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Quantitative Methods for Estimating the Distribution of Soil Fungi

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By J. E. C. Aberdeen

The applications of the species-volume curve and frequency percentages in studying the ecology of soil fungi are discussed.

Frequency percentages are used to develop a new method for estimating the average size and density of the colonies, or the volume of soil occupied, for any particular species of fungus.

The species-volume curve is used to give an estimate of the extent of aggregation for the entire community of fungi present in a soil sample.

To facilitate the above studies modified methods were developed in the cultural technique. A weak agar solution was used for all dilution work to assist in even distribution of soil particles. A simple rapid method was introduced for measuring out soil quadrats of known volume.

Introduction

The outstanding difficulty in the study of any soil micro-organism is our inability to see it and to investigate it *in situ*. Added to this there are the problems associated with the ecology of the higher plants, such as the meaning to be attached to the word "individual" and the extent to which "individuals" are aggregated. The few direct methods available for the study of soil fungi are very limited in their scope so indirect methods are more commonly used in ecological studies on these communities. Quantitative estimations are usually expressed in terms of the number of colonies which develop when a known amount of soil is mixed into a particular medium and incubated within a certain temperature range. The interpretation of these results is difficult as all soil microbiologists realise because all cultural methods are selective and there is no certainty that the number of colonies measures, even relatively, the amount of active mycelium present.

The present paper shows that this uncertainty can be overcome to some extent by developing the methods applied to the quantitative study of the distribution of the higher plants, particularly those based on the presence or absence of a species in a quadrat of known size. This approach has already been utilised by Warcup (1951) in a number of clear articles on fungal ecology, but only in a general way, no attempt being made to put the results on a sound foundation. The two quantitative concepts discussed in the present paper are frequency percentages and the species-area curves.

Materials and Methods

Culture techniques

The estimation of both frequency percentages and species-area curves requires a convenient method for measuring out varying sized samples of the population under consideration. For higher plants this is based on area, on the assumption that the number of individuals per sample is proportional to area. For soil work volume or weight can be used in the same way. The weighing out of small soil samples, such as several milligrams, is far too tedious for routine work when considering a relatively large number of samples, though this combined with dilution techniques, has been the usual basis for all quantitative estimations for soil samples up to the present.

The mixing of a soil sample in sterile water or other dilutent prior to the final extraction of the required amount of soil is also undesirable as a freely sporing species is given an undue advantage. A suitable method for quadrats in soil microbiology must utilise an unmixed

sample of soil and yet measure the amount of soil with reasonable accuracy. Warcup (1951) emphasised the first point but did not develop the second. In the present experiments, brass plates of varying thickness were taken and holes of different diameters bored through them. In this way a series of measures from approx. 0.5 cu. mm. upwards was obtained. The plate containing the required hole was placed on a flat solid metal surface and a sample of soil pressed firmly into the hole, the soil sample being distributed as little as possible. The soil was then smoothed off level with the surface of the metal plate. For experimental purposes each sample was regarded as a sphere of volume equal to the sample and its equivalent radius was calculated and used for further estimations. With the smallest quadrats the soil particle size in sands and sandy-loams is comparable to the dimensions of the measuring device which would increase the variation between samples for those soils as compared with those of a high clay or silt content. Whenever colony counts would allow it each quadrat of soil was plated out in a 10 cm. petri dish, the cultures being poured as outlined by Warcup (1950). With large counts, however, distribution over a number of plates was necessary.

Warcup (1950) also demonstrated the importance of the soil fragments as a source of a number of species, which point is readily confirmed by experience. To achieve an even distribution of solid particles throughout a number of plates the sample was taken up in a weak sterile agar solution (0.15–0.20 %) instead of water before being divided. The strength used varied with the atmospheric temperature, the objective being to keep the particles suspended or to reduce the falling rate to such a low figure that sufficient time was allowed after shaking to ensure a reasonably even distribution of the soil particles throughout the plates. Similarly if dilutions were made for quantitative work the same medium was used. Relatively large rock fragments still caused trouble but in most soils these can be neglected.

