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# Some edaphic factors related to the distribution of flora (including soil fungi) along the bank of the hiver wear at Shincliffe, county Durham

Wong, Ming-Hung

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WONG Ming-Hung, B.Sc.

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Thesis submitted as part of the requirements for the Degree of Master of Science in Ecology in the University of Durham. September, 1969.

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### Introduction

The environment of a plant is the sum of all external factors and substances which affect its growth, structure, and reproduction.

Plant distribution is primarily controlled by the interaction of climatic and edaphic factors. It would appear that in the life history of organisms there are times when it is in some critical phase of its development which results in a narrow tolerance range for a particular factor in the environment. The narrower the range of tolerance, the more critical the factors become. Such factors become controlling according to their duration, summation or extremes, and any individual factor either of climatic or edaphic origin may in itself become limiting to one or more species. Thus any one factor or combination of several factors may serve to restrict the Fange of a single species, group or association. Edaphic factors are most likely to show sharply defined patterns covering over small areas.

Under natural conditions a plant cover develop under the balance of physiological and ecological conditions with climatic and edaphic conditions. However, the chemical differences in the soil may produce a marked change in the vegetation even within fairly uniform climatic areas.

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In general, major plant community groups are often closely correlated with the developed soil type and independent of such factors as physical texture of the soil. The smaller or minor communities are often directly affected by such factors.

Soil factors become controlling according to their duration, summation or extremes, and any individual factor either of physical or chemical origin may in itself become limiting to one or more species. Therefore we must understand what influences seems to be dominant or in what phase in the plants' development the individual factors assume a leading or limiting influence, and what combination or summation of the environmental entities become significant, both for single species and for group or association.

As there are four obvious zones of vegetation along the slopes of the river bank at Shincliffe, a vertical gradient of edaphic factor is expected.

This study attempts to interpret the distribution of higher plants and fungi in terms of soil factor variation. For this purpose, several physical and chemical characteristics of the soil were examined.

#### Review of Literature

Billings (1951) studied the vegetation of the Great Basin of western North America, and concluded that large vegetational zones were connected with climatic and soil, the mosaic of smaller vegetational differences within each zone may be caused by edaphic factors, topography, or successional stages of the vegetation itself.

In the study of soil-vegetation associations of northern Tanzania, Africa, Anderson and Talbot (1965) found that wind erosion and soil depth, texture and salt concentration, all of which affect moisture availability, largely determine the grassland patterns. Levels of available nitrogen, manganese, and magnesium play a minor role.

Loach (1966) in North-east Hampshire, noted three adjacent and closely related plant communities in Bramshill Forest: each community was restricted to certain areas according to the levels of nutrient and water table.

Van der Maarel (1966) studied several dune and salt marsh communities and discovered that the sharp boundaries between communities were related to variation of pH, soil moisture and topography.

West and Kamal (1968) in south-eastern Utah, found

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that the discontinuity between four distinct plant communities is correlated with edaphic discontinuities.

In the study of vegetation in Upper Teesdale, Marshall and Bridgewater (1969) stated that the boundaries of the plant communities was found to be correlated with simple edaphic factors, water holding capacity, Ca<sup>++</sup>, Na<sup>+</sup>, Mg<sup>++</sup> as total concentrations in the soil, and the organic content of the soil.

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### Description of Study Site

The study site was on the west bank of the River Wear near Shincliffe, about one and a half miles from Durham City. The area, which belongs to the Durham Agricultural College, is at the edge of a ploughed field. The slope is about 45°, facing east. Four zones of vegetation, each dominated by one or more oharacteristic species can be seen easily.

In this report the zone nearest the river is described as zone 1, the adjacent one zone 2, the next zone 3 and the highest zone 4.

Marked rises of the river level usually occurs in raining seasons and especially during snow thaw, causing flood waters to reach the top of zone 2. This is shown by the accumulation of alluvial material in these zones.

In general, the weather and river conditions are correlated and are comparatively unsettled throughout the summer, the river falling to its lowest level in the autumn (Fifteenth Annual Report of Wear and Tees River Board 1965).

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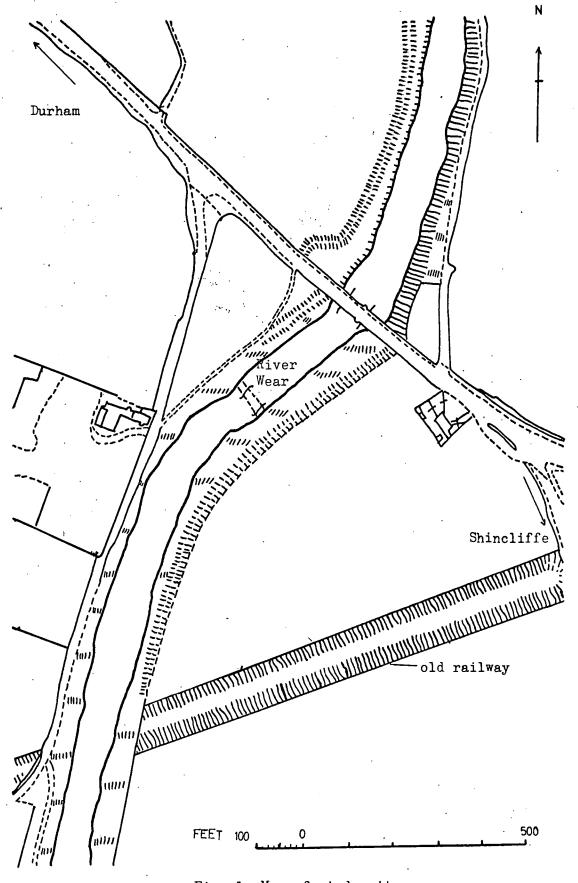
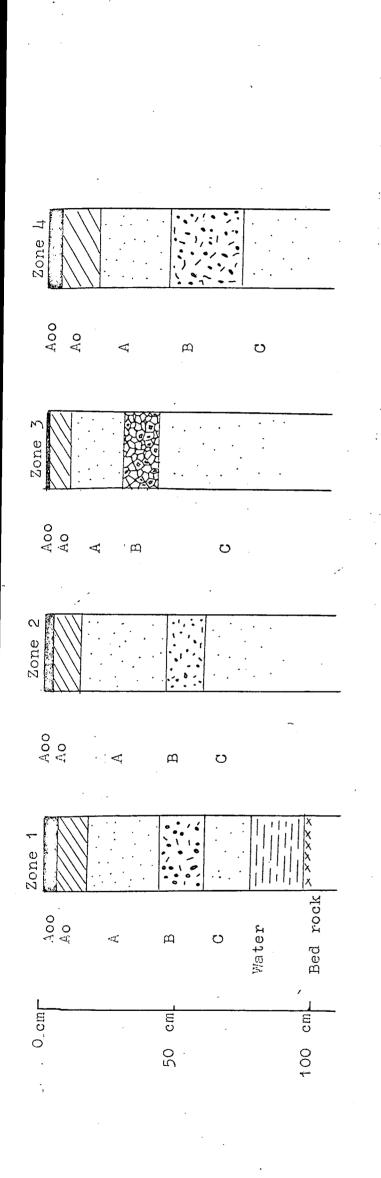


Fig. 1: Map of study site.

	Table 1 : Results of edaphic	HKA 80.1 3.14 16.76 19 B 80.2 0 19.8 14 C 77.46 3.09 19.45 15 Ave 79.25 2.07 1.8:67 16₀	85.5 1.037 84.82 0 82.71 4.07 84.34 1.705	61.79 2.02 16 79.84 0 20 87.8 3.02 9 83.14 1.68 15	86.9 3.03 10.07 88.84 2.02 9.14 85.85 2.02 13.14 87.19 2.02 10.78	MECHANICAL ANALYSIS SAND, CLAY, SILT,	
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Aco Leaf and litter layer highly developed.	Dark humified organic material.	Greyish brown sand of loose texture.	Dark and yellowish brown highly enriched layer, with vast amounts of earth worms, insects and dead roots.	Greyish brown silty sand.
Ac	AO	V.	р Д	σ
This layer is the thinnest among 4 zones.	Compacted sandy soil.	Dark brown with light brown sandy soil, showing a high degree of compaction.	Yellow brown with orange brown compacted layer, a clear transition to next horizion is shown.	Dark brown with light brown clayish sand.
A00	Аo	, W	ъ	Ö
Aoo Litter layer.	Dark humified layer, similar to Zone 1.	Light greyish brown sand of loose texture.	Dark brown, highly humified, rocks and dead roots distributed regularly through out the horizon.	Light yellowish grey sand of loose texture.
Aoo	A0	A	ф	Ö
o Leaf and litter layer mixed with sand.	Dark humified layer mixed with bleached sand grain.	Humus incorporated grey layer, with many roots.	Very dark brown (nearly) black under poor drain- age condition), with plenty of rocks and rotten roots.	Parent material, light, Creyish brown sand of loose texture.
o Leaf an mixed w	Dark humifi mixed with sand grain.	Humus ir grey lay roots.	Very dark bro black under p age condition plenty of roc rotten roots.	Farent <i>C</i> reyish Loose t

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Fig. 2: Soil profiles, diagrams and descriptions.

## Analysis of Soil Factor

I. <u>Method</u> Details of the method are contained in Appendix I.

## II. Results

The results of soil analysis are represented in Table 1. Analysis of variance were calculated for the average value of each soil characteristics from 3 depths (surface, 30 and 80 cm.).

