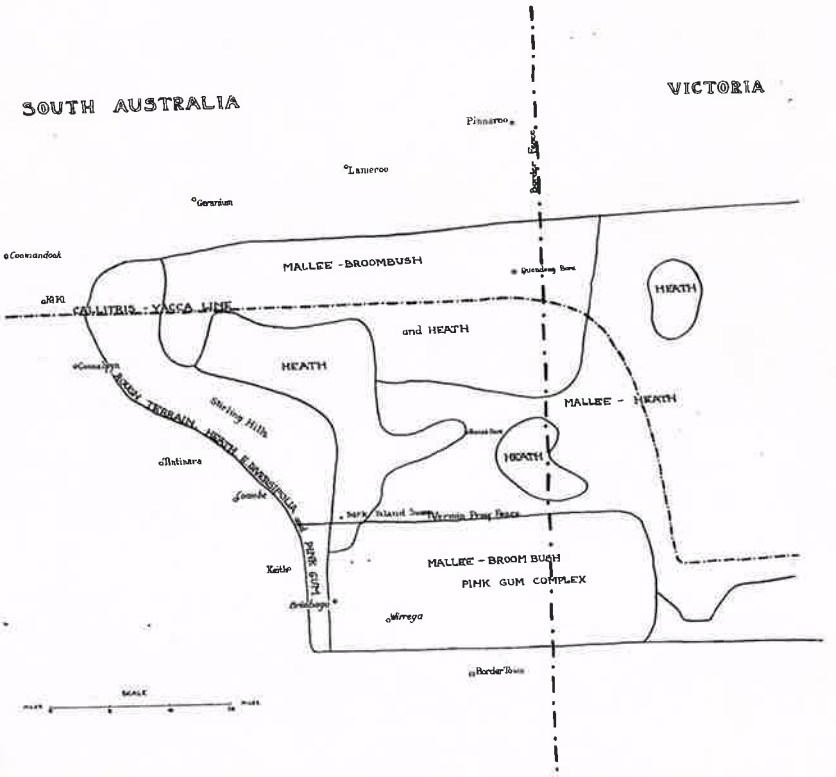
	A_1	A_2	B_1
pH	5.86	6.31	7.41
N	0.013	.003	.005
P	.004	.005	not done
TSS	.010	.006	" "
NaCl	.002	.001	" "

Exchangeable Cations

	me%	%
Ca	2.5	32
Mg	4.0	51
K	.6	7
Na	.7	10
	7.8	100



Map I. Location of the Dark Island Swamp Area in relation to County Buckingham



Map II (After Coaldrake) Vegetation boundaries in the County of Buckingham

(3) Physical Characteristics

The following table expresses as percentages the composition of the Makin Sand.

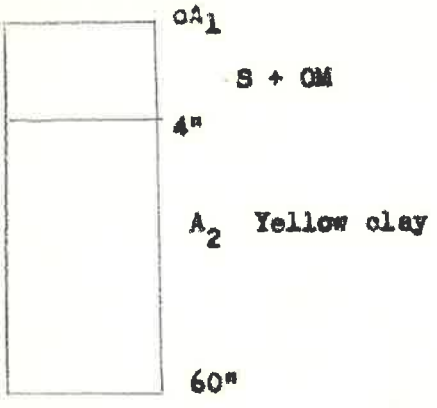
Buckingham Suite

Makin Sand

	A_1	A_2	B_1	B_2/C
Depth	0-4"	4-30-50"	50-60"	60-70"
Coarse sand	46.8	38.5	19.5	7.2
Fine sand	50.4	60.0	58.4	21.6
Silt	-	-	0.9	3.0
Clay	-	0.5	22.8	61.8

Vegetation - Heath

Soil Profile may be represented as follows



S = sand
OM = organic matter

(4) Soil Moisture

The water holding capacity of the top 6" was 26.87% as determined by the method given by Piper (1947). In winter at the junction of the clay and sand surface the water content of the soil reached 21%. In summer the moisture content of the soil surface may be as low as 0.5%. In winter it may be 6.8% under plants or 3.5% in more open areas. Day conditions in summer were unfavourable to micro-organisms.

(5) Soil temperatures

During summer the ground temperatures during the day reach 45°C and fall to as low as 5°C at a depth of 3" in winter. In summer, the soil temperature remains high in the top 6" of soil and must have a great affect on the soil population.

5. Vegetation

The area is covered by a heath type vegetation. The dominant species are: -

Xanthorrhoea australis (R.Br)

Casuarina pusilla (Macklin)

Casuarina mucelleriana (Miq)

Phyllota pleuiandroides (F.v.M.)

Banksia marginata (Cav).

" ornata (F.v.M)

Hibbertia stricta (R.Br)

Grasses grow during the wet season. Specific features of the vegetation are that the majority of the plants have extremely long tap roots reaching into the clay. Even small seedlings have immense tap roots. Lateral roots were abundant in the top 6" where organic matter was located. The type of root systems probably has some effect upon the distribution of micro-organisms.

The return of nutrients to the soil from the organic matter was of interest hence some characteristics should be reported here regarding the leaf drop of the vegetation. Certain plants have one leaf fall per year,

e.g. Banksias, Casuarina and Phyllota. The yearly layers of litter can be distuinguished under the plants. The Banksia species carry 2 years growth of leaves and the age of the plants can be determined from this and the mode of yearly growth.

III COMPOSITION OF THE MICROFLORA

1. Introduction

Interest in soil micro-organisms arose about 1880 following work on nitrogen bacteria of the soil. During the following 25 years spectacular discoveries were made by Beijerinck, and Schloesing and Muntz (1877; 1878) and by Winogradsky, (1890; 1891; 1892). Winogradsky isolated in pure culture the organisms concerned in the process of nitrification. Following the introduction of selective media the isolation of nitrogen bacteria was simplified. Various reviews are available on early soil microbiology up to the present time. Waksman (1931) adequately reviewed the literature on microbiology. Waksman (1936) reviewed the literature and work between 1932 and 1936. Norman (1946) and Smith (1948) reviewed the literature up to 1948.

The whole of Winogradsky's work is collected together in the publication "Microbiologie du sol" and his interest in microbiology is fully revealed. Smith (1948) stated that since 1936 emphasis has been on nitrification and factors affecting it. Work on the rhizosphere and mycorrhiza has progressed favourably in the last few years.

Certain controversies have arisen regarding the classification of soil micro-organisms. Lockhead developed a system of classification for bacteria on the basis of their amino acid requirements as a contrast to the usual morphological and biochemical basis but as yet no completely satisfactory method of classification is available.

In the present study the general nature of the microflora has been considered as more important than the actual identification and isolation

of single species except in very particular circumstances.

2. Materials and Methods

Waite virgin soil was used as a control soil and Makin Sand used as an experimental soil.

(1) General sampling technique

(a) Using sterile scoops the top $\frac{1}{8}$" of soil was scraped away and samples from the required depths collected into clean containers. Where the soil samples were intended for use in the isolation of particular species in pure culture, numbers of samples were bulked, and soil was then used from this bulked sample. Where the samples were required for estimation of the numbers of organisms in the soil the samples were kept separate. Samples from depths greater than 12" were taken from the sides of soil pits after the outer layer of soil had been removed.

All soil samples were taken at random.

(b) Sample preparation

Soil samples were returned to the laboratory and sieved through a 2 mm sieve. Roots and other organic matter were broken up in a mortar and pestle to enable them to pass through the 2 mm sieve.

Samples to be used for nitrification work described later were air dried.

All samples were used within 48 hours.

(2) Estimation of total numbers of organisms in the soil

(a) Bacteria

The Plate count method

Ten grams of sieved soil were added to 100 mls of sterile distilled

water (S.T.W.) Sterile glass beads were added and the bottles shaken for 5 minutes in a mechanical shaker. After standing for 5 minutes, 10 mls of this $\frac{1}{10}$ dilution were pipetted into 190 mls S.T.W. and 490 mls S.T.W. respectively giving dilutions of $\frac{1}{200}$ and $\frac{1}{500}$ respectively. Tenmbbs of the $\frac{1}{200}$ dilution were pipetted into 90mls S.T.W. giving a dilution of $\frac{1}{2000}$ and 10 mls of the $\frac{1}{500}$ dilution into 90 mls giving a $\frac{1}{5000}$ dilution. The $\frac{1}{500}$ dilution was diluted to give $\frac{1}{25000}$ dilution. One cc of each of the required dilutions ($\frac{1}{2000}$ and $\frac{1}{5000}$ and $\frac{1}{25,000}$) was pipetted into 9 cc of molten agar (for bacteria Waksman's egg albumen agar and Thornton's asparagine mannitol agar were used. (Waksman 1931).) After mixing by rotation the 10 cc mixture was poured into a petri plate.

After incubation at 37°C for 2 days the plates were counted, reincubated for 3 days and recounted.

3 samples for each soil level were taken and from each sample 3 dilutions were made. Each dilution was triplicated.

(b) Fungi

The plate count method was used for fungi using Waksman's fungus agar and potato Marmite dextrose agar (Waksman 1931)) as the plating media. (Potato Marmite dextrose agar is referred to as PMDA). The same technique was followed as for bacteria and the same dilutions used after a preliminary experiment.

The plates were incubated at 25°C for 2 days, counted, incubated for 3 days and recounted.

(3) Isolation of organisms in pure culture

(a) Algae

Soil collected from the soil surface was plated onto

Bristol Roach medium and incubated for 3 weeks at 25°C. Soil was also taken from 3" and 6" and scattered onto Bristol Roach agar. (Fred & Waksman 1928).

(b) Actinomycetes

No specific attempt was made to isolate actinomycetes but large numbers occurred on the plates used for the estimation of bacterial and fungal numbers.

(c) Bacteria

(i) Azotobacter

Liquid culture and solid media were used. Jensen's medium (1940) was used and also Ashby's (1907). The soil was inoculated into liquid medium and was sparingly sprinkled onto the agar plates. Cultures were incubated at 28°C for up to 21 days.

(ii) Anaerobes

Soil was mixed with nutrient agar and also inoculated into cooked meat medium, and in the former case incubated at 37°C in a Fildes's McIntosh anaerobic jar.

0.5 gms. soil were sprinkled over 75 mls of glucose agar in a 20 cm petri plate. A further 100 mls of medium at 80°C were added and the plate incubated for 6 days at 25°C. After this time, the plates were flooded with dilute iodine solution which stains young colonies of Clostridium butyricum red and older colonies yellow.