The medium used was a modified Czapek's similar to Warcup's except that the sugar was reduced to 0.1–0.3 % and rose-bengal (60 p.p.m.) was used as the bacteriostatic agent. Rose bengal was later added to the weak sterile agar solution as the dilution of the medium on pouring the plate sometimes reduced the concentration of rose bengal to such an extent that an excessive number of bacterial colonies developed.

Mathematical treatment

The Use of Frequency Percentages: The relative rapidity with which frequency percentages can be obtained has led to their wide use in plant ecology. Unfortunately, however, the lack of understanding of the conditions underlying this method has led in many cases to a false picture of the plant association under consideration. The defects have been pointed out clearly by Blackman (1935) and Ashby (1935) among others and more recently by Goodall (1952), while Williams (1950) and Preston (1948) have discussed the relationship of these values to certain theoretical populations. Goodall (1952) summarises his section on frequency as follows: "Certainly the frequency found reflects certain absolute characteristics of the vegetation, as well as the size and distribution of the quadrats used; but it combines so many (density, distribution, and in many cases size of individuals), and unites them in so complex a fasion, that it is not usually possible to argue back from the frequency to the features of the vegetation on which it depends." Aberdeen (1954) listed the underlying factors and shows how plant cover and size of quadrat and density of individuals are related to frequency figures, assuming a random distribution. In cases of extreme departure from random distribution, *i.e.*, very obvious aggregation, this last formula would then apply to group units rather than units of one individual. With fungi any fragment of mycelium is capable of developing into a new colony. The word "individual" cannot be used in its proper sense to include all such fragments so the term "unit" will be employed to cover any fragment or life form of a fungous species which is capable of growth.

In spite of the complex relationships involved frequency figures have been recommended or used for estimates of population composition by a number of workers. The methods suggested are as follows:

(i) Curtis and McIntosh (1950) suggested that up to values of about 80 %, frequency

percentages can be used to estimate approximately relative densities assuming random distribution. Quadrat sizes giving 70–80% frequency for dominant species are suggested.

(ii) Blackman (*ibid*) showed that density is inversely proportional to the logarithm of absence, *i.e.* (1 minus the frequency value as a proportion) both theoretically and in practice even if there are departures from a purely random distribution. The slight deflection of the regression line from the zero in fig. 11 of Blackman's article for *Trifolium repens* is probably due to plant unit size.

(iii) By using two or more different sized quadrats some estimate of cover, etc., can be obtained Archibald (1952), Aberdeen (*ibid*).

A method similar to the first has already been utilised in a simple way in soil microbiological work by Warcup (*ibid*) and Tresner *et al* (1954) without any attempt to use quadrats of known size. It appears to be the obvious method for exploratory work on a particular problem. It is better not to interpret the figures as a measure of densities, but to retain them as frequency percentages or proportions. As such they can be put on a statistical basis.

Method (ii) is a refinement of the previous and puts the work on a sounder theoretical foundation as an estimate of density. Blackman (*ibid*) discusses the relationship with density of individuals and density of cover. It has not been used in soil work.

The third enables an analysis of the population to be made and a somewhat clearer view of the individual or unit to be obtained. The note by Aberdeen (1954) only mentions its application to quadrats of two dimensions. Considering now the third dimension necessary for soil work, and again assuming that Poisson's distribution is applicable the proportion of samples not containing a particular species is obtained from the equation:

$$a = e^{-m}$$

where m = average number of individuals, or units per sample
a = proportion of samples containing no individuals or units.

therefore $a = e^{-vd}$
where v = volume of sample or quadrat
d = density of the species