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#### A. Physical Character

### 1. Soil Profile

The soil profile of each study zone (Fig. 2 to 5) depicted the morphological characters of soils. The number of horizons were found to be the same in different zones, and most of them are similar in their origin and development. As zone 1 is near water level, the condition of poor drainage caused much black humified material to develop in the B horizon. A similar condition was found in the adjacent zone 2 but to a lesser degree. In zone 3 a high compaction was shown, probably due to treading, as two foot paths were laid along this zone. The B horizon of zone 4 contained highly humified organic material together with vast amounts of earthworns, insects and dead roots.

## 2. Mechanical Analysis

The mechanical composition of soil has a decisive influence on many of the soil properties:, on the moisture-air and thermal regimes, on the absorption capacity of the soil, on the accumulation of humus and elements of plant nutrients, etc. All these in turn determine the type of vegetation that occupies the soil.

Of the 4 zones, zone 1 has the highest content of sand (87.19%) as expected, as it was deposited by the water currents. Zone 4 has the highest percentage of silt and clay (2.07% and 18.67% respectively) and lowest of sand (79.25%). The values for zone 2 and 3 are in between those of the two extremes. As to the textural classification, zone 1 can be classified as sandy, zone 2 and 3 sandy loam, and zone 4 loamy sand (Bowen-Jones: An Atlas of Durham City).

3. <u>Natural Moisture</u>

Soil moisture is related to plant growth in many ways, directly and indirectly. Directly its effects pertain to the adequacy of the moisture supply. Indirectly, it influences plant growth by its effects on properties of soil.

The natural moisture was found to be relatively high in zone 1 and the value increased with depths (20, 24, 32%). In other zones it varied from 15.5 - 16% (Fig. 3a).

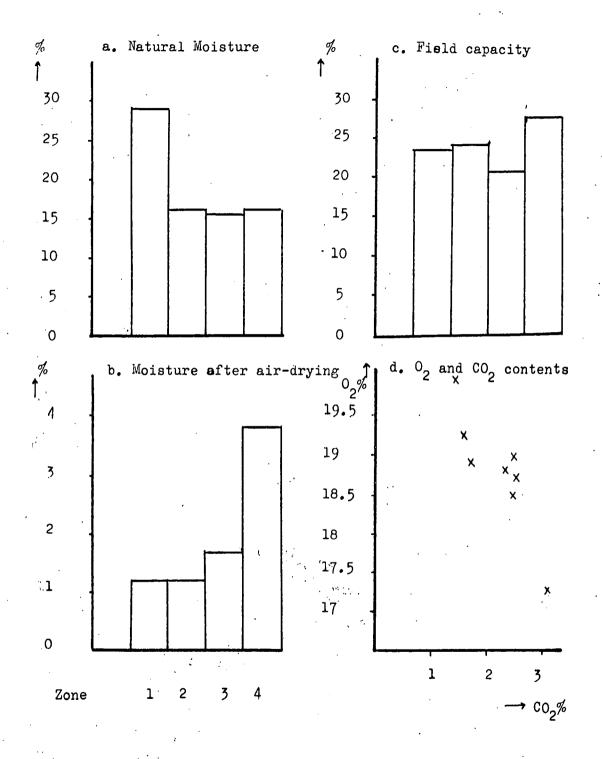


Fig. 3: The average value of several physical analyses in each zone.

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## 4. Moisture after Air-drying

As to moisture after air-drying, a reverse situation to natural moisture was found. Zone 4 had the value of 3.7% due to its comparatively peculiar texture. No significant difference was noted between the others (1.23 - 1.53%) (Fig. 3b).

#### 5. Water Table

The postion of water table depends on profile differentiation, topography and aeration. The water table as shown in Fig. 7 a was more or less parallel to the soil surface.

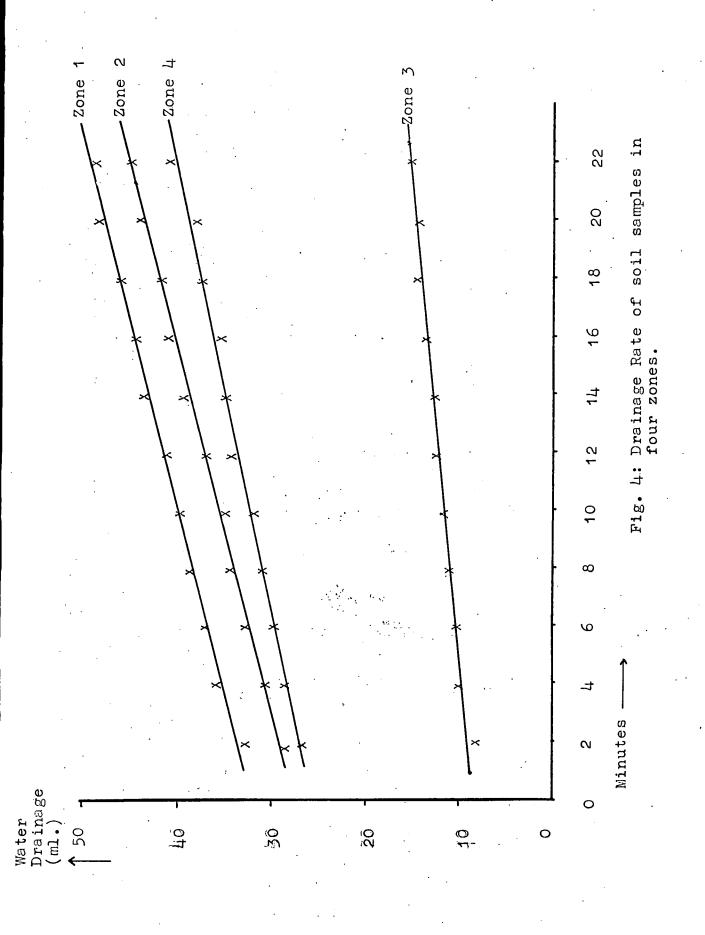
## 6. Drainage Rate and Field Capacity

Field wapacity is the moisture content of soil two or three days after the drainage of heavy rain has progressed. The results showed correlation with texture and moisture after airdrying (zone 4 27.3%, the others varied from 20.3 - 24.3%) (Fig. 3c).

Drainage rate may be affected by texture, structure and orgainc matter content etc. There appeared to be no considerable variation between depths in each zone, but significant difference between zones. Zone 1 is the highest and zone 3 lowest. The former is mainly affected by the higher content of sand and the latter by compaction of soil (Fig. 4).

## 7. Carbon Dioxide and Oxygen Contents

The soil atmosphere occupies that part of the space in the soil not taken up by soil solutes and the balance between



the two will fluctuate with rainfall and drainage. Prolonged heavy rain can lead to the displacement of much of the soil atmosphere by water, but as this drains away fresh supplies of air will be drawn into the soil behind it. Drainage and the soil atmosphere are thus inseparably linked.

Variation in CO<sub>2</sub> and O<sub>2</sub> contents between soils in different zone appeared to be relatively minor and inconsistent. But the concentration of CO<sub>2</sub> (2.52 - 3.1%) slightly increased and O<sub>2</sub> (17.25 - 19.82%) slightly decreased with depth. (Fig 3.4)

## B. Chemical Analysis

## 1. Organic Matter Content

Organic matter consists mainly of plant and animal residues in all stages of decomposition. Its composition produces substances that increase available plant nutrients. From a physical point of view, organic matter improves the aeration of soils and the water holding capacity.

The results of organic matter content correlated with the feature of the soil profiles, zone 4 had the highest value of 7.32%, the others are as follow:- zone 2, 5.36%; zone 1, 5.1%; and zone 3, 4.5% (Fig. 5a).

#### 2. Carbon Content

The amount of carbon constituted the main part of the organic mather, thus the data of carbon content in each zone was

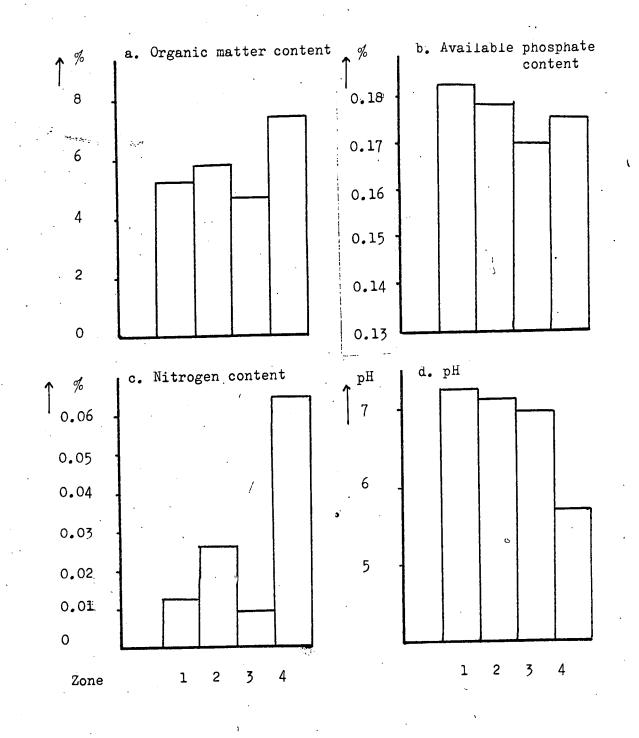


Fig. 5: The average value of several chemical analyses in each zone

proportional to organic matter content.

#### 3. Available Phosphate

Phosphate plays a fundamental role in the very large number of enzymic reaction that depends on phosphorylation. It is a constituent of the cell nucleus and in essential for cell division and for the development of meristem tissue. Available phosphate is part of the total phosphate available for the use of plants. The amount of available phosphate is evenly distributed in different depths as well as in different zones (average: 0.272%) (Fig. 5b).