(iii) Nitrite and nitrate forming organisms

Isolation of nitrite formers

The method used was after Meikejohn (1950). The method is a

modification of Winogradsky's original method (1949). Due to low numbers of organisms the following procedure was adopted.

Soil was freshly collected and 1% calcium carbonate added. This soil mixture was then alternately percolated with a 1% $(NH_4)_2SO_4$ solution, and air drawn through with a vacuum pump. The treatment was such that percolation and aeration alternated every 2 hours. Percolation was carried out for 5 days and 5 gms. of this soil was then used to inoculate Meikeljohn's medium A (1950). The progress of nitrite formation was followed by means of Griess-Ilosvay reagent (Topley & Miles 1945). Nitrite built up fairly rapidly to about 20 ppm in 10 days. After 14 days all this nitrite had disappeared and nitrate appeared and its presence identified by the α naphthylamine method (appendix). The method from here followed Meikeljohn's method (1950) but alternative methods for preparing the silica gel required, were used. Following isolation on silica gel the colonies of the nitrite former were picked off with a finely drawn glass needle using a dissecting microscope the colonies were reinoculated into medium A given by Miekeljohn and their capacity to convert ammonia to nitrite measured after 14 to 21 days. The complete isolation took 15 months.

The nitrate forming organisms

These were isolated by inoculating soil - 5 grams of the enriched soil into a medium containing nitrite. This medium is given in the appendix under Salle's sodium nitrite medium (1943)

Following various serial subcultures the organisms were plated onto salt enriched silica gel medium. Following isolation the colonies

were picked off from the silica gel and reinoculated into sodium nitrite medium and the nitrate production tested.

(iv) Fungi

Isolation of fungi was attempted from litter collected on the surface of the soil and from the soil itself.

Litter was plated onto potato Marmite dextrose agar, Waksman's fungus agar (1931) and Warcup's agar (1950).

Similarly soil was scattered on the surface of agar plates and mixed with the medium before pouring the plates.

(v) Protozoa

Isolation and identification of Protozoa has been carried out by Mrs. R. L. Specht, Zoology School, Adelaide.

3. Results and Discussion

The following results were obtained for the Makin Sand for fungal counts using PMDA as the medium. Soil was collected 20.4.51.

TABLE I

Dilutions

0-6"	Sample	1/500		1/2000		1/5000	
	1	210)		72)		26)	
		215)	208	73)	71	28)	26
		198)		68)		24)	
	2	208)		67)		22)	
		180)	182	70)	67	15)	18
		187)		61)		16)	
	3	215)		79)		26)	
		230)	221	72)	75	29)	26
		278)		73)		22)	

Table I continued

6-12"	1	38)		12)		3)	
		44)	41	11)	11	5)	5
		40)		10)		5)	
	2	48)		12)		8)	
		40)	48	10)	11	6)	6
		55)		12)		4)	
	3	31)		9)		7)	
		45)	39	12)	11	4)	6
		42)		11)		8)	
12-24"	1	26)		6)		1)	
		22)	24	10)	7	4)	3
		25)		5)		5)	
	2	22)		7)		2)	
		26)	23	4)	7	4)	2
		22)		10)		1)	
	3	21)		8)		4)	
		27)	25	11)	9	6)	4
		26)		8)		4)	
24-48"	1	— sample not done					
	2	16)		5)		2)	
		14)	16	3)	4	4)	4
		19)		4)		5)	
	3	19)		5)		-)	
		13)	17	4)	4	2)	2
		30)		4)		2)	

The average of these readings gives

0 - 6"	1.2×10^5	organisms	per	gram	of	soil
6-12"	2.4×10^4	"	"	"	"	"
12-24"	1.6×10^4	"	"	"	"	"
24-48"	1.0×10^4	"	"	"	"	"

Estimates made on the same soil on 1.7.51 gave the following results on PMDA. Only 2 replicates per dilution were made.

TABLE II

0 - 6" sample		Dilution 1/500		1/2000		1/5000	
	1	113)		39)		21)	
		131)	122	47)	43	23)	22
	2	109)		45)		17)	
		110)	109	43)	44	22)	19
	3	115)		51)		23)	
		110)	112	55)	53	16)	19
6-12"	1	45)		15)		4)	
		44)	44	14)	14	3)	3
	2	49)		13)		9)	
		34)	41	14)	13	10)	9
	3	42)		10)		7)	
		33)	37	15)	12	10)	8
12-24"	1	21)		11)		3)	
		28)	24	4)	7	4)	3
	2	22)		7)		4)	
		22)	22	9)	8	5)	4
	3	27)		9)		3)	
		18)	22	5)	7	1)	2
24-48"	1	12)		2)		2)	
		10)	11	3)	2	1)	1
	2	7)		3)		1)	
		5)	6	5)	4	1)	1
	3	8)		2)		0)	
		11)	9	2)	2	3)	1

The average of these readings gave

0 - 6"	8.3×10^4	organisms per gram of soil
6-12"	2.5×10^4	organisms per gram of soil
12-24"	1.3×10^4	organisms per gram of soil
24-48"	4×10^3	organisms per gram of soil

These counts do not show any great variation between the readings for different times of the year. Bacteria numbers of course vary in the soil from day to day and this technique would not be sufficiently accurate to pick up such variations hence counts were not made through a continuous period of time. The fungal count was not particularly high but was similar to the results given by Waksman (1931) for a sandy soil of low fertility. The counts obtained on fungus agar medium were similar to those given for PMDA and for that reason were not quoted here.

The bacterial counts gave the following results.

TABLE III

	Sample 1	2	3	Average
0 - 6"	1,500,000	1,250,000	980,000	1.24×10^6 oz/gm
6 - 12"	950,000	723,000	895,000	8.56×10^5 "
12 - 24"	500,000	565,000	480,000	5.09×10^5 "
24 - 48"	465,000	355,000	210,000	3.43×10^5 "

These results were averages of triplicate plates per dilution. There were 3 dilutions per sample and the dilutions used were $\frac{1}{2000}$, $\frac{1}{5000}$, and $\frac{1}{25000}$. The bacterial counts were found to be only slightly greater than the fungal counts. In a fertile soil containing large amounts of organic matter the bacterial count when using the plate count method may reach 300-400 x 10^6 organisms per gram soil while the fungal count may be 40 - 60 x 10^6 organisms per gram of soil.

According to Russell (1951), Adametz and Waksman recorded finding 320,000 - 500,000 bacteria per gram in sandy soil by the plate count method. The numbers of bacteria obtained for the Makin Sand were a little higher than this estimate.

The results indicated that the highest numbers of bacteria and fungi occurred in the top 6 inches of soil but the bacterial and fungal numbers did not decrease very rapidly. Due to the light sandy soil which provides aeration to a greater depth than in a clay soil the organisms are capable of growth at a greater depth.

Although no attempt was made to count actinomycetes present in Makin Sand they occurred very frequently on the plates used for estimating numbers of fungi and bacteria and may be assumed to be frequent in this soil. Estimation of their numbers is complicated by their hydrophobic spores which float on the surface of the water used for soil dilutions. Actinomycetes are frequently found in warm sandy soils (Russell 1951). The actinomycetes isolated resembled Streptomycetes micromonospora sps.

The virgin Waite soil is known to have a bacterial count at least 100 fold greater than the Makin Sand (student estimation) and the Makin Sand simulates a warm sandy soil in its fungal and bacterial population.

Regarding the plate count method for estimating the numbers of the fungal and bacterial population the following should be noted .

The method if based on several assumptions.

(1) Each colony is assumed to develop from one bacteria only.

(2) All bacteria in the soil suspension are assumed to be brought into suspension.

(3) All bacteria present in the suspension are assumed to be capable of growing on the nutrient medium used.

All these assumptions do not hold at the one time. In the case of fungi also the assumptions do not hold at the one time. The plate count

method tends to underestimate the numbers of organisms present and counts only aerobes. Another method for the estimation of organisms in the soil is the direct microscopic method. The stains employed in this method however stain both dead and living cells and hence overestimate the number of organisms in the soil.

However since some idea of the genera of organisms present in the soil was required it was decided to use the plate count method so that isolations could be performed from these plates and hence the method served a double purpose.

Upon examination of the composition of the microflora the bacterial and fungal population were found to be a heterogeneous collection and due to the virginity of the soil no introduced soil members were found.

Classification of soil bacteria has been attempted on morphological bases but the more recent attempts at classification have been on the basis of the nitritional requirements of the bacteria with particular reference to their amino acid requirements. The time involved in identification of bacteria is enormous and hence no attempt was made to classify bacterial genera except where they had particular interest in the nitrogen cycle.

Many attempts to isolate Azotobacter species from Makin Sand were made but no positive results were obtained. However they could be isolated from an enriched garden soil and therefore the technique appeared sound. Collins (1951) has isolated to Azotobacter from Kangaroo Island soil in numbers far in excess of anything so far reported in the literature. His isolations however were all made on solid medias and not in liquid media. He has not attempted to prove that the organisms fix

atmospheric nitrogen in pure liquid culture.

Anaerobes were found to be present in the Makin Sand but their numbers appeared few. Clostridium butyricum was isolated in glucose agar medium.

Bacteria concerned in the conversion of ammonia to nitrite to nitrate were found to exist but their numbers were small. By trial and error they could be isolated from 20 gms. of soil but not 15.

Organisms resembling Nitrosomonas and Nitrobacter were isolated from the Makin Sand. They were not identified further. The absence of Azotobacter species and the low numbers of organisms capable of carrying out nitrification i.e. conversion of $NH_3 \longrightarrow NO_2 \longrightarrow NO_3$ allows very litte build up of nitrate in this soil.

The presence of algae has been noted on the surface of the soil at Dark Island Swamp at certain times of the year. During autumn the surface was covered by a dense mat which when examined and grown on Bristol Roach agar was identified as Cylindrocapsa species. Various Chro-ococcale sps. were present. No blue greens were isolated and hence there was no possibility of nitrogen fixation by algae. This mat of algae filaments even when dry in summer helps to bind the surface sand. This is one of the 4 ways in which algae may benefit soils.

(1) They may add organic matter to the soil

(2) Algae may help bind the surface particles

(3) They may improve aeration of swamp soils

(4) They may fix atmospheric nitrogen.