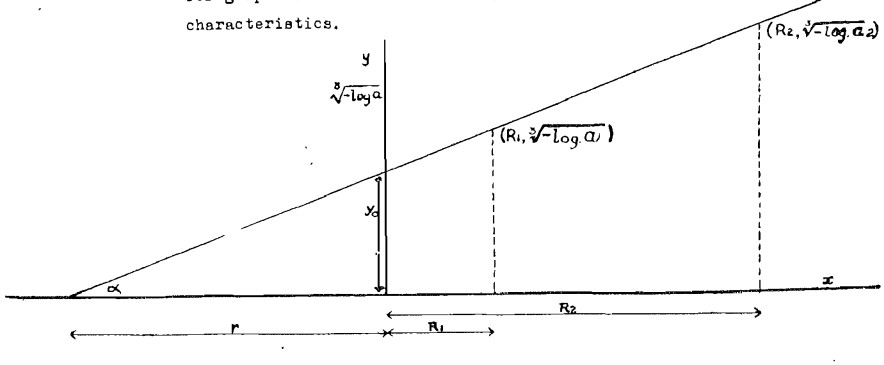
therefore $a = e^{-4/3\,\pi\,(R+r)^3 d}$
where R = radius of quadrat
r = radius of plant or fungal unit.

This equation develops from following the same line of reasoning as given by Aberdeen (*ibid*).

therefore $\log a = -4/3\,\pi\,(R+r)^3 d \log e$ (1)
therefore $\log a = k\,(R+r)^3 d$,
where $k = -4/3\,\pi \log e$.

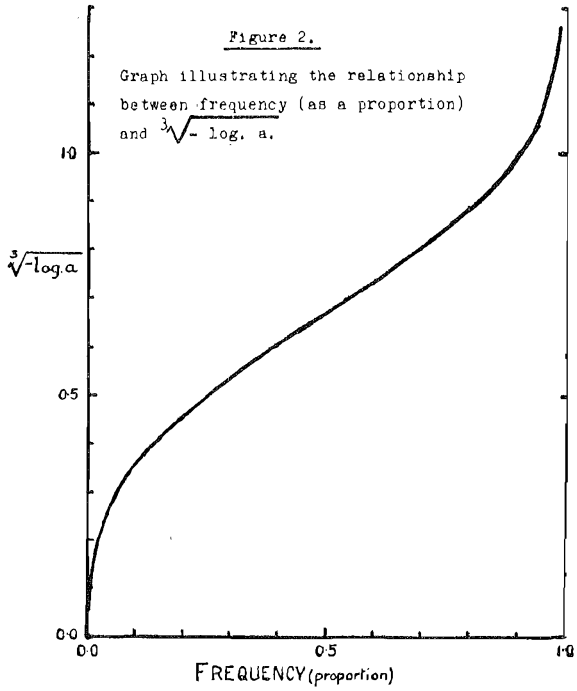
Figure 1.

Diagram illustrating the relationships used for graphical estimation of fungal colony characteristics.



If the size of the fungal unit is to be neglected the relationship used by Blackman (*ibid*) holds, *i.e.*, $log\ a \propto d$, for a particular sized quadrat. If point quadrats are used, then R becomes negligible and $log\ a$ is directly related to volume, or cover for two dimentions.

For rapid estimation of fungal unit size a straight line relationship suitable for graphing was deduced as below. From equation (1) above



$$R + r = \sqrt[3]{\frac{1}{4/3\ \pi\ log\ e}} \cdot - \sqrt[3]{\frac{log\ a}{d}}$$

therefore for a constant density

$$R + r \propto \sqrt[3]{-log\ a}.$$

Figure 1 shows the form of graph that can be used. The radius of the fungal unit, r, is unknown, but by plotting $\sqrt[3]{-log\ a}$ as "y" against R as "x" and continuing the regression line until it cuts the negative x axis it can be seen that this intercept will be $-r$. The relationship between frequency as a proportion and $\sqrt[3]{-log\ a}$ is shown in figure 2.