#### 4. Nitrogen

Nitrogen is also essential for plant growth as it is a constituent of all proteins and hence of all protoplasm. Nitrogen is accumulated in the soil profile in accordance with the distribution of soil organic matter. Thus zone 4 had the value of 0.063%, which was several times higher than the other zones (Fig. 5c).

## 5. <u>pH</u>

There was an obvious vertical gradient of pH variation with depth. This was mainly due to the leaching process of the soil. The average pH values for each zone were as follow: zone 4, 6.217; zone 3, 6.85; zone 2, 7.05; zone 1, 7.2. Thus zone 1 had accumulation of leached material which had been washed down (Fig. 5d).

The pH value of the water at Shincliffe Bridge fluctuates between 7.4 and 7.9 (Fifteen Report of Wear and Tees River Board 1965). applying a second department of the standard second second second second second second second second second sec (a) A second difference of the second s second s second s second se and the second secon and the state of the second state of the second state of the second state of the second state of the and the state of the second A galaxies and a straight straight and and a second • . . . . .

#### Study of Higher Plants

## I. Method

There was a significant difference between types of soil characteristics: texture, moisture content, organic matter content and pH etc. in the four zones. In order to understand better the effect of soil upon the composition and density of vegetation, two methods were employed to study the higher plant distribution.

1. Braun-Blanquet Scale (1932)

Floristic analysis was made by the eye. Fifty-two quadrats (one meter each) were examined at random in the study site using Braun-Blanquet scale (Appendix II).

2. BeltTransect

A belt transect which consisted of 23 quadrats (70 cm<sup>2</sup> each) were laid down perpendicularly from the water level up to the top of the bank. The percentage of occurrence of each species was estimated.

#### II. Results

The results of the two methods of study were closely correlated. The data of the first method are shown in a phytosociology table (Table 2), and the percentages of occurrence of 25 main species are represented as histograms in 41 52 3 50 1 23 4 51 41 2 48 45 46 53 55 20 54 56 5 44 8 41 43 6 42 7, 23, 11 10 18, 28, 29, 35, 36, 31, 38, 39, 9, 12, 24, 27, 26, 31, 39, 17, 21, 15, 40 25, 33, 30, 16, 13, 14, 34, 32,

rits purpurea	49 52 3 50 1 25 4 51 47 2 48 45 45 45 46 122 11 43 22 32 11 23 11 22 11 43 22 32 11 23 11 22 43 32 32 32 32 32	53, 55, 20,54, 56, 5, 44, 8, 41, 43, 6, 42, 7	23,11,10,18,28,29,35,36,51,38,39,9,12,24,2	23,11,10,18,28,29,35,36,31,38,39,9,12,24,27,26,31,39,17,21,15,40,25,33,30,16,13,14,34,32,
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estuca rubra		+ 1-1 1-1 1-1 + 1-1		
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annoulus repens		+ + + + + + + + +		г
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icia sativa			+ + + +	1++++++++++++++++++++++++++++++++++++
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"alcus lanatus		+	21 + 11 11 +	+ + 1:2 + +
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Inanthus cristi-gallii		11	+	
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scinculus acris				
eranium do.		•		
. alba		+		
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"ar Jrium album		+		+
11		+		+ 15t
-1. hee un missbulum		+		+

- 11-1 - -" a sign of a physical of the charle charles the distribution of i Fig. 6. The results of the second method are in table 3 and represented as ladder transect histograms in Fig. 70.

<u>Poa trivialis, Angelica sylvestris</u> and <u>Arrhenatherum</u> <u>elatius</u> were the most common species widely and evenly distributed throughout the bank. The second important group was <u>Cirsium arvense, Rubus fruticosus, Artemisia vulgaris</u> and <u>Rumex</u> <u>obtusifolius</u>. They were not so common, as they showed only scattered appearance in zone 3. The distribution of <u>Uritica</u> <u>diocia</u> was restricted to zone 2 and 4, <u>Vicia sativa</u> to zone 2, 3 and 4, whereas <u>Lolium perenne</u> and <u>Holcus lanatus</u> to zone 3 and 4 only.

Apart from these widely distributed taxa, each zone was dominated by a characteristic community which was named in this study as A, B, C and D respectively:-

A. <u>Phragmities communis</u>, <u>Salix purpurea</u> and <u>Impatiens</u> <u>glandulifers</u> in zone 1.

B. <u>Tanacetum vulgare</u>, <u>Equisetum arvense</u>, <u>Festuca rubra</u>, <u>Dactylis glomerata</u> and <u>Ranumculus repens</u> in zone 2.

C. <u>Phleum pratense</u>, <u>Trifolium repens</u> and <u>T. arvense</u> in zone 3.

D. <u>Galium aparine</u>, <u>Lamium album</u> and <u>Alopercurous pratensis</u> in zone 4.

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1 Phragmities communis

2 Salix purpurea

3 Impatiens glandulifera

4 Tanacetum vulgare

5 Equisetum arvense

6 Festuca rubra

7 Dactylis glomerata

8 Ranunculus repens

9 Phleum pratense

10 Trifolium repens

11 T. arvense

12 Galium aparine

13 Lamium album

14 Alopercurous pratensis

15 Poa trivialis

16 Angelica sylvestris

17 A-rrhenatherum elatius

18 Cirsium arvense

19 Rubus fruticosus

20 Artemisia vulgaris

21 Rumex obtusifolius

22 Urtica diocia

23 Vicia sativa

24 Lolium perenne

25 Holcus lanatus

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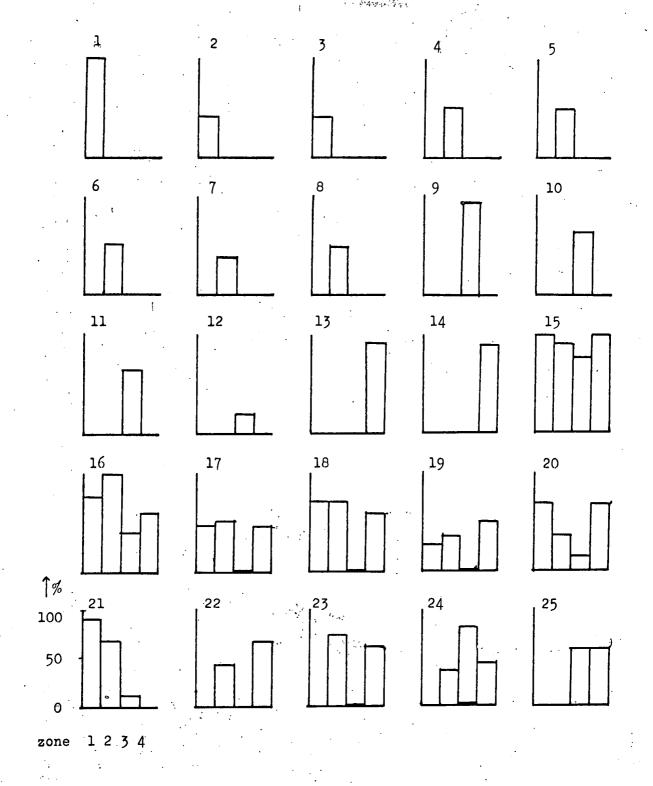
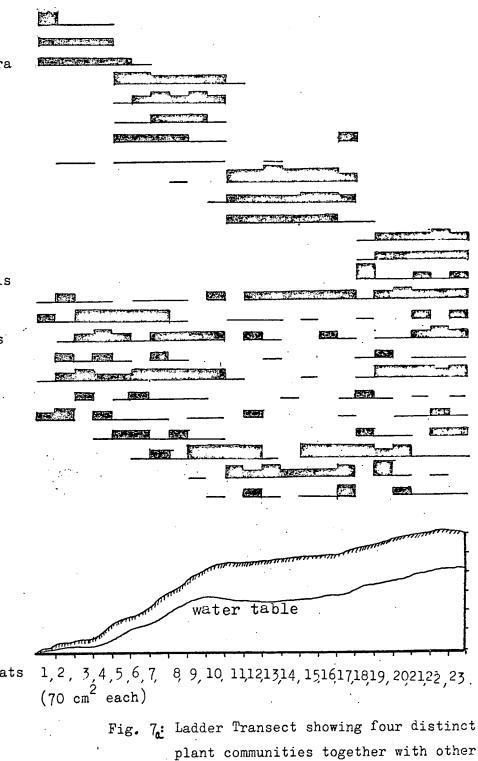


Fig. 6: % occurrence of 25 main species.in each zone

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Phragmities communis Salix purpurea Impatiens glandulifera Tanacetum vulgare Equisetum arvense Festuca rubra Dactylis glomerata Ranunculus repens Phleum pratense Trifolium repens T. arvense Galium aparine Lamium album Alopercurous pratensis Poa trivialis Angelica sylvestris Arrhenatherum elatius Cirsium arvense Rubus frutcosus Artemisia vulgaris Rumex obtusifolius. Urtica diocia Vicia sativa Lolium perenne Holcus lanatus



important vegetations along the river

Quadrats

over 40 %
20-39 %
1-19 %
sparse, very
low cover

III. Van der Maarel's Group Analysis (1968)

In order to locate the exact position and the nature of the boundaries within the four different zones, this sensitive method was employed (Appendix III).