Salisbury (1915) has suggested that they may be of some importance in the recolonization of bare burnt ground. This has not been observed in the Dark Island Swamp area but will be looked for in the future.

Protozoa were found in the Makin Sand and the species isolated by Mrs. R. L. Specht (1952. private communication) were 2 species of Colpoda and a Uroleptus sps.

The fungi appeared prevalent in the soil and could be readily isolated both from litter and the soil itself.

Species isolated included Rhizopus, Mucor, Trichoduma, Aspergillus and Penicillium sps. The Aspergillus species were extremely prevalent and produced a wide variety of diffusable and non diffusable pigments. From the organisms isolated on the plates used for plate counts there appeared no differentiation in the species of organisms isolated at different levels but there was a slight difference in the frequency of these species at different soil levels.

Fungi isolated from litter included, Chaetomium, Alternaria, Cladosporium, Syncephalastrum, FusariumsPestalozzia, Penicillium, Mucor, Stemphyllium, Acrosphaera and Aspergillus species.

From the exposed surface of the litter bacteria were the main organisms isolated and fungi were isolated from the undersurface of this top layer.

No attempt was made to isolate Basiodioycetes in culture but during autumn the following species were isolated. Polyporus oblectans, Flammulla paludosa, Continarius fibrillosus, Psilocybe subammophila.

Other species are no doubt present but no fruiting bodies were collected.

An ascomycete, a Sarcosphaera sps. not previously recorded in South Australia was found.

From these experiments and observations the microflora of the Makin Sand resembled the warm sandy soils as described in the literature.

The actinomycete and fungal numbers approached the bacterial numbers since the actinomycetes and fungi particularly thrive in warm dry soils. No introduced species were recorded and hence the microflora consists of autochthonous members. The microfloral count was low indicating that in all probability processes such as organic matter decomposition will be slow and all other processes involving micro-organisms in the soil will likewise be slow.

IV RELATIONSHIP OF MICRO-ORGANISMS TO SOIL PROBLEMS

A. DECOMPOSITION OF SOIL ORGANIC MATTER AND THE RETURN OF NITROGEN TO THE SOIL FROM THE ORGANIC MATTER.

1. Introduction

Plants consist essentially of proteins, sugars, starches, fats, celluloses, lignins, pectins, gums and mucilages. When plant material decomposes, the above substances are broken down in the soil and important elements such as carbon, nitrogen and phosphorus are returned. Of these elements returned to the soil by decomposition, nitrogen is of particular interest in the Mekin Sand in which total nitrogen percentage is only 0.013.

The biochemistry of the rotting of plant residues has never been studied in detail because of the lack of methods and also, rotting processes include the synthesis of microbial protoplasm. All decomposition processes of the organic matter are carried out by micro-organisms and for this reason a study of the organisms themselves would be of interest. However such a study would be too involved for purposes of the present project and so only the decomposition itself and its end results were considered.

Where decomposition is microbial there is an evolution of carbon dioxide since all heterotrophic aerobic microbial processes are accompanied by the production of carbon dioxide which can be taken as an index of the microbial activity in soil. As early as 1915 Russell and Appleyard found that the curves for bacterial numbers, nitrate content and carbon dioxide in soil were sufficiently similar to justify the view that all 3 phenomena were related; for example, a rise in bacterial numbers was accompanied by a rise in carbon dioxide in the soil air, and was

later followed by a rise in CO_2 (Russell 1950).

During decomposition various substances important to plant growth are returned to the soil.

Carbon dioxide evolution was measured as an indication of organic matter decomposition; and some estimate of the return of nitrogen to the soil from the organic matter was made.

2. Materials and Methods

(a) CO_2 evolution measurements

200 gm lots of freshly collected soil were placed in sterilised Agee Pyrex Jars. The moisture content of this soil was determined (Piper 1947) and the moisture made up to 60% water holding capacity (Piper 1947). The soil was incubated at 25°C in a constant temperature room throughout the experiment.

Each jar contained a 100 ml. beaker holding 10 mls. .1N caustic soda.

Makin sand and Waite virgin soil were used as the test soils.

(i) Various carbon and nitrogen sources were added to the soil. All treatments were triplicated.

The following substances were added to the soil.

Ammonium sulphate	4.75 ccs. of .5% solution
Sodium nitrate	15 ccs. of .5% solution
Asparagine	9.6 ccs. of .5% solution
Urea	10.65 ccs. of .5% solution
Glucose	5.0 ccs. of .5% solution

The varying amounts of nitrogenous substances were added to give equal concentrations of nitrogen.

Glucose was added both separately and in combination with the nitrogenous substances.

(ii) Sodium nitrite and potassium nitrate were added at the following concentration.

Sodium nitrite	1 cc.	of 5% solution
	2 cc.	of 5% solution
	3 cc.	of 5% solution
	5 cc.	of 5% solution
	8 cc.	of 5% solution
	10 cc.	of 5% solution
	15 cc.	of 5% solution

Potassium nitrate

	1 cc.	of 7.2% solution
	2 cc.	of 7.2% solution
	3 cc.	of 7.2% solution
	5 cc.	of 7.2% solution
	8 cc.	of 7.2% solution
	10 cc.	of 7.2% solution
	15 cc.	of 7.2% solution

Controls were set up in each experiment.

After 24 hours incubation the excess caustic soda was neutralised with .1N hydrochloric acid using phenolphthalein as indicator for (i) and (ii).

(b) Estimation of total nitrogen returned by litter to the soil.

Total leaf litter was collected from under the following plants:-

Banksia ornata and Banksia marginata

In collecting, the litter was kept separate in yearly layers as nearly as possible. The total percentage of nitrogen was estimated in this material using Pipers' method (1947). The total dry weight of total litter for 6 plants of each species was obtained by weighing the oven dried litter. Hence the average dry weight of litter for one plant was obtained.

By drying and weighing 100 leaves taken at random from each yearly layer of litter a ratio of the dry weights of the yearly litter to each other could be obtained. From this ratio the total dry weight per yearly litter

could be estimated from the total dry weight of litter per plant. From this and the nitrogen estimates the total percentage of nitrogen returned by the litter per year could be obtained for one plant.

3. Results and Discussion

The results in Tables IV and V were obtained for the Makin Sand and Waite Virgin Soil when carbon dioxide evolution was measured under given conditions.

The technique used is not as accurate as desired since there is bound to be some little gas leakage although the jars were made as air tight as possible. No fresh supply of oxygen was fed to the soil to replace oxygen removed as carbon dioxide and therefore conditions were not as balanced as could be wished.

From the results the rate of evolution of carbon dioxide was found to be slow from the Makin Sand indicating that the activity of the micro organisms was slow.

Stoklasa (1922) stated that a soil of low fertility evolved 8-14 mgms. carbon dioxide in 24 hours while a good soil evolved 58-68 mgms. CO_2/24 hours. The Makin Sand obviously lies in the category of a soil of low microbiological activity.

Waite Virgin soil has a low carbon dioxide evolution rate but if cultivated with no nutrients added and then tested it gave 40-60 mgms. carbon dioxide per 24 hours. Waite soil which was cultivated appeared to have a high micro organism activity but Makin Sand similarly treated did not increase in micro organism activity. The addition of nitrate and ammonium sulphate did not increase the micro organism activity; in fact in higher concentrations the nitrate appeared to

MAKIN SAND

Expt. I	Treatments									
	Glucose	Asparagine	$NaNO_3$	$(NH_4)_2SO_4$	Urea	Glucose + Asparagine	Glucose + Sod.nit	Glucose + ammon. sulphate	Glucose + Urea	Control
NaOH cc's neutralised	3.3	2.9	2.4	1.5	4.1	3.7	2.8	2.2	5.2	1.2
millions CO_2/24 hours	7.3	6.38	5.28	4.1	9.02	8.14	6.16	4.84	11.44	2.64
Expt. II	3.3	3.1	1.5	1.2	3.9	3.8	2.5	3.1	5.6	1.3
	7.3	6.82	3.30	2.64	8.58	8.36	5.50	6.82	12.32	2.86
Expt. III	3.2									1.2
	7.0									2.64
Expt. IV	3.2									1.2
	7.04									2.64
Expt. V	3.2									1.3
	7.04									2.86

WAITE SOIL (Virgin)

Expt. I	6.1	6.3	5.3	5.8	7.2	7.1	5.3	6.2	7.6	5.4
	13.4	13.9	11.7	12.8	15.8	15.6	11.7	13.6	16.7	11.6

TABLE V

MAKIN SAND

milligms CO_2 / 24 hours

$NaNO_2$ 5% solution	1 cc	2.2
	6 cc	1.8
	9.5 cc	2.0
	Control	1.0
5% solution	1 cc	3.1
	2 cc	3.4
	3 cc	3.2
	5 cc	3.5
	8 cc	3.7
	10 cc	3.8
	15 cc	4.0
	Control	3.2

$NaNO_3$ 5% Solution	5 cc	1.6
	7.5 cc	1.4
	15 cc	1.1
	Control	1.0
7.2% solution	1 cc	1.9
	2 cc	1.9
	3 cc	1.8
	5 cc	2.0
	8 cc	1.5
	10 cc	1.5
	15 cc	1.5
	Control	2.4

depress the carbon dioxide evolution. Urea did increase the activity of the organisms. That this increase in carbon dioxide evolution was due to an effect on the micro organisms rather than a spontaneous decomposition of the urea was proved by adding urea to a sterile soil. No carbon dioxide was evolved. Asparagine and glucose both increased the CO_2 evolution rate.

Plate counts of bacteria were made 48 hours after the addition of glucose and it was found that the bacterial population had increased to 5×10^6 bacteria per gram of soil. The addition of glucose plus urea or asparagine increased the carbon dioxide evolution rate indicating that a carbon source alone was not sufficient to increase the micro-organism activity and that nitrogen was limiting also.

Since the carbon dioxide evolution was low in the Makin Sand we assumed the micro organism activity was low. This assumption followed work by Russell and Appleyard (1915). They found that the curves for bacterial numbers, nitrate content and carbon dioxide in the soil were sufficiently similar to justify the view that all are related. A rise in bacterial numbers gives a rise in carbon dioxide followed by a rise in nitrate.

Hence in a soil such as the Makin Sand where carbon dioxide evolution was low we assumed micro-organisms to be limiting and hence activities such as decomposition of organic matter and nitrate formation were limiting.