The heterogenity between contiguous quadrats was obtained using the differential occurrence of the species. This gave the differential profile shown in Fig. 7<sup>b</sup>, with three acute peaks: between quadrats 4 & 5, 10 & 11, and 17 & 18. This implied that there were spatially abrupt changes while the remainder of the graph profile showed a rather variable and disturbed form indicating gradual change. These boundaries were correlated with the edaphic factors in each zone (Table 1, Fig. 3 & 5). Zone 1 had the highest natural moisture content, zone 2 had a relatively high organic matter and nitrogen centent, zone 3 was a trodden area, showing a high compaction of soil; zone 4 had the highest contents of moisture after air-drying, field capacity, organic matter and nitrogen. These characteristics could be closely related to the existence of the floral groups.

#### IV. Discussion

and the first states of the

Narrowly restricted species are unable to grow under condition other than those to which they are restricted. Theoretically it can be the best indicator of conditions in natural environment. Zone 1

<u>Phragmities communis</u> had the highest percentage cover in community A as well as in the whole zone. It dominated completely the area near water level but only showed slight occurrence in other places within this zone. However, <u>Salix</u> <u>purpurea</u> and <u>Impatiens glandulifera</u> were evenly distributed. They were both characteristic of <sup>S</sup>alici-populetum. In general, the species in this community were widespread in wet conditions, expecially near river and stream. The highest percentage of the natural moisture in this zone established an ideal condition for such a demand.

Besides community A, <u>Angelica sylvestris</u>, <u>Rubus</u> <u>fructicosus</u>, <u>Arrhenatherum elatius</u>, <u>Rumex obtusifolius</u> are the most important taxa. The former three increased percentage cover with distance from the water level, while the situation was different in <u>Rumex obtusifolius</u>.

#### Zone 2

<u>Tanacetum vulgare</u> and <u>Equisetum arvense</u> were the most dominant and widely occurred species in community B in this zone. They were characteristic of Artemisietea. While the others, <u>Festuca rubra</u>, <u>Dactylis glomerata</u>, <u>Ranunculus repens</u> are widespread on meadows, waste places, hedge banks and dunes.

Besides this characteristic community, zone 2 also had the same dominant species as the previous one.

<u>Urtica diocia</u> and a kind of moss <u>Brachythecium</u> <u>rutabulum</u>, which are both indicator of nitrogen, appeared in this zone. It seemed to be correlated with the relative high organic matter and nitrogen content.

Zone 3.

The community C in this zone consisted of <u>Phleum</u> <u>pratense</u>, <u>Trifolium repens</u> and <u>T. arvense</u>. As the former two are characteristic of Cynosurion, they showed a good association and are evenly distributed in the area.

The vegetation in this zone was rather peculiar when compared with the others. The dominant species in the other zones were found to be absent or had only scattered occurrence here.

The newly established species, <u>Vicia sativa</u>, <u>Lolium</u> <u>perenne</u> and species in community C usually occurred in grassy places after cultivation, and the vast amount of <u>Vicia sativa</u> in this zone probably indicated a relict of cultivation. <u>Trifolium repens</u> and <u>Lolium perenne</u> were the most abundant species found on the more heavily trodden part of the road verge or pathway (Davies 1938). Thus the footpaths of this zone gave them an ideal location. An intensive trampling effect was demonstrated by the high compaction of soil in the soil profile (Fig. 2c).

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Zone 4

Community C contained the characteristic species in this area, they were <u>Galium aparine</u>, <u>Lamium album</u> and <u>Alopercurous</u> pratensis.

The other dominant species became restablished in zone 4 after their disappearance in zone 3. <u>Poa trivialis</u> together with these dominant taxa had the highest percentage cover when compared with the other zones (except <u>Vicia sativa</u> and <u>Holcus</u> <u>lanatus</u>).

The higher value of <u>Urtica diocia</u> together with <u>Galium</u> <u>aparine</u> (usually occur in base-rich soil) in community D suggested a high base content in the soil, with high organic matter and nitrogen contents in this zone. Nevertheless the pH value was found to be comparatively low.

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# Study of Soil Fungi

## I. Method

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For the isolation of fungi, a modification of Warcup's soil plate method (1951) and a modification of Chester's immersion method (1940) (Appendix IV) were employed. The distribution of fungi was studied by using Kulczynski's square (coefficient of similarities) (1939) (Appendix V).

## II. Results

Slow-growing colonies on petri-dish were found to be overgrown by bateria when 2% malt agar was used. The situation was much better in Rose Bengal medium, because Rose Bengal eliminated all the actinomyces and most of the bacteria and reduced the spreading of fungal colonies to a minimum (Smith & Dawson 1944).

Table 4 shows the distribution of the fungal species in the four zones of soil. There was a gradual falling off in the number of species from zone 1 to 4, and also fewer number of species in depth 30 cm. than in 80 cm. below the soil surface.

Three groups of species appeared in the soil plate method (Fig. 8, and Table 5). Group 1: <u>Penicillium brevi</u>-<u>compactum</u>, <u>P. raistrickii</u>, <u>Trichoderma lignorium</u> and <u>T. koningi</u>

						Zone	1	2	3	4
Transect	I	80	cm	below	soil	surface	A	Ε	I	М
		30	cm	below	soil	surface	В	F	J	N
Transect	II	80	cm	below	soil	surface	C	G	K	0
		30	cm	below	soil	surface	. D	H	L.	P
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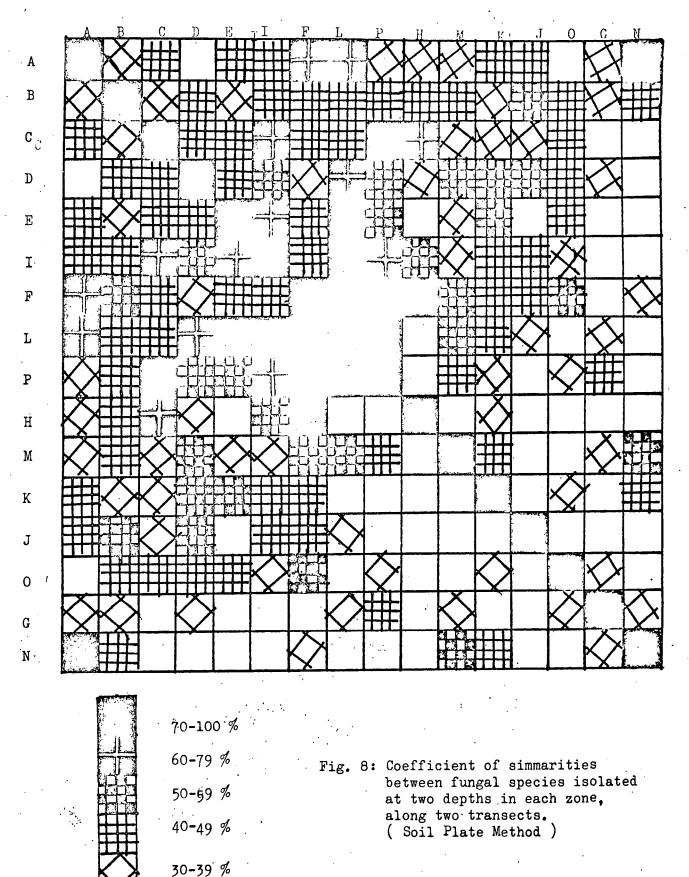
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## I. Soil Plate Method