Following work on the biological activity of the soil a report on the actual return of nitrogen to the soil from the leaf litter

by decomposition seemed logical.

The decomposition rate of any organic matter is governed by moisture, temperature and the presence of the requisite organisms. Since the climate at Dark Island Swamp is typically "mediterranean", it is unlikely that decomposition proceeds during the summer months. However during autumn, winter and spring when temperatures and moisture are favourable decomposition may be more rapid. That this is true was proved by the fact that litter present in March and April had disappeared after the June - July period. The cycle of return of organic matter to the soil was found to be an interesting one for the plants of the heath vegetation at Dark Island Swamp. Using Banksia sps as an example we found that the plants carried 3 years growth of leaves during the summer and dropped the oldest yearly leaf growth during February leaving 2 years growth on the plants. These leaf drops could be distinguished in the litter where each yearly drop of leaves existed in a quite distinct layer.

Following the method described the nitrogen return per Banksia plant could be estimated. Banksia sps. were used due to the ease with which their litter could be handled. Banksias however are not the most frequent plants of the heath vegetation as the following figures compiled by R. L. Specht show.

Analysis of heath vegetation 17th March 1950.

6 years after burning	Mean no. of plants per 22 sq. yards
Banksia ornata	2.0 ± 1.5
" marginata	1.2 ± 1.7
Casuarina pusilla	24.5 ± 4.5
Xanthorrhoea australis	10 ± 5.7

8 years after burning	Mean. no. of plants per 22 sq. yards
Banksia ornata	1.7
" marginata	2.0
Casuarina pusilla	24.4
Xanthorrhoea australis	9.3

The following figures were obtained for the total nitrogen returned per plant through the litter. The litter was collected from plants selected at random from a stand 6½ years after burning.

	Banksia ornata	Banksia marginata
Total dry weight total leaf litter per 6 plants	250 grams	690 grams
Average dry weight total leaf litter per plant	41.7 grams	115 grams
Dry weight of 100 leaves 1951-2 litter	3.944	13.25
1950-1 "	3.340	9.9

From the ratios (3.94 : 7.28 and (13.25 : 23.15
(3.34 : 7.28 (9.90 : 23.15 ,

The total dry weight of each year's litter can be estimated

	Banksia ornata	Banksia marginata
Total dry weight 1951-52 leaf litter	24.4 grms/plant	62.3 grams/plant
" " " 1950-51 " "	17.3 grms/plant	52.7 grms/plant
" % N for 1951-52 " "	.23%	.29%
" " " " 1950-51 " "	.24%	.43%

From the given figures the grms. of nitrogen returned by litter per year for 1 plant was:-

Banksia ornata	litter	1951-52	= .056 grms
	"	1950-51	= .042 grms
Banksia marginata	"	1950-52	= .186 grms
	"	1950-51	= .227 grms

The return of nitrogen from the litter for 1 plant appeared extremely small.

It was interesting to compare the total nitrogen that was present in the leaves prior to their fall. We know that the dry weight of leaves varies throughout the growth cycle of plants. The following table shows the dry weight of the whole of each year's leaves taken from 10 plants selected at random. Results given in grammes.

The data was collected by R. L. Specht, 18th March 1952.

Banksia ornata	1951-52	1950-1	1950-49
Plant No. 1	130.5	40.1	20
2	195.8	40.0	19.7
3	168.5	36.1	19.8
4	121.6	23.2	6.1
5	189.8	46.8	45.7
6	80.8	15.7	11.5
7	67.3	11.4	10.9
8	102.3	17.2	14.4
9	108.9	29.4	24.4
10	70.1	16.4	11.6

The 1950-49 leaves fell during the next February. Obviously as the leaves aged, their dry weight decreased and the 1950-49 leaves had an average total dry weight per plant of 18.5 grams. The total percentage nitrogen was .62% $\pm$ .023. This gave a total of .54 grams of nitrogen returned per plant which is nearly tenfold greater than the nitrogen returned in the fallen litter. Obviously further translocation of nitrogen takes place from the leaves before leaf fall.

When the nitrogen is returned to the soil not all is available to plants but is competed for by micro organisms which are capable of utilising available nitrogen more rapidly than plants. Not all the nitrogen will be available to plants and micro-organisms, some may be bound in the soil in an unavailable form.

Work on this subject by Stoklasa (1922) stated that in the case of a loess soil with a total of 0.150%Nitrogen, 0.04% Nitrogen was active while in a sandy soil with a total of 0.015% nitrogen all the nitrogen was active. These facts could apply to the Makin Sand where all nitrogen returned to the soil may become available for use by micro-organisms and plants.

The slow decomposition of the organic matter and the small return of nitrogen to the soil indicates that micro-organism activity in the soil is low. However micro-organism activity may not be the only factor limiting decomposition. The type of organic matter itself may affect the rate of its decomposition. The percentage of lignin may affect cellulose decomposition e.g. 15% lignin reduces the rate of decomposition of cellulose; 20-30% (as is common in wood) slows up decomposition to such an extent that they have no agricultural value as a source of humus; 40% renders the fibre extremely resistant according to Fuller and Norman (1943).

Since the Dark Island Swamp vegetation is sclerophyllous and has a high lignin and cutin content (up to 30%) the decomposition may be retarded for this reason.

However since the lignin content alone is probably only about 6% for this Sclerophyllous vegetation (Wood 1933) this explanation may not hold.

The lack of nitrogen in this Makin Sand is apparently part of a vicious cycle of slow decomposition of organic matter already low in nitrogen, due to lack of micro-organisms which in turn are hampered by lack of nitrogen. That nitrogen is necessary in organic matter decomposition is known from work reported by Russell (1951). He stated

that organic matter containing less than 1.2 - 1.3% nitrogen on a dry weight basis when rotting in the presence of ammonium salts caused some of the ammonia to be taken up and converted into organic nitrogen compounds. The rotting of nitrogen-poor remains in a soil will lower its mineral nitrogen, i.e. ammonium and nitrate ion whilst the rotting of nitrogen-rich soils will increase it. However these effects depend on the organic matter decomposing and neither the nitrogen content alone nor the ratio of the carbon : nitrogen in the material is a safe guide to the effect of the rotting process on the mineral nitrogen level in the soil. The nitrogen demands during the rotting of a material can be specified by its nitrogen factor which is defined as the number of grammes of nitrogen in the form of ammonium or nitrate ions immobolised during the decomposition of 100 gms. of the material. The nitrogen factor is not constant for the given material but depends on the conditions prevailing during the decomposition. Lack of other nutrients may retard decomposition but whether this occurs in a soil such as Makin Sand already so low in nitrogen that decomposition of substances such as lignin is extremely slow is not known. All these factors and their effect on decomposition of organic matter require further investigation.

It is established that decomposition of organic matter returned to the Makin Sand is slow and that the return of nutrients to the soil is limiting. The decomposition of organic matter and micro-organism activity are the only ways in which nutrients are returned to the soil. Nutrients required for micro-organism growth are limiting hence the cycle is inhibited at all points. The lack of nutrients, causing a low micro-organism population in turn slowing up organic matter, together with other factors existing

at Dark Island Swamp in part explains the low fertility and low nitrogen status of the soil.

B. NITRIFICATION AND PROBLEMS ARISING FROM A STUDY OF NITRIFICATION IN THE MAKIN SAND.

1. Introduction

Interest in nitrogen bacteria constituted some of the first studies in soil microbiology. Pasteur (1862) was the first to suggest that conversion of ammonia to nitrate was due to micro-organisms. This was confirmed by Schloesing and Muntz. (1877, 1878, 1879). Winogradsky (1890) commenced work on the nitrogen assimilating organisms and published a large number of articles on the subject.

Barthel discussing nitrification said, "It must be admitted as a quite outstanding fact, that the biochemical explanation of the course of nitrification which was put forward by Winogradsky over thirty years ago has been maintained almost unchanged to the present day. In actual practice all subsequent researches on this subject have only served to confirm Winogradsky's classical researches both on the morphology and the physiology of the bacteria active in the process and on the chemical progress of the nitrification." (Winogradsky "Microbiologie du Sol," Preface)

Nitrification, i.e. conversion of $NH_3 \longrightarrow NO_2 \longrightarrow NO_3$ is known to occur in the majority of soils but the rate at which nitrate is produced varies considerably. In South Australia there are certain soils having a very low total nitrogen content. Of these soils the Makin Sand has a nitrogen content as follows:

0 - 6"	-	0.032%
6 - 12"	-	0.013%
12" - 24"	-	0.007%
24 - 48"	-	0.004%

It was proposed to investigate the nitrification rate of this soil to determine if the low nitrification rate and problems related to nitrification in any way accounted for the low nitrogen status of the soil.

2. Materials and Methods

(1) Estimation of Nitrification Rate

The soil sampling technique was described in a previous section.

Fresh soil samples were collected and air dried for 48 hours, then sieved through a 4 mm. sieve.

Soil equivalent to 100 grams of oven dry soil were placed in screw top bottles, and the moisture content adjusted to 60% water holding capacity (Piper 1947). The soils were thoroughly agitated and then incubated at 25°C in a constant temperature room. Series were set up allowing 3 replicates to be sampled per sampling day when the nitrate or nitrite was estimated as the experiment demanded. From each bottle of soil, duplicate estimations were made. Various experiments were carried out using this basic method. Waite virgin soil was always used as a control.

(a) Experiment 1

Ammonium sulphate was added at the rate of 100 ppm of nitrogen. Calcium carbonate to give a final concentration of 1% was added together with ammonium sulphate.

(b) Experiment 11

Potassium nitrate and sodium nitrite were each added at the rate of 100 ppm nitrate and nitrite nitrogen respectively. The levels of nitrite and nitrate were estimated at regular intervals.

(c) Experiment 111

Sodium nitrite at 100 ppm nitrite nitrogen was added to Makin Sand with

various carbon sources. Carbon sources were added to give 1000 ppm of carbon. Carbon sources added were glucose, sucrose, maltose and glycerol. The nitrite and nitrate levels were estimated at regular intervals.

(d) Experiment IV

Sodium nitrite at 100 ppm nitrite nitrogen was added to autoclaved Makin Sand and the nitrite level followed at regular intervals.

(e) Experiment V

Sodium nitrite at 100 ppm nitrite nitrogen was added to Makin Sand together with the following enzyme inhibitors at 10^{-3}M concentration. Sodium azide, sodium iodoacetate and chloroform, and 8 hydroxy quinoline at 10^{-3}M concentration was added because of its chelating powers. Nitrite and nitrate levels were estimated regularly.

(f) Experiment VI

To an actively nitrifying sample of Waite Garden Soil 10 cc of an extract of Makin Sand was added. The extract was made by shaking 100 grms of soil with 100 ccs of water for 48 hours. One extract was Seitz filtered and another was autoclaved before addition to the soil. A 10 gm. sample of Makin Sand was added to 100 gms of the Waite garden soil. After incubation at 25°C and at 60% water holding capacity the nitrate level was estimated at regular intervals.

(g) Experiment VII

Makin Sand was treated with concentrated hydrogen peroxide to destroy the organic fraction washed thoroughly and sodium nitrite added at 100 ppm nitrite nitrogen. Another sample of soil was ignited in a furnace and then had sodium nitrite added. A further sample of soil was treated with

strong caustic and after thorough washing, sodium nitrite added. The nitrite level was estimated at regular intervals.

2. Method of estimation of nitrate in the incubation series.

The method used was after Lewis (1950) (Private communication).

Reagents

Concentrated sulphuric acid (5 + 1)

Added 5 volumes of pure sulphuric acid to 1 volume of distilled water.

Xylenol solution

Made a 1% solution of xylenol (3 : 4 xylon - 1 - ol) using acetone as solvent.

Caustic Soda Solution - approximately N/5

Dissolved 8 grams of sodium hydroxide pellets in a litre of distilled water.

Silver Sulphate Solution

Dissolved 5 grams of pure nitrate-free silver sulphate in 60 ml. of concentrated ammonia. Boiled off excess ammonia and diluted to 100 ml with water.

Apparatus

A glass distillation unit with a standard ground glass joint was used. To the outlet of the double surface condenser was fused a piece of glass tubing long enough to reach to the bottom of the 100 ml. collecting flask and the tube connecting the boiling flask to the condenser was also fused to the inlet of the condenser so that the only detachable joint was that between the boiling flask and the unit.

Method

To 15 ml. of nitrate solution (containing less than 200 micrograms of nitrate nitrogen) in a 500 ml. boiling flask added 50 ml of the sulphuric acid (5 + 1), swirled until thoroughly mixed and then placed in a waterbath

maintained at 20 - 25°C. If the solution contained more than 10 micrograms of chloride excess silver sulphate was added. When the contents of the flask had reached the temperature of 20 - 25°C the flask was removed from the bath, 1 ml of the xylenol reagent was added, thoroughly shaken and allowed to stand on the bench to nitrate for 20 - 25 minutes, then 150 ml of water was added and the nitroxylenol distilled over into 10 ml of the caustic soda solution (N/5) (contained in a 100 ml volumetric flask) until 80 - 85 mls of distillate had been collected. The condenser water was run out and the steam allowed to remove any xylenol which may have solidified in the condenser. The solution was allowed to cool to room temperature, diluted to the mark and mixed thoroughly. Within 2 - 6 hours of distillation the light transmission of the solution was measured in a photo-electric colorimeter using Ilford colour filter 1601.

Standard curves prepared by Lewis using this method gave a straight line curve. The method was sensitive to small amounts of nitrate.

For each series of estimations a blank and appropriate standard were used.

The colorimeter readings were read off from standard graphs to give \ / ml of solution which were then converted arithmetically to ppm.

3. Method of Estimation of Nitrite

Reagents

1. 0.5% sulphanilamide in 1 : 1 HCl (stored in a refrigerator). Used 2 ml per 100 ml solution.

2. 0.1% N (naphthl) ethylenediamine dihydrochloride in water- stored in a dark bottle. Used 1 ml per 100 ml.

Method

Took a sample of nitrite solution containing less than 20 γ NO_2'N in

100 ml volumetric flask and diluted to 90.95 ml. Shook to mix and added 2 ml of sulphanilamide solution and allowed to stand in a cool place away from direct sunlight for 5 minutes then added 1 ml of N (1 naphthyl) ethylene diamine dihydrochloride solution and allowed to stand for 10 minutes while colour developed. The colour developed remained stable for about 140 minutes after which slight fading began.

Read the percentage transmission in a colorimeter using distilled water as 100% transmission using a green filter such as I 605.

The following are the figures for a standard curve obtained by the above method.

Solution	%Transmission	Average	No_2 N
1	99.15)	99.18	Blank
2	.20)		
3	91.81)	91.88	.5 gm
4	.89)		
5	85.10)	85.09	1.0
6	.08)		
7	73.40)	73.42	2.0
8	.43)		
9	59.11)	59.08	3.5
10	.05)		
11	47.72)	47.77	5.0
12	.82)		
13	41.03)	41.31	6.0
14	.59)		
15	34.00)	33.98	7.5
16	33.96)		
17	29.84)	29.74	8.5
18	.63)		
19	24.10)	23.93	10.0
20	23.75)		
21	47.50)	47.51	5.0
22	.51		

This gave a graph which is not quite a straight line but no better method could be evolved. The portion of the curve used was a straight line.

3. Results and Discussion

The nitrification rate for the Makin Sand is compared in Graph 1 with that of Waite virgin soil. The figures obtained by addition of ammonium sulphate and calcium carbonate are plotted on the same graph.

The results obtained were as follows:-

Makin Sand (Keith soil)

NO_3N in ppm

Time (in days)	Control	Ammonium sulphate added	Ammonium sulphate + calcium carbonate
0	2	-	3
10	2	-	19
20	2.5	2	38
30	5	4	52
40	10	6	72
50	12	9	not done
60	13	10	" "
70	14	11	" "