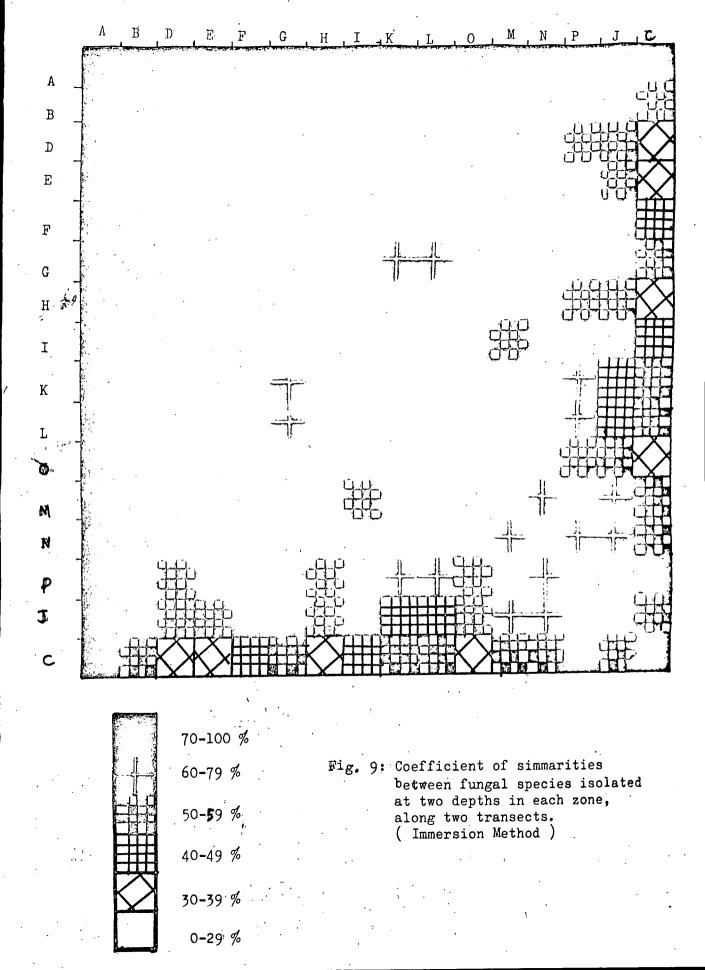
•	. ~		-	**	-	~ **	-	+		-				~	
Alternaria tenuis	AE	3 C	D	E	문 ( + .	GH	1	Ļ	K	L	M	N	0	9	
Circinella sydowi	+.			+			+		+						
Cephalosporium coremioides	+ •	. +				+ .	+				+	+		•	
Cladosporium herbarum	+ -			+						•		•	•		
Hel-minthosporium microsorum		· +	+			. +					•		+		
Humicola grisea	. +		+								-		+		
Monilia sitophila	•		+			+ .		+	+		+		•		
Mortierella sp.	•••	•											+	Ì	
	•••	• •				+ .	+	•	•					+	
Mucor jansseni	••	, ,	•	•	•			+	+			•			
Mycogone nigra var. minor	• •	• •	• -	•	•	• • + +	+		+	+	+	•	•	+	
Penicillum brevi-compactum	• •	г т 	т	т		· ·	'	'	•	•	•	•	•		
P. frequentans		· •	•	٠	• +	' • • +	•	•	•+	•	•	•	•	•	
P. raistrickii	.+ 1		•	-	т	• •	•	т	т	•	•	•	•	• .	
P. spinulosum	• •	. +	+	+	•	••	•	•	•	•	•	•			
P. sp.	• •	· +	•	+	•			. <b>•</b> .	Ŧ	•	•	•	•	•	
Rhizopus cohnii	• •	•	+	•	•	+ •	+	•	•	•	•	•	•	•	
Spondylocladium fumosum	• •	•	•	•	•	+ .	•	•	•,	•	•	.•	+	•	
Sporotrichum chlorinum	• •	, +	•	+	•	••	•		•	•	•	•	+	•	
S. laxum	+.	, +	•	•	+	• +	-		•	•	•	•	+	+	
Trichoderma lignorum	+ +	••	+	+	+	••	+		·ŀ	+	+	Ŧ	Ŧ	Ŧ	
T. koningi	+.	, +	+	· +	+	• •	.+	+	•	+	•	•	•	٠	
Trichothecium roseum	• +	۰ ۱	+.	•	•	••	•	•	٠	٠	•	٠	٠	•	
Verticillium terrestre	:+ .	•	•.	•	+ .	• •	•	٠	•	•	•	•	•	•	
V. glaucum	• •	i i	•	•	٠	••	•	•	•	•	٠	+	٠	•	
Zygorhynchus heterogamous	• •	•		•	•	+ .	•	+	•	•	٠	٠	•	٠	
Streptomyces	• •	•	•	•	•	• • +	5 i j e	v •	r: •	٠	٠	•	•	•	
Yeast	• 1	• +	٠	٠	•	+ .	•	•	•	۰.	•	.•	٠	•	
			:												
II. Immersion Method															
		•	•										•.		
Aspergillus clavatus	• +	•	•	• •	. +		٠	+	•.	•	+	•	• '	+ 	
Cylindrophora hoffmanni	+ .	+	٠	• •	, +	-	•	+ "	•	• •	•	+· .	•	+.	
Monilia humicola	+ +	٠	+	+ .	, +	+	•	•	+	+ .	+ ·	+ ·	+	•	
Rhizopus cohnii	+ +	+	+ ·	+ +	+ +	+	+	+	+	+ ·	+	+ .	+	Ŧ	
Trichoderma koningi	• •	٠	•	• •	•	•.	•	+	•	ė.	•	•	•	•	
Verticillium candelab rum	+ +	•	+	+ +	+ +	+	+	+	+	+ ·	+ ·	+ '	<b>T</b>	Ŧ	
Sterile white mycelium	+ +	+	•	• •	•	٠	• .	•	+	+	t	•	•	+	
										•					

Table <u>4</u>: The Occurrence of fungal species at 80 and 30 cm below soil/surface in each zone.



0-29 %

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#### ABCDEFLPIHMKJOGN

Trichoderma lignorum T. koningi Penicillum brevi-compactum P. raistrickii P. spinulosum P. frequentans P. sp. Trichothecium roseum Circinella sydowi Cephalosporium coremioides Cladosporium herbarum Helminthosporium microsorum Humicola grisea Sporotrichumechlorinum Monilia sitophila Alternaria tenuis Mortierella sp. Mucor jansseni Mycogone nigra var. minor Rhizopus cohnii Spondylocladium fumosum Sporotrichum laxum Verticillium terrestre V. glaucum Zygorhynchus heterogamous Streptomyces Yeast

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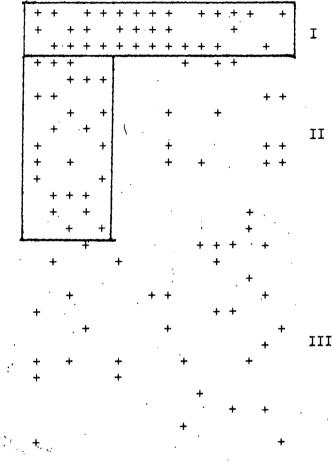


Table <u>5</u>: Phytosociology table showing the distribution of soil fungi.

were widely distributed. The occurrence of species in group 2 (Table 5) were more or less restricted to zone 1 which had a relatively high natural moisture content whereas species in group 3 (Table 5) were restricted to 80 cm. below soil surface.

As to the immersion method, the occurrence of species did not show any restriction between depths as well as zones (Fig. 9). <u>Rhizopus cohnii</u>, <u>Verticillium candelabrum</u>, <u>Monilia humicola</u> and sterile white mycelium were the most abundant species isolated by this method.

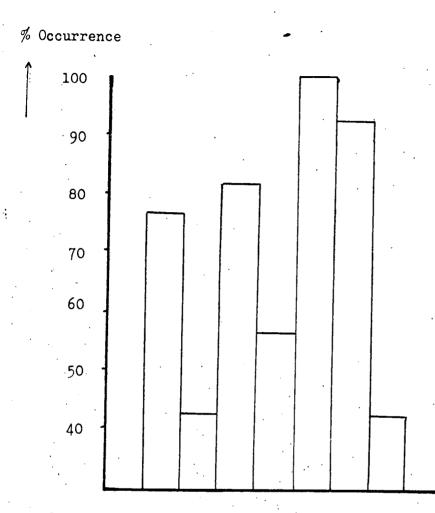
The percentage of occurrence of several abundant species in the two methods of isolation were represented as histograms in Fig. 10.

#### III. Discussion

### 1. Comparison between Methods of Fungal Isolation

The overall composition of the fungal flora, as demonstrated by the soil plate and immersion method is represented respectively in Table 6 and 7. It became apparent that the two methods gave different pictures of the fungal flora. Only two species were common to both isolation. It also showed that a higher total number of species were recorded from the first than the second method (4:1 in proportion).

As a nonselective method for general isolation of soil



Fungal spp P.b. P.r. T.1. T.k. R. V. S.

Fig. 10: Total % occurrence of seven abundant fungal species.

P.b.=Penicillum brevi-compactum T.l.=Trichoderma lignorum V. =Verticillium terrestre R. = Rhizopus (chn:; P.r.= P. raistrickii T.k.="T. koningi S. = Sterile white mycelium

Transect	I	· II
Zone	1,2,3,4	1, 2, 3, 4
Depth	ABABABAB	ABABABAB
No. of spp Soil plate	10 9 8 7 8 7 4 4	10 9 9 5.7 3 6 4
Immersion	5 5 3 2 2 5 5 4	3 13 5 3 4 4 3 5
Total	15 14 11 9 10 12 9 8	13 12 14 8 11 7 9 9

Table 6: Number of fungal species isolated in each transect

Transect 1+11 Zoneh		1.		2		L		2
Depth	Ā	В	A	В	A	В	A	B
No. of spg Soil plate	20	18	17	12	15	10	18	8
Immersion	8	8	8	5	6	9	10	9
Total						19		
. %	16	15	14	10	12	11	10	10

Table 7: Total Number of species isolated in two transects

Not any seas

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A=80 cm below soil surface B=30 cm below soil surface

fungi, soil plate method probably represents the best compromise. It is possible by this method to culture fungus mycelium embedded in organic material and cemented to mineral particles and it also seems to reduce the competitive advantage given to fastgrowing sugar fungi by Chester's method. Evidence as to the practical success of this method is supported by the greater range of species being isolated.

Chester's own conclusions (1940, 1945) concerning the scope of his method are summarized as "the limits of the immersion tube method is that it easily isolates active spreading mycelium, or active localized mycelium which happens to come into contact with the capillaries". (In this study, several holes were used instead of the capillaries and provided a more direct contact between fungal mycelium and agar medium). Chester has thus emphasized current mycelial activity as the characteristic reflected by isolations of soil fungi in his immersion method.

In general, the first method favoured the isolation of heavily sporing fungi, particularly <u>Penicillium</u> spp. It is suggested that a physical 'rhizosphere' effect might have been produced by the immersion in soil of solid objects, which stimulated the growth of certain fungi, and competition between species was a major factor in determining the results obtained by all methods  $(S_{well}^{e})$ .

#### 2. Factors related to Fungal Distribution

The fungal mycelium presents a large surface area to the influence of external factors. The survival of such mycelia is dependend upon high humidity in the atmosphere or water content of the substrate, flavourable temperature and a continuous supply of suitable organic matter at a favourable pH.

Among the edaphic factors which were tested in this study, pH and moisture were probably the major factors determing the range of species. The percentage of occurrence of species was positively correlated with the moisture and pH of the soil (Figs. 11, 12, 13).