Waite Soil

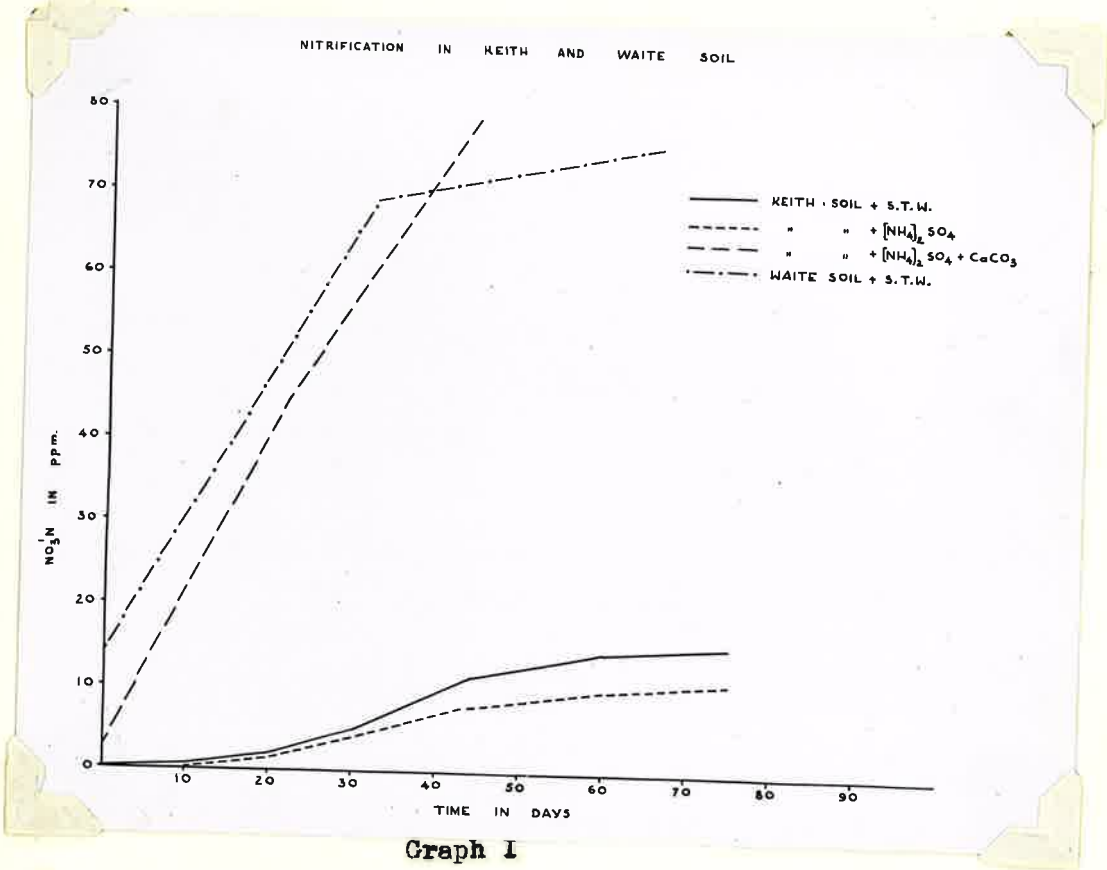
NO_3'N in ppm

Time in days	Control
0	14
20	43
30	65
40	70.5
60	72.1

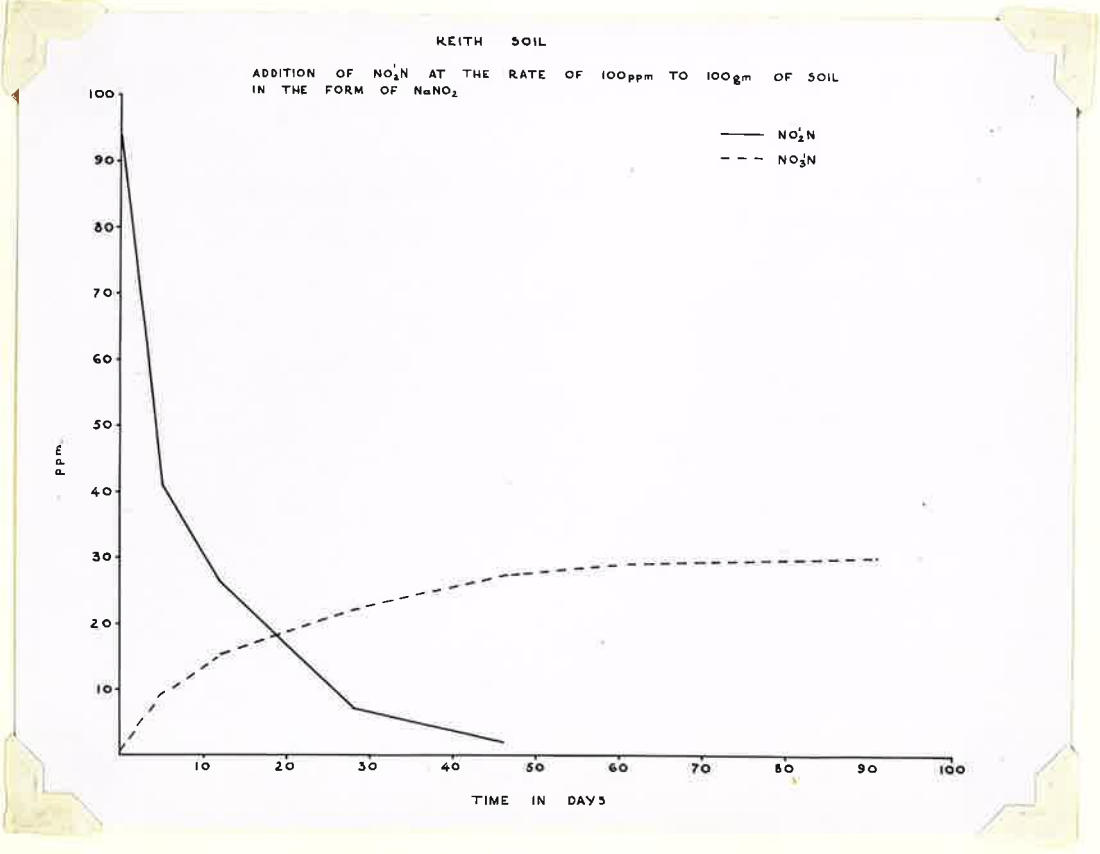
These results were after incubation at 25°C and at 60% water holding capacity.

The addition of ammonium sulphate did not give an increase in nitrate nitrogen but rather caused a decrease in nitrification. However the addition of ammonium sulphate together with calcium carbonate caused a rapid rise in nitrification. The effect of ammonium sulphate on nitrification may be explained as follows.

Following addition of ammonium sulphate to the soil a base exchange



Graph I



Graph II

reaction takes place; NH_4^+ ions enter the exchange complex and an equivalent amount of base usually calcium is displaced. This displaced calcium then washes out of the soil in association with the SO_4' ions introduced in the ammonium sulphate. If the ammonia is nitrified the nitrate produced is neutralised once again by calcium. If the nitrate is taken up by a plant or utilised by micro-organisms the calcium is free to enter the system again. If the nitrate is leached out the calcium is also leached. Hence it can be seen that it is possible to deplete the soil quite seriously of calcium. So long as the soil contains a reserve of calcium carbonate the loss of calcium is not serious since the calcium is taken from the excess reserve and not the exchangeable calcium. In the Makin Sand however where there is no excess calcium carbonate, replaceable calcium is removed from the soil. The exchangeable calcium in the Makin Sand is extremely low (2 Me%) and its depletion is therefore rapid. After serious loss of exchangeable calcium the soil becomes acid. It is this acidity which probably affects nitrification and causes a decline in rate of nitrate production. Nitrification functions best between pH 6 - 9. The Makin Sand has a pH of 5.9 - 7. With the addition of ammonium sulphate and the lack of calcium the pH is probably lowered to a point below that favourable to nitrification.

That this state of affairs held for the Makin Sand was borne out in part by the results obtained when calcium carbonate was added with the ammonium sulphate.

The addition of calcium carbonate increased the free calcium content of the soil which affected the pH and probably also acted in the following manner. Bacteria concerned in nitrification in pure culture grow in a film

around the calcium carbonate particles. Hence the addition of free calcium carbonate to the soil may provide a favourable medium for the growth of these bacteria.

The nitrification rate was shown to be low in the Makin Sand and the addition of ammonium sulphate and calcium carbonate increased the production of nitrate. Whether lack of raw materials necessary for the process was the only limiting factor was not known.

Attempted isolation of the organisms concerned in nitrification showed that their numbers in the soil was low but this of course may be governed by the lack of nitrogenous materials necessary for their growth. Whether any biological or chemical block to the nitrification process was present in the soil was not known. For this reason experiment VI was carried out. However the addition of extracts of Makin Sand and Makin Sand itself, added to an actively nitrifying sample of Waite garden soil did not cause any decrease in the nitrification rate of the Waite soil.

The lack of raw materials apparently affected the bacterial population thus slowing down nitrification. Since the Makin Sand is limiting in copper, zinc and phosphorous, their addition would enhance nitrification further. This was found by Lewis (private communication) for Seddon gravelly sandy loam, a soil of poor fertility on Kangaroo Island and by Lees and Meikeljohn (1948 (1); 1948 (2)) for English soils.

While adding various nitrogenous substances to Kangaroo Island soils Lewis (private communication) noticed that when nitrite was added to his soils, after given intervals, he could no longer estimate nitrite and the nitrate present did not indicate that there was a corresponding conversion of nitrite to nitrate.

When nitrite was added to Makin Sand the same phenomenon was noticed. The following results were obtained:-

ADDITION OF NO_2'N AT THE RATE OF 100 ppm TO 100 GMS OF AIR DRIED SOIL IN THE FORM OF $NaNO_2$

Time	Keith Soil (Makin Sand)		Waite Institute Virgin Soil	
	NO_2'N ppm	NO_3'N ppm	NO_2'N ppm	NO_3'N ppm
Original value	93.8 ppm to oven dried soil	.2	90.8	.5
5 days	41.2	9.1	13.8	16.0
12 days	26.2	15.2	.02	24.4
28 days	7.4	22.1	.06	26.9
46 days	2.3	27.1	.06	37.1
61 days	-	29.1	-	43.4
91 days	-	30.3	-	70.9

These results are plotted to give graphs II and III. This experiment was repeated six times and each repetition gave replicas of the above results to an accuracy of $\pm$ 5%.

It was proposed to investigate whether the phenomenon of nitrite utilisation was due to micro-organisms. The method for estimating nitrite was first checked carefully. We assumed that if the nitrite was being utilised by micro-organisms and was not being converted to nitrate, denitrification may be taking place. If this were so then nitrate added to the soil under the same conditions of moisture and temperature should be utilised by micro-organisms.

Potassium nitrate was therefore added at the rate of 100 ppm NO_3'N to 100 gms. of air dried soil which $\equiv$ 95.2 ppm to 100 gm of oven dried soil. The following results were recorded.

ADDITION OF KNO_3 TO SOIL AT THE RATE OF 100 ppm OF NO_3'N

Time	Keith	White Institute Virgin Soil
	NO_3'N ppm	NO_3'N ppm
Initial	95.2	92.2
5 days	98.0	98.1
12 days	98.9	112.7
28 days	102.0	134.8
61 days	104.6	171.0

The results when plotted gave graph IV. It can be seen that the potassium nitrate can be measured even after 60 days. The process by which the nitrite was being utilised did not appear to be denitrification.

Still assuming that the process was due to micro-organisms several experiments were set up simultaneously. These were experiments III and IV. In experiment III various carbon sources were added together with sodium nitrite on the assumption that if the action was due to micro-organisms the addition of a readily available carbon source would increase the rapidity with which the nitrite was utilised.

The following are the results for these experiments.

ADDITION OF 100 ppm NO_2'N AS $NaNO_2$ TO 100 GMS OF AIR-DRY SOIL AND 1000 ppm of C AS GLUCOSE

Time	Keith Soil		Waite Institute Virgin Soil	
	NO_2'N ppm	NO_3'N ppm	NO_2'N ppm	NO_3'N ppm
Calculated value	93.8	Orig. .2	90.8	0.5
½ hour	92.4	-	79.7	-
4 days	67.8	3.77	5.25	19.69
14 days	22.2	22.4	.07	23.1
28 days	12.7	27.1	.03	18.8
51 days	-	26.5	-	37.9

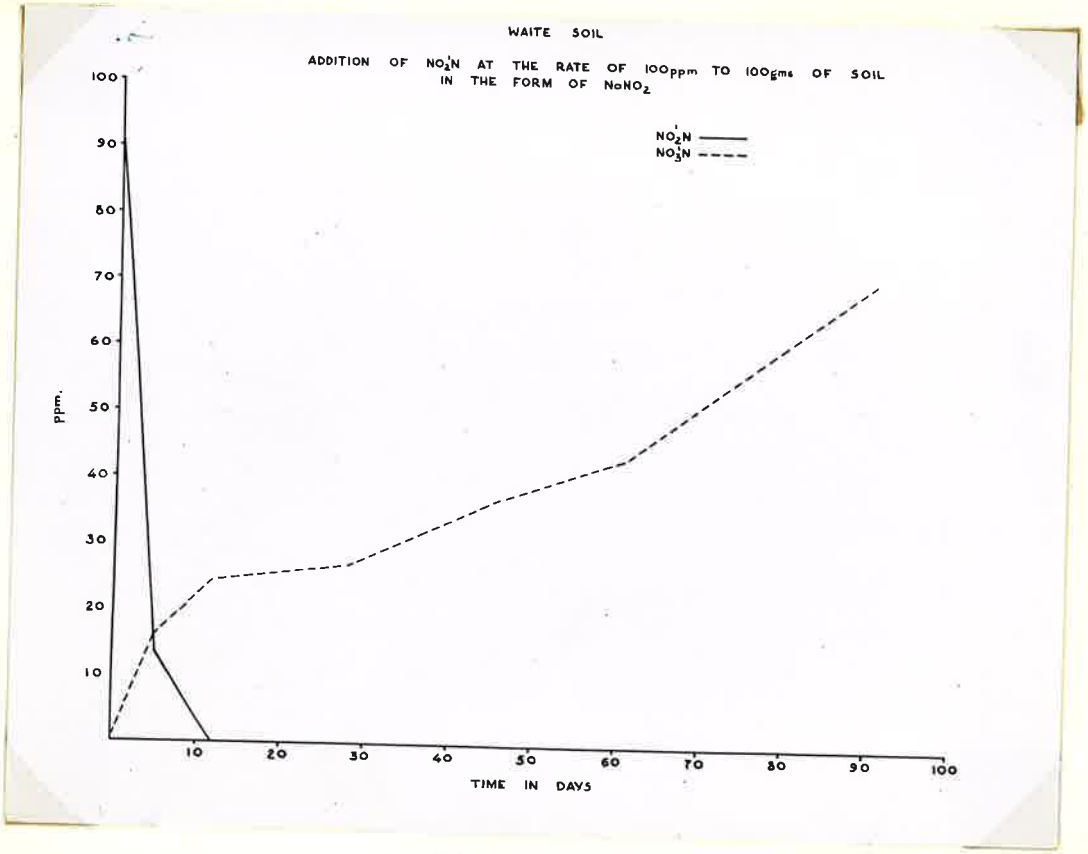
These results are plotted to give graphs V and VI.

Results for the other carbon sources were:

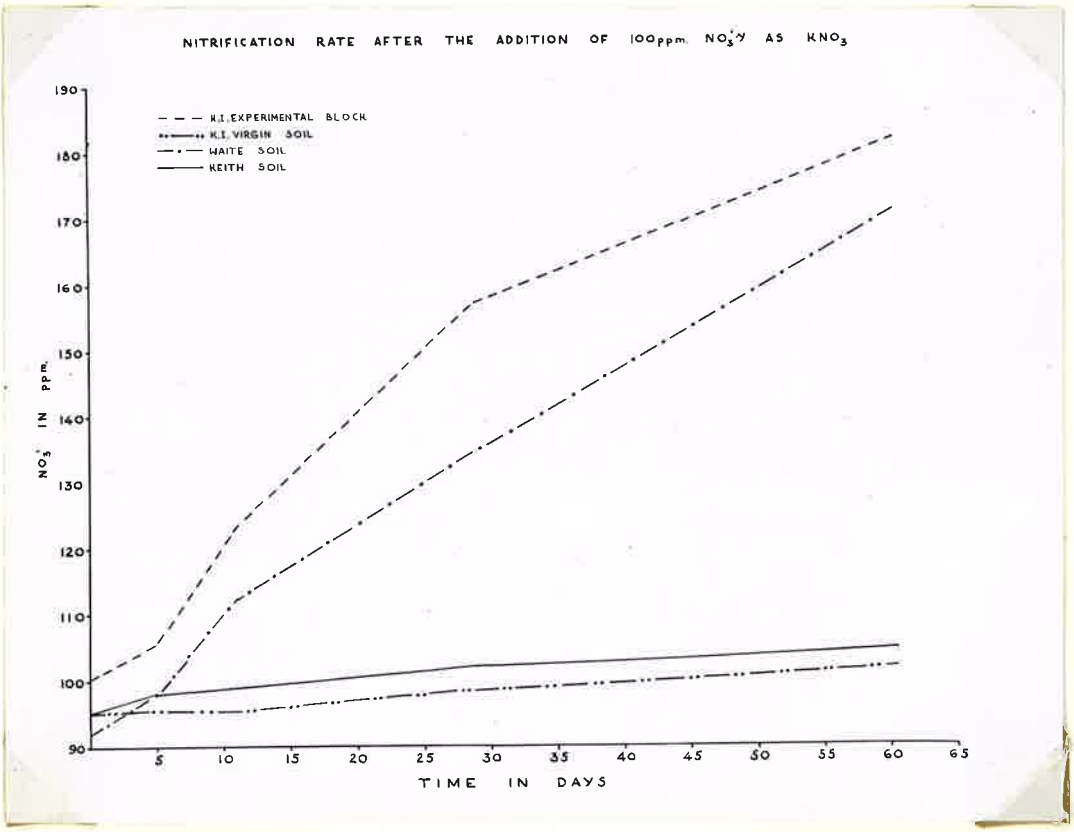
MAKIN SAND

Time in days	Sucrose ppm		Glucose ppm		Maltose ppm		Glycerol ppm	
	NO_2'N	NO_3'N	NO_2'N	NO_3'N	NO_2'N	NO_3'N	NO_2'N	NO_3'N
0	91.7	.2	91.3	.2	89.9	.2	90.3	.2
2	72.3	-	72.3	-	72.0	-	72.9	-
6	45.9	10.2	43.3	10.8	41.2	11.6	46.6	9.6
14	15.1	20.5	13.6	19.8	15.9	21.4	4.7	18.2

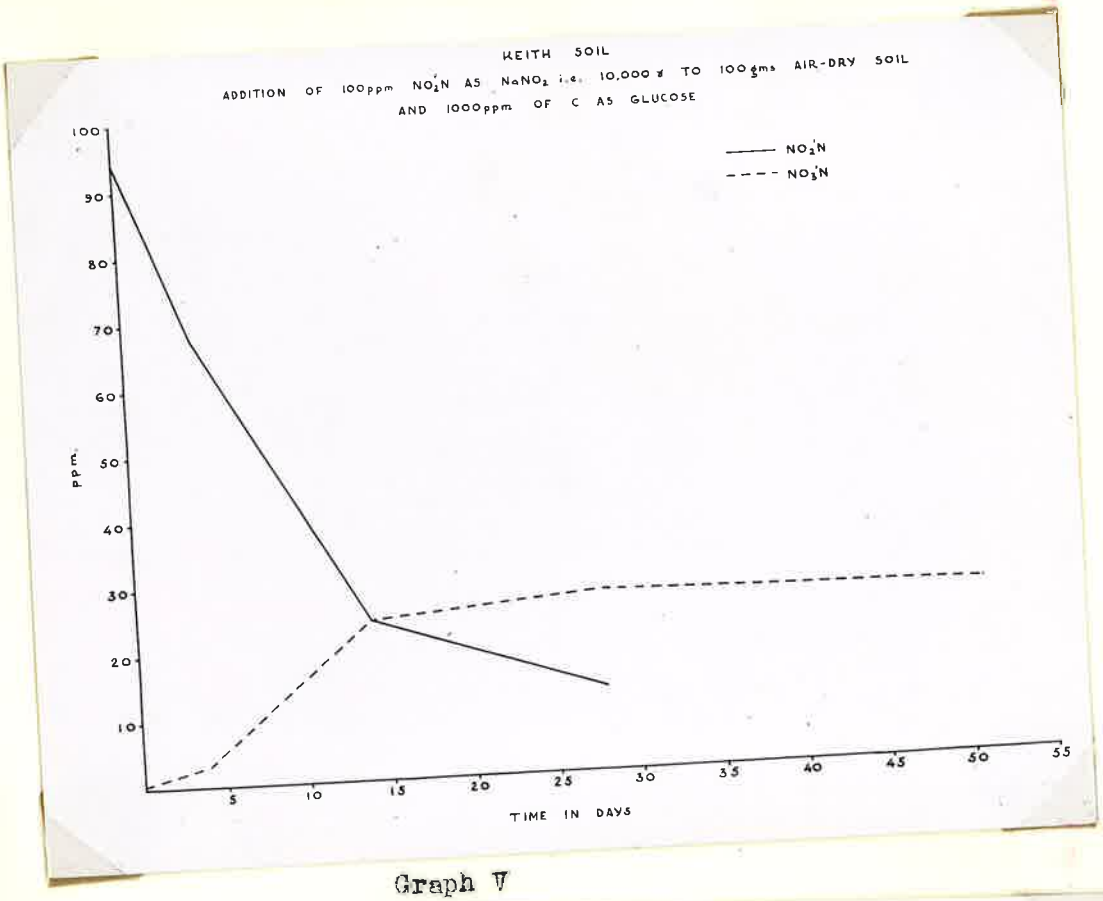
Comparing these results with the control curve plotted to give graph II it can be seen that the addition of these carbon sources did not increase the rate with which the nitrite was utilised.



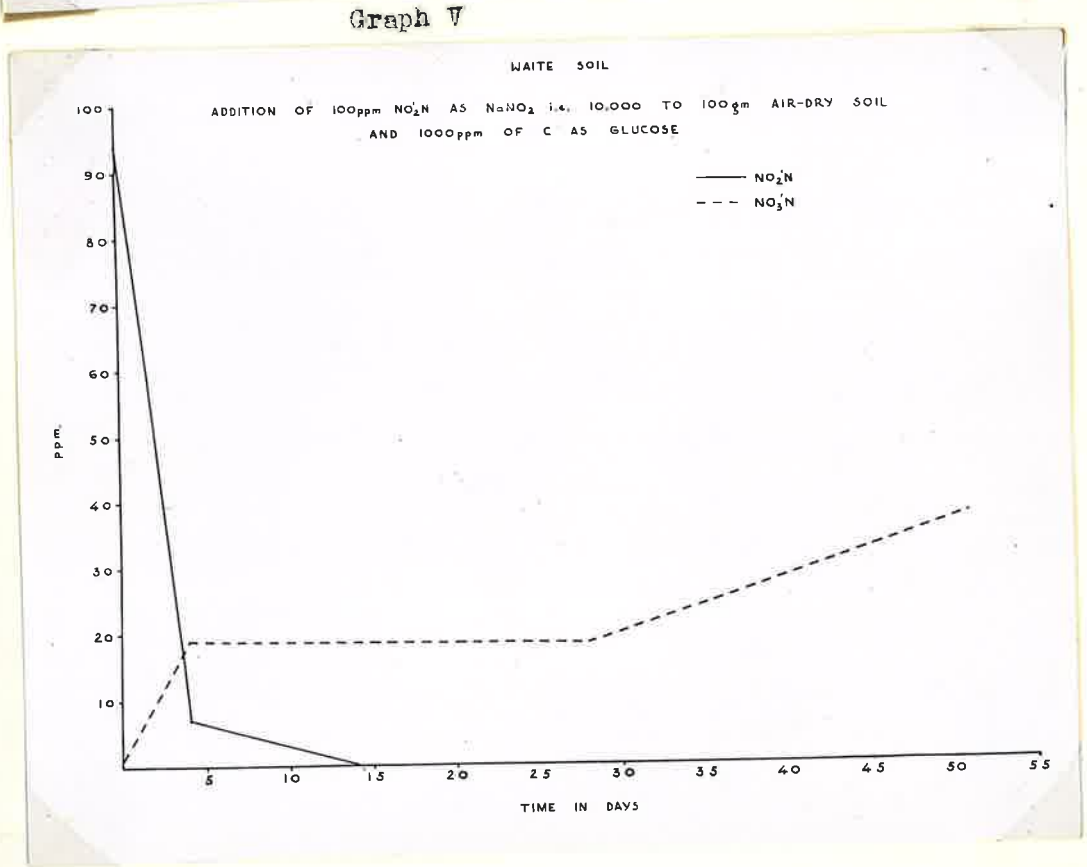
Graph III



Graph IV



Graph V



Graph VI

It did not appear that the nitrite loss was due to micro-organisms. The addition of nitrite to an autoclaved sample of soil proved fairly conclusively that the nitrite was not being used by micro-organisms. The results obtained were as follows.