When the water content of a soil either falls below the wilting percentage or becomes sufficiently great to impede soil aeration there is a great reduction in the active growth of fungal hypgae. Warcup (1957) in the study of soil fungi in a wheat field, obtained the data which showed that there was a marked seasonal distribution of fungal flora related to soil moisture, with the highest number of viable units in the soil during the wet winter period.

Soil pH affects the availability of nutrients in the soil. It is possible that it also influences the physiological functioning of the fungal thallus, possibly through its effects on the reactions occurring on the cell surface rather than through

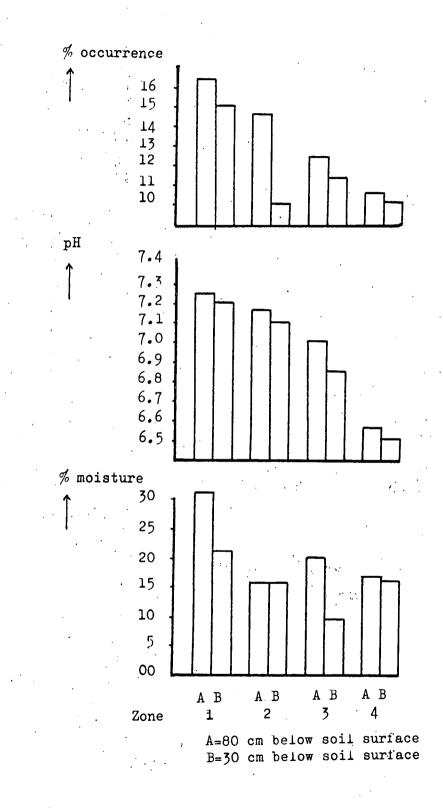
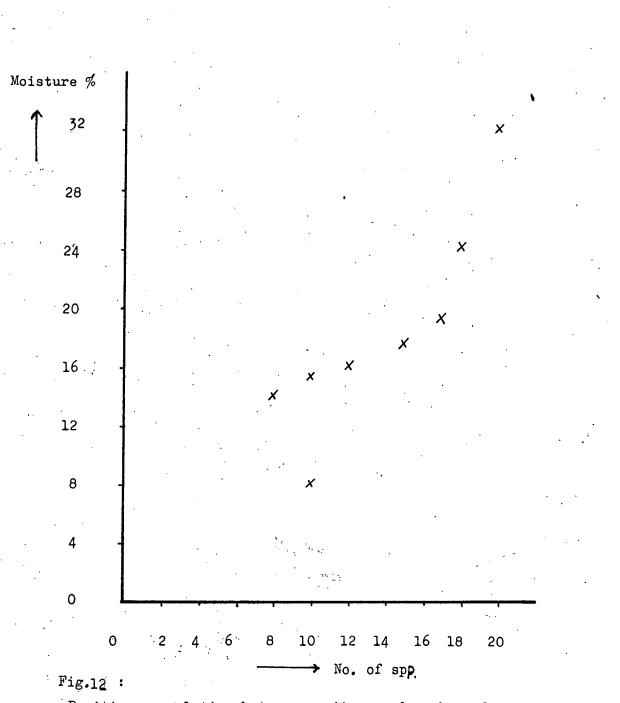


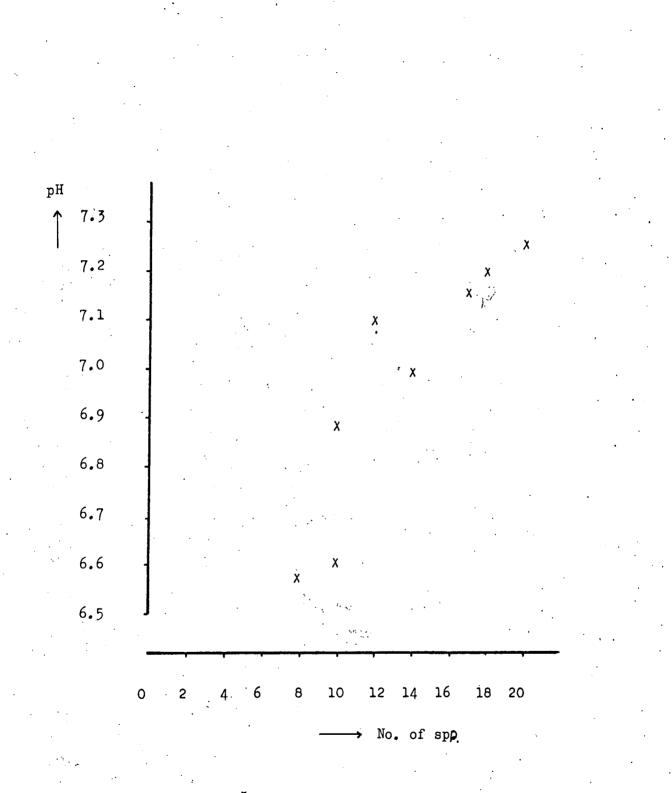
Fig. 11: % occurrence of number of fungal species at two depths in each zone. (compare with pH and moisture)

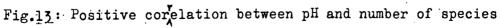
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Positive coryelation between mositure and number of species.

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a direct influence on the internal processes of the cell. Warcup (1951) recorded that more species of fungi grew in neutral than in acid or alkaline grassland soils.

At the beginning of this study, it was expected that oxygen concentration would limit the occurrence of fungus species. . . . However, it was soon found that there was no great correlation . . between these two. This was due to the relative low carbon dioxide concentration (1.62-3.1%) which was not harmful to fungal In the study of the effect of carbon dioxide on the growth. growth of certain fungi, Burges & Fenton (1953) found a high concentration of carbon dioxide developed in the soil after rain (3.5 - 9.2%), and the tolerance of high carbon dioxide rather than of low oxygen content may determine the occurrence of fungi. The organic matter content seemed to play a minor role in the appearance of fungues species in this study.

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# General Conclusion

 $\sigma_{\rm eff} = \frac{1}{2} \left[ \Delta \phi_{\rm eff} + \phi_{$ 

The ecological explanation of plant distribution is based primarily upon environmental factors. From the stand point of highly restricted distributional patterns in plants it is evident that of all the environmental influences, the edaphic factors are the greatest, as they frequently occur in sharply defined patterns and often vary within a very small area. It is because of the great local variation that exists in the physical and chemical nature of the substratum and soils, the edaphic factors represent the possibility of enermous diversity of habitats in any given area. Thus the breaks or transitional zones between communities usually follow observable changes in the soil, indicating that local edaphic factor and plant communities are closely associated.

The discontinuities of higher plant communities were found to be correlated with topography, moisture, nitrogen and organic matter contents. As to the occurrence of fungi, moisture and pH seemed to play a major role, and soil atmosphere and organic matter content were relatively less important.

Tansley (1949) stated that the higher plant community may be defined as a collection of plants growing together and having a 'certain unity', a common habitat --- the soil. The

determination of comparable micro-fungal community growing and flourishing in a common habitat, however, presents considerable technical problems.

#### Summary

Four obvious zones of vegetation were evident on the bank of River Wear at Shincliffe, County Durham. The edaphic factors were considered of primary importance in the determination of the distribution of these different vegetational groups as well as the soil fungi. To determine whether differences in soil characteristics occurred concomitantly with differences of vegetational types, intensive soil studies were made in each zone. The soil samples were subjected to extensive chemical and physical analyses.

Higher plant analysis was carried out by the methods of Braun-Blanquet scale and Belt transect. The same picture was obtained: four distinct communities were, found, restricted one in each zone. By using the group analysis of Van der Maarel, the actual boundaries between communities were found to correlate with topography, moisture, nitrogen and total organic matter contents of the soil.

Two methods were employed in the isolation of fungi, a modification of Warcup's soil plate and a modification of Chester's immersion method. Two distinct fungal groups were obtained, the first being dominated by higher sporing capacity species, and the second higher mycelial growth species. The occurrence of

them were correlated to the soil moisture and pH. Soil atmosphere and organic matter contant seemed to play a relatively less important role.

No correlation seemed to be apparent between the distribution of macro- and micro-flora in this study.

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### Acknowledgement

I wish to thank Mr. G.H. Bambury for his help with the mycological aspects of this project and Dr. D.J. Bellamy for his advice on the phytosociology, and to Mr. Stephens for permission to use the soil analysis labofatory in the Geography Department.

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### Appendix I

### Analysis of the Soil Factor

#### I. Method

Twelve samples, from three depths (surface, 30 and 80 cm.) in each of the four zones, were collected. Each sample consisted of five sub-samples from that zone in order to reduce. the error caused by chance. All samples for laboratory analysis (except natural moisture) were air dried, crushed gently and passed through a 2 mm. mesh sieve.

#### A. PHYSICAL CHARACTER

1. Soil Profile

Soil profile, one from each zone was exposed by excavating a trench sufficiently deep to include the C horizon. The diagram and description was shown in p.

2. Mechanical Analysis --- Bouyoucos Method

Fifty grams of sieved soil were weighed into a 500 ml. flask, 400 ml. of distilled water and 10 ml. of / N NaOH added, and the whole shaken overnight. The slurry was then transferred to a litre measuring cylinder making sure that all the mineral matter was washed out of the flask. A hydrometer was inserted and the volume made up to one litre with distilled water. The hydrometer was withdrawn and the suspension shaken throughly by hand for one minute. The hydrometer was inserted again. The first reading was taken after 40 seconds and the second reading after 4 minutes and 48 seconds. The temperature of the suspension was also taken. After allowing the suspension to stand for two hours, the hydrometer and temperature readings were taken again.

Calculations:-

1. Correction for temperature readings.

For each degree above 19.5°C, 0.4 of a division of the hydrometer scale was added, and for each degree below 19.5°C, 0.4 of a division of the hydrometer scale was subtracted.

% sand =  $100 - (\frac{1 \text{ st corrected reading}}{50 - 1/2 \text{ moisture}} \times 100)$ 

This gave reading for percentage of sand after 40 seconds (U.S.D.A. limits) and by substituting the second corrected reading into the formula the percentage of sand after 4 minutes and 48 seconds (International limits) was obtained.

$$\%$$
 clay =  $\frac{3rd \ corrected \ reading}{50 - 1/2 \ moisture} \times 100$ 

The percentage of silt for both limits was found by calculation.

% silt = 100 - (% sand + % clay)

### 2. Correction for organic content.

This method was not used when the loss on ignition exceeds 25%. Where loss on ignition was less than 4% no correction was applied. Where loss on ignition was 4-15% half of the value of the loss on ignition was deducted from the the percentage of sand. Where loss on ignition was 15-25% three-quarters of the value of the loss on ignition was deducted from the percentage of sand. Where total sand exceeds 70% no correction needed be made unless this would result in bringing the sand content appreciably below 70%.

#### 3. <u>Natural Moisture</u>

Ten grams of soil which had not been previously dried in air was weighed and dried in an oven at 100°C overnight,

cooled in a dessicator and reweighed.

#### Calculation:-

% natural moisture = <u>lst weight - 2nd weight</u> x 100 lst weight

#### 4. Moisture after air-drying

Ten grams of air dried soil were placed into a weighed silica crucible, maintained in an oven at 105°C for 4 hours, cooled in a dessicator and reweighed. This gave the percentage moisture in the soil calculated as a percentage of the air-dried soil.

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Calculation:-

% moisture =  $\frac{1 \text{ st weight} - 2 \text{ nd weight}}{10} \times 100$ 

5. Water Table (Rutter 1954)

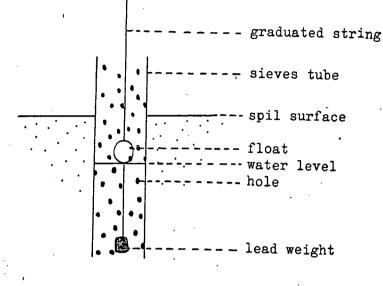
Four different lengths of plastic splieve tubes were inserted into holes excavated with an auger to the appropriate depths (100, 150, 200 and 250 cm. approx.) in each zone. The water level was measured at 4-day intervals over a month's period by using a float with a graduated rod fixed to one end and a lead weight to the opposite end (Fig. 14), (Table 8)

6. Drainage Rate and Field Capacity

Fifty grams of soil were weighed and placed in an extraction tube. A cotton wool ball was used as a plug to prevent the soil from washing through. 100 ml. of water was poured onto the soil and the water draining through was collected in a measuring cylinder. The amount of water draining through at 2-minute intervals was recorded. The results were plotted on graphs as shown in fig.

Calculation:-

field capacity = 100 c.c. - vol. of water drained through



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Fig. 14: Appantus for measuring water table.

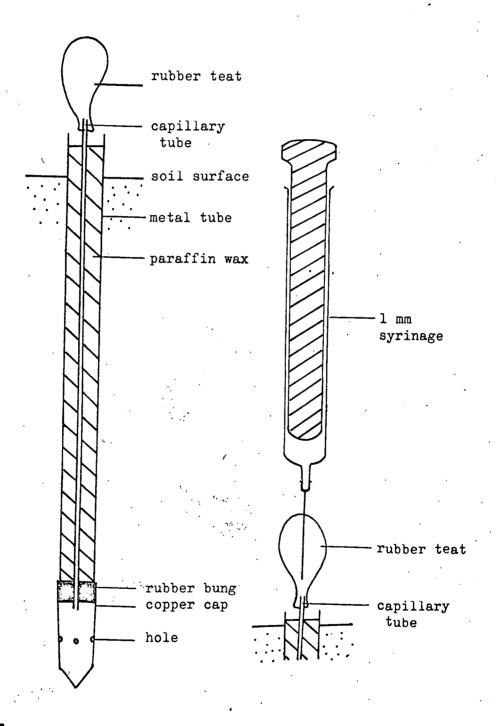
			/		
Zon	e:	1	2	3 ъ	4
Four	1、	70	142.4	161	140 cm
day <del>n</del>	2	75 <sup>°</sup>	142	162	139 cm
inter <del>y</del>	3	50	100.5	125.5	118.1 cm
vals	4	60.5	108	113.2	121 cm
	5	70.7	110.2	134.6	128.5 cm
	6	76.5	129.5	156.2-	142 cm
•	7	77.1	135	155.5	139.4 cm
Avera	ge	68.5	120.9	144	132.3 cm

Table 8: Results of water table measurment.

#### 7. Carbon Dioxide and Oxygen Contents

#### a. Gas Sampling

Two transects were selected, running from the height of the adjacent field down towards water level in the river valley. Samples were taken at depths of 30 cm. and 80 cm. (surface gas was not sampled) from four points, one in each zone, along each of these transect lines at the time of sampling for fungi. A gas sampling tube was used for each sampling site. The sampling tube was made in the following way  $(\mathcal{F}_{ig}, I_{f})$ : capillary tube of convenient length was put into a wider but shorter metal tube and passed through the rubber bung at its end. The space between the two tubes was filled with paraffin wax poured in while molten. A copper cap with 4 small holes (about 1 mm. diameter) at its sides was placed at the stoppered end of the metal tube for protection. A rubber teat was placed on the free end of the capillary tube to close it. The apparatus was buried vertically in the sampling site at the required depth, copper cap down and rubber teat exposed. Four days later gas samples were withdrawn into a 1 ml. syringe by piercing the rubber teat. The first sample was discarded and the composition of the second sample analysed for carbon dioxide and oxygen contents. The sampling and analysis were repeated at 4-day intervals for 2 times. (Table, 9)



# Fig. 15: Gas sampling tube

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C	03	I O	2		20 <sub>2 B</sub> I	I O <sub>2</sub>	,	
Α.	В	. A '	<u>в</u> .	A	∠B	A <sup>2</sup>	В	Transect
lst 2.8	3.4	18.32	16.8	1.5	2.7	19.2	18.3	•
2nd 2.4	2.9	18.4	17.62	2.14	2.8	18.4	18.0	1
lst 3.0	3.2	18.4	17.0	1.9	2.07	19.5	18.1	
2nd 2.9	2.9.	18.9	17.43	1.6	2.48	18.2	18.7	2
ave 2.78	3.1	18.52	17.25	1.76	2.58	18.82	18.25 g	6
	co <sub>2</sub> B	III 0,		co	IV	0		
Α	B	A 02	В	Α	<sup>2</sup> B	A 2	В	
lst 1.84	2.7	19.7	18.2	1.61	2.6	19.88	18.7	
2nd 1.7	2.4	18.8	18.8	2.0	2.9	20.45	19.2	1
lst 1.7	2.6	19.5	19.0	1.41	2.9	19.55	17.7	2
2nd 1.64	2.38	18.9	18.4	<b>1.</b> 46	2.4	19.40	20.0	. 2
ave 1.72	2,52	19.2	18.6	1.62	2.7	19.82	18.9 %	, , )

A=30 cm below soil surface B=80 cm below soil surface lst=First sampling 2nd=Second sampling I, II, III, IV=Zone 1, 2, 3, 4 ave=Average value

Table 9: Results of gas sampling

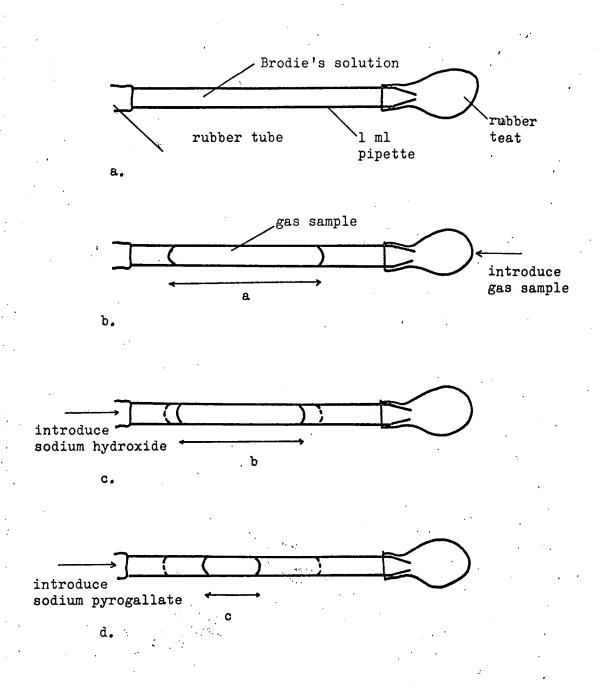
### b. <u>Gas Analysis</u>

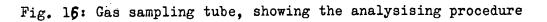
The gas analysis tube consisted of a 1 ml. pipette with a rubber teat at one end and a rubber tube at the other. (Fig. 15 a) The whole apparatus was filled with Brodie's solution and immersed in a water bath maintained at constant condition of temperature.