ADDITION OF 100 ppm NO_2'N AS $NaNO_2$ TO 100 GM OF AIR-DRY AUTOCLAVED MAKIN SAND

Time in days	NO_2'N ppm	NO_3'N
0	93.8	.2
5	43.1	10
12	25.1	16
28	7.9	21.5
46	3.1	28.2

The results are similar to those plotted to give graph II.

To further substantiate evidence that the utilisation or loss of nitrite was not due to micro-organisms experiment V was carried out. Enzyme inhibitors added at 10^{-3} M concentration together with sodium nitrite gave the following results:

100 ppm NO_2'N + ENZYME INHIBITORS AT 10^{-3} M CONCENTRATION TO KEITH SOIL MAKIN SAND

Results expressed as ppm NO_2'N

Time (in days)	Chloroform	sod. iodo acetate	sod. chlorate	8 hydroxy-quinoline	Control
0	93.8	93.8	93.8	93.8	93.8
4	73.1	18.1	53.9	55.3	71.8
11	38.9	.75	17.7	31.0	33.4

These results are plotted to give Graph VII.

With the exception of sodium chlorate all the inhibitors gave an apparent enhancement of the nitrite utilisation rather than a depression as would be expected if the nitrite were being attacked by micro-organism.

It appears then from the results that the nitrite is being utilised in a chemical reaction rather than in a reaction concerning micro-organisms.

Experiment VII in which Makin Sand samples were (1) Ignited (2) washed with peroxide and (3) washed with caustic soda and then had nitrite added gave the following results

TREATMENT Results as ppm NO_2'N

Time (in days)	Ignited	Peroxide washed	Caustic washed	Control
0	101.2	101.2	101.2	93.8
3	101.8	101.6	101.1	89.8
7	101.3	101.1	101.0	69.8

The results indicate that there is some reaction between nitrite and the organic fraction of the soil.

The reported nitrite behaviour need not necessarily mean that the nitrite is lost to the soil altogether. The nitrite may simply be tied up for a transitory period and then released to enter into the nitrogen cycle again. However the problem of this nitrite behaviour is an interesting one and several suggestions are made to account for it. Further work would prove interesting on this subject.

Temple (1914) reported that nitrites were decomposed by an acid soil with formation of nitrous oxide. Similarly Turtschin (1936) found that acid soils react with nitrites to produce gaseous nitrogen compounds, with consequent losses of nitrogen. Fraps and Sterges (1939) observed

that even when they added 1% $CaCO_3$ to acid soils nitrite was still lost. Franklin, Allison and Doetsch (1950) have suggested that loss of nitrogen gas may occur in acid soils by:-

(1) volatilization of compounds such as ammonia, nicotine, and possibly oxides of nitrogen.

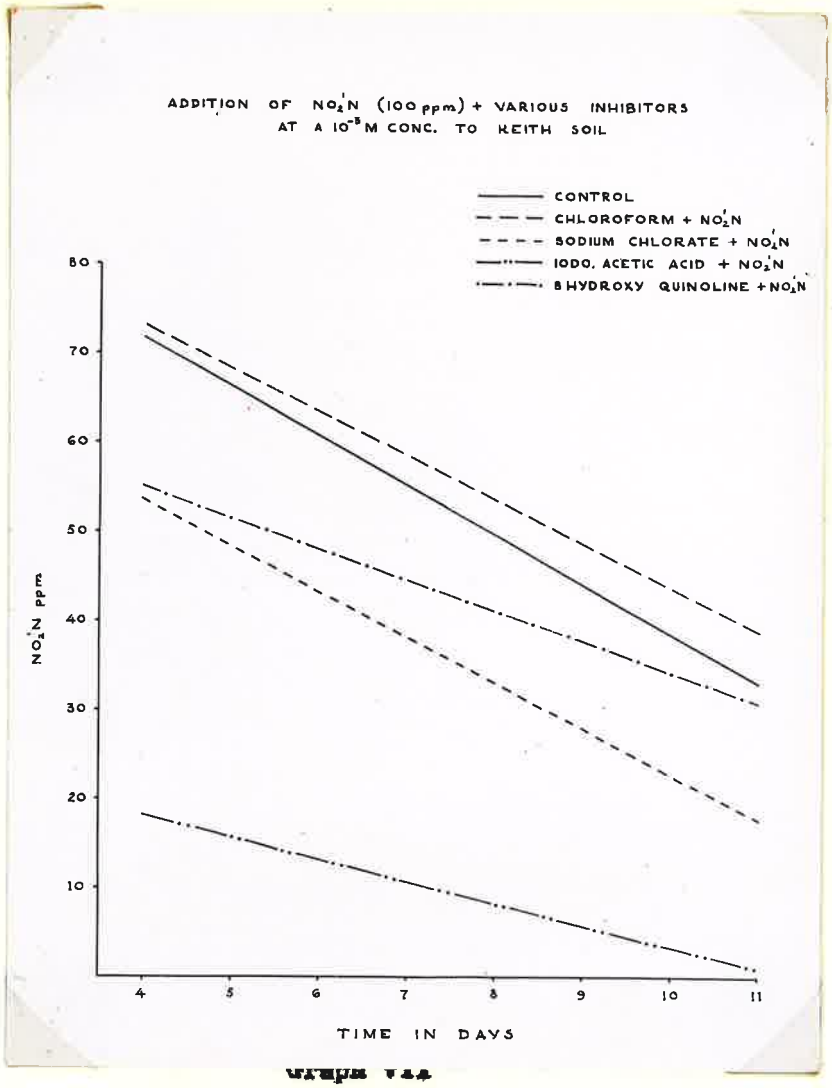
(2) formation of elemental nitrogen through the reaction of denitrifying bacteria.

(3) interaction of nitrous acid with ammonia or a-acids with the resultant gas being given off.

They claimed these reactions took place only up to a pH of 5. The pH of the Makin Sand ranged between 5.8 - 7.5 and conditions do not appear to be sufficiently acid to allow the above reaction to take place. Denitrification does not appear to be the explanation as previously discussed.

No measurements were made of gases evolved due to technical difficulties and lack of time to verify any of the above suggestions.

It was suggested that nitrite may be reacting with ascorbic acid to give oxides of nitrogen. However it is not known as yet whether there is any free ascorbic acid in the soil and if it is present what quantity exists. Shorey (1930) reported that nitrogenous substances were involved in the break-down of lignin to humic acids. He considered that lignin decomposition involved (1) an oxidation (2) a process during which enrichment in nitrogen occurred. This process is of interest where we are considering a soil such as the Makin Sand which is high in lignin and cutin. It was reported that in an alkaline medium, in the presence of ammonia some of the ammonia was converted into a part of the new oxidation product, although the way in which the nitrogen was linked


ADDITION OF $NO_2'N$ (100 ppm) + VARIOUS INHIBITORS
AT A 10^{-5} M CONC. TO KEITH SOIL
CONTROL
CHLOROFORM + $NO_2'N$
SODIUM CHLORATE + $NO_2'N$
IODO. ACETIC ACID + $NO_2'N$
8 HYDROXY QUINOLINE + $NO_2'N$
$NO_2'N$ ppm
80
70
60
50
40
30
20
10
4
5
6
7
8
9
10
11
TIME IN DAYS

Graph VIII

up was not known.

Mattson and Anderson (1943) have shown that the reaction was not confined to lignin but applied to polyphenols, resorcinol, hydroquinone, gallic and tannic acids in much the same way.

Further Laatsch (1948) has shown that actinomycetes in particular, but fungi also, produced quinone and related compounds that behave much like lignin to give on further autoxidation in the presence of amines and amino acids, substances very similar to soil humus. These conversions were usually performed by micro-organisms in the course of their metabolism. Finally Reesema (1942) has shown that autoxidised nitrogen-rich humic like compounds are very resistant to decomposition in the soil. (Russell 1950).

Whether any of these reactions cited above offer an explanation of the free nitrite loss in the soil needs further investigation.

The Makin Sand is not the only soil showing the loss of free nitrite since the phenomenon was shown by Waite and Kangaroo Island Soils. It is probable that other soils show this phenomenon.

V. SUMMARY

It has been established that the Makin Sand which is a soil of relatively low fertility supported a microflora in which fungi and bacteria occurred in comparable numbers (of the order of 1×10^6 orgs/gm of soil) and the actinomycete population was high by comparison. These conditions also apply to all sandy soils of low fertility so far investigated throughout the world.

Since the soil under study was one of low nitrogen status the nitrogen cycle in the soil has been of particular interest. Organisms responsible for nitrification were low in numbers and no organisms capable of fixing nitrogen were isolated. The decomposition of organic matter returned to the soil was very slow; each year's litter taking up to three years to be decomposed. The nitrogen returned to the soil from this litter was low; 0.05 gms / plant of Banksia ornata. The nitrogen returned to the soil being limited is rapidly used by micro-organisms and plants and hence the cycle is inhibited at all stages.

Nitrification within the Makin Sand was extremely slow but could be increased by the addition of $CaCO_3$ and nitrogenous substances. Substances such as phosphorus, copper and zinc compounds also increased nitrification. The effect of all these substances was to act upon the micro-organisms involved and to increase their numbers.

While adding nitrogenous substances to the soil to test their effect on nitrification it was found that if nitrite was added no nitrite could be detected after 14 days but there was no corresponding increase in nitrate. The nitrite was not removed by denitrification and the removal was not due to micro-organisms and was confined to the organic fraction of the soil.

The pH of the soil does not favour any interaction with amino acids or the loss of nitrogenous substances by way of ammonia or oxides of nitrogen. A suggestion has been made that the nitrite may combine with polyphenols and may even be combined in an unavailable form. The nitrite loss provides a problem for further work. It is interesting that this nitrite phenomenon also occurred in Waite and Kangaroo Island Soils.

Since the Makin Sand was already low in nitrogenous substances, micro-organisms were low in number and hence processes involving micro-organisms were slow and limited. Nitrification and decomposition of organic matter was slow in the Makin Sand and hence nitrogenous substances were limited.

All these factors help to account for the low nitrogen status of the Makin Sand and help to explain its low fertility. Problems suitable for further work have arisen from the present study. The study also provides microbiological information toward the completion of the studies on the heath vegetation commenced by the Botany School.

VI ACKNOWLEDGMENTS

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