The gas sample from the syringe was introduced through the rubber teat and the volume (a) noted (Fig. 6b). Half ml. of 10% NaOH was injected into the gas analysis tube through the rubber teat to react with the carbon dioxide. The analysis tube was shaken lightly until a constant volume of gas was obtained and the reading (b) then taken (Fig. 5c). Half ml. of 10% sodium pyrogallate was then similarly introduced to absorb the oxygen. The analysis tube was again lightly shaken and a reading (c) was taken after a constant volume was obtained (Fig. 6d)

Calculation:-

 $\% \ CO_2 = \frac{a - b}{a} \times 100$  $\% \ 0_2 = \frac{b - c}{a} \times 100$ 





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### B. CHEMICAL ANALYSIS

### 1. Organic Matter Content

Oven-dried soil was weighed and placed in a muffle furnace (at about 800°C) for 2 hours, cooled in a dessicator and reweighed. The percentage loss on ignition was calculated as a percentage of the oven-dried soil. As loss on ignition consists of organic matter and combined water, it is usual in practice to deduct a certain aliquot of the loss on ignition as combined water, the aliquot depending upon the mechanical composition of the soil: for clayey soils, one half; for loamy soils, one third; and for sandy loam or loamy sand, one quarter.

Calculations:-

% loss on ignition = <u>oven-dried wt. - ignited wt.</u> x 100 ignited wt.

organic content = % loss on ignition - corrective factor for combined water

## 2. Carbon Content --- Walkley & Black

One gram of soil was placed in a 500 ml. conical flask, and to this 10 ml. of potassium dichromate and 20 ml. conc.  $H_2$ SO4 were added. After 30 minutes, 200 ml. distilled water and 10 ml. phosphoric acid were added, followed by 20 drops of 0.5 N Diphenylamine. This resulted in a black liquid, which was titrated with 0.5N FeSO<sub>4</sub> until the end point (green colour) was obtained. A blank titration was carried out as above without the soil.

Calculation:-

carbon content =  $\frac{(blank - titration) \times 0.0039}{weight of soil} \times 100$ 

3. Available Phosphate

Ten grams of soil together with 100 ml. 0.5N acetic acid were placed in a 500 ml. flask and shaken overnight. The contents were filtered into a 100 ml. measuring cylinder, and made up to 100 ml. with distilled water. A sample of 10 ml. of this was taken and added to 50 ml. of water in a 100 ml. cylinder, to this 10 ml. 5% ammonium molybdate and 10 ml. 1% SnCl<sub>2</sub> were added and the volume was made up to 100 ml. with distilled water. The cylinder was placed in a dark cupboard for 20 minutes to allow a blue colour to develop.

Three phosphorus standard solutions were made up by using 0.01, 0.02 and 0.03  $ppm_5$  KH<sub>2</sub>PO<sub>4</sub> respectively. The optical density readings measured by a spectrometer were plotted in a graph (Fig. ). The optical density readings of the test samples were referred directly to this calibration curve to obtain the ppmg of phosphorous in solution.

#### Calculation:-

% available phosphate =  $\frac{X \text{ mg.}}{1000} \times \frac{100 \text{ ml.}}{10 \text{ ml.}} \times \frac{100}{10 \text{ gm.}} \times \frac{94.97}{136.07}$ 

4. Nitrogen --- Kjeldahal procedure

Ten grams of soil were weighed into a Kjeldahal flask, 20 ml. water, 10 Kjeldahal tablets and 30 ml concentrated  $H_2SO_4$ were added. The flask was placed on a Kjeldahal rack and digested at low heat until the liquid changed to a green or straw colour. It was then washed out into a 250 ml. volumetric flask and topped up to the mark with distilled water.

A Markham 'still' was set up. Into the still 10 ml. 40% NaOH and 10 ml. of the solution out of the volumetric flask were placed. This was heated and allowed to bubble into a beaker containing 10 ml. 4% boric acid and mixed indicator. The indicator turned blue and was left until approximated 30 ml. had accumulated. This was titrated with 0.02 N HCl until the colour returned to red.

Calculation:-

% N<sub>2</sub> = X ml. HCl x  $\frac{0.28}{1000}$  x  $\frac{250}{10}$  x  $\frac{100}{10}$  gm.

I-9

5. <u>pH</u>

Ten grams of soil were mixed with 25 ml. of distilled, water. The suspension was stirred by a glass rod and left to stand for 2 hours. The suspension was stirred again and the pH of it measured by a pH meter.

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### Appendix II

Braun-Blanquet Scale (1932)

### A. Cover-abundance scale

lst --- 1 plant

+ --- sparse, very low cover

1 --- common, small scale

2 --- either 5 - 25% cover or cover low, but high numbers

3 --- 25 - 50%, any number of individuals

4 --- 50 -75%, any number of individuals

5 --- 75 - 100%, any number of individuals

(+) --- occur just outside record area

### B. Sociability

1	single stem or shoot
2	small tufts or loose tufts
3	smaller patches or cushions
4	extensive patches or carpets
5	plants in great crowds giving complete

dominance

1. A. A.

### Appendix III

Van der Marrel's Group Analysis (1968)

A simplification of the summary of "Small-scale vegetational boundaries, on their analysis and topography" is given below:

Small-scale vegetational boundaries may be considered of special importance in a general approach to ecological boundaries, since they determine the basic structures in vegetation on which the entire structure of vegetation is built.

According to the Information Theory the difference between two quadrats may be measured as the heterogenity in the set of two quadrats. Each species occurring in only one of the two quadrats contributes one bit of selective information or, preferably, half bit/quadrat. When quantitative data are a available the heterogenity contribution of a species may be approximated by dividing the difference of performance in the two quadrats by the maximum difference. This lead to:-

$$H = \frac{1}{2} \times \sum_{i=1}^{G} \left( p(g_{i,a}) - p(g_{i,b}) \right) / p_{max}$$

where H = hterogenity between two adjacent quadrats G = no. of species  $p(g_{i,a}) = performance of the ith species in$ quadtrat a

III-1

 $p(g_{i,b}) = performance of the ith species in quadrat b$  $<math>p_{max} = maximum$  difference of performance

The valus p can be measured as frequency, coverage, abundance or with a combined scale, e.g. the Braun-Blanquet scale.

For qualitative data this formular reduces to:-

$$H = \frac{G_{B} - G_{C} + G_{b} - G_{c}}{2}$$

1.11

where  $G_{B}$ ,  $G_{D}$  = numbers of species in quadrats a and b

respectively

 $G_c$  = number of common species.

#### Appendix IV

#### Analysis of Soil Fungi

### 1. Isolation of Fungi

### A. Modification of Soil Plate Method (Warcup 1951)

A soil profile was exposed by digging a pit about 30 cm. square, 100 cm. deep. Two soil samples were collected, one from 30 cm. and the other 80 cm. below surface. This was repeated at four points, one in each zone, along each of the two transect lines running from the height of the adjacent field down towards water level in the river valley.

Small samples (0.005 - 0.015 gm.) of soil were taken from the main samples by means of a sterile nichrome needle with a flattened tip, which was then used to disperse the soil aggregate in a drop of sterile water on the surface of the agar medium. The particles were distributed evenly throughout the surface by shaking and rotating the dish.

Two media, rose bengal malt agar and 2% malt agar were used for each sample for isolating purpose. Two duplicates for each medium, each sample were set up. The soil plates were then incubated at room temperature (about 20°C) and examined at intervals during two months for isolating and recording of fungi. The examination was carried out under the microscope.

### B. Modification of Immersion Method (Chester 1940)

50 ml. plastic beakers were used instead of glass tubes. Several 1 mm. holes were bored through the sides of the beakers They were wrapped tightly in aluminium foil and sterilized by putting into boiling water before the agar medium was introduced. 0.2% malt agar was used at the beginning but it was soon discovered that the concentration of nutrient was too low for soil fungi. 2% malt agar was used instead. The unwrapped beakers of medium were buried underground at 30 cm. and 80 cm. levels in each zone along the two established transect lines. The beakers were removed after 2 weeks. The agar medium was cut and transfered individually to petri dish for culturing and isolation.

For each method the fungus species were recorded against the depth and region of the profile in which they occured in each zone. A fungus was given a positive record if it occured on one or more of the duplicate plates.

#### 2. Method of Slide Mounting

For accurate identification, the fungi were examined on slides using a sticky mixture and Amann's medium. A drop of sticky mixture was spread out on a slide and left until it was dry. The slide was pressed gently on the culture with the sticky surface downwards. The fungi appeared nicely under the

IV-2

microscope after staining in Cotton Blue in Amann's medium. This method was developed specially for fungi having powdery spores.

3. Media employed

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r medium	· .
	02 gm.
	20 gm.
	10 gm.
	1000.ml.
<u>m</u>	
	2 gm.
	20 gm.
	1000 ml.
	20 gm.
	20 gm.
· ·	1000 ml.
	<u>m</u>

d. sticky mixture

Approximately 1 yard of sellotape was placed in a beaker of 50 ml. of xylol. The un-dissolved part of the sellotape was removed after 20 minutes. : . \*

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Phenol (pure crystal)	• •	'20 gm.
 lactic acid		20 gm.
glycerine	•	40 gm.
distilled water		20 gm.
Cotton Blue		trace amount

### Appendix V

Kulczynski's Square (1939)

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The method employed was the simple self structuring, agglomerative technique (Lambert and Dale 1964) of Kulczynski (1939).

This is based on an index of similarity using the

## formula:

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$$S = \frac{c/a + c/b}{2} \times 100$$

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where S = similarity

a = number of species in sample 1

b = number of species in sample 2

c = number of species common to both samples

A matrix of their coefficient are calculated and then rearranged to give the best pattern of similarity. The resulting figure is called a Kulczynski's square, or triangule when a half matrix is used.