From equation (1) and figure 1

$$d = \frac{1}{4/3\ \pi\ log\ e} \cdot \tan^3 \propto$$

If y_0 is the y intercept of the regression line

$$\text{then } y_0^3 = \frac{4}{3}\ \pi\ r^3\ d\ log\ e$$
$$= V_f\ log\ e$$

where V_f = volume of soil occupied by the fungal species under consideration. Thus estimates can be obtained for the average radius of the fungal unit, the average density of the units and the proportion of the soil occupied by the fungus.

The species-area curve: Goodall (1952) has reviewed succinctly the published work on the species-area curve for higher plant populations. In discussing its application to soil fungi ecology the term "species-volume curve" however, will be used. This curve has two possible uses in the latter sphere, (1) to estimate the index of diversity as outlined by Williams (1944) for higher plant associations and (2) to give an estimate of the overall effect of aggregation in a soil population. The latter is a new application and will be discussed in more detail.

The species-area curve for a randomised population reflects the influence of two factors: (1) the relative density of the species, and (2) the rate of increase in numbers of species with the size of sample (the index of diversity). In practice the relationship approximates a straight line for both animal and plant populations when the number of species is plotted against the logarithm of area, volume or time. This holds quite well for higher plant populations, *e.g.*, Pidgeon and Ashby (1940) and Myers and Chapman (1953). The writer has found it approximately true for soil fungi populations (fig. 6). The slope of the line now gives a direct reading of the index of diversity. After discussing the application of his basic theory to very large animal populations and showing that it may not be correct, Williams (1951) points out that the index can be retained in an empirical form, *e.g.*, as the relative increase in number of species for a tenfold increase in sample size, and so be read off from the straight line relationship. This empirical approach is equally applicable to plant populations.

The extension of this work in the treatment of plant associations in terms of Williams' theory, demonstrated a number of exceptions, *e.g.*, Jones (1945) obtained two different values for the index of diversity from the same population by using different sized quadrats and Myers and Chapman (*ibid*) found no consistent correlation between actual numbers of individuals and estimations made by Williams' method. In discussing Jones' results, Williams (1945) points out that the differences are due to aggregation of individuals of species and that the individuals of some species were relatively large compared to the quadrat size, and indicated that the true index of diversity was obtained by using quadrats large enough to eliminate the effect of aggregation and individual size. No doubt these same factors influenced the results of Myers and Chapman (*ibid*). Gleason (1922) noted that the species-area curve rises more steeply if the larger samples were made up by combining a number of smaller ones selected at random than if separate determinations were made for each sample or quadrat size. He then points out that the former method gives a curve that approaches nearer to that which would be developed if the population was truly randomised. This was verified by Blackman (*ibid*). This same relationship between the two curves was also found to be present by the writer in those built up from soil fungi populations (see fig. 6). The true species-volume curve therefore must be developed from separate determinations for each size of quadrat. Thus it is now clear that a third factor, the extent of aggregation including displacement of species, must be added to the previous two as influencing the form of the species-area and species-volume curve for plant and fungal communities, and that a measure of this factor is possible if the curve for a truly randomised population can be established.

Given a community of higher plants that show aggregation or contagious distribution it is not possible to convert that population into a community with random distribution. A sample of soil, however, can be thoroughly mixed, the aggregates of soil particles broken down, and a number of small samples of uniform size taken from it. Then the species-volume curve built up from a combination of these small volumes must approximate very closely to that obtainable from a completely random distribution of the individual species present. All fungal species are now most likely present as approximately equal sized units, *i.e.*, mycelial fragments and spores. So the departure of the true species-volume curve (obtained from separate determinations for each quadrat size) from this curve can be used as a measure of the aggregation present in the population as a whole. If the results are plotted with number of species against the logarithm of the volume the regression line for the true species-volume data first indicates a lower index of diversity than, but should later curve upwards to meet the regression line obtained from sampling the random distribution. The point at which it rejoins is the point above which Williams (1945) recommends that quadrats be selected.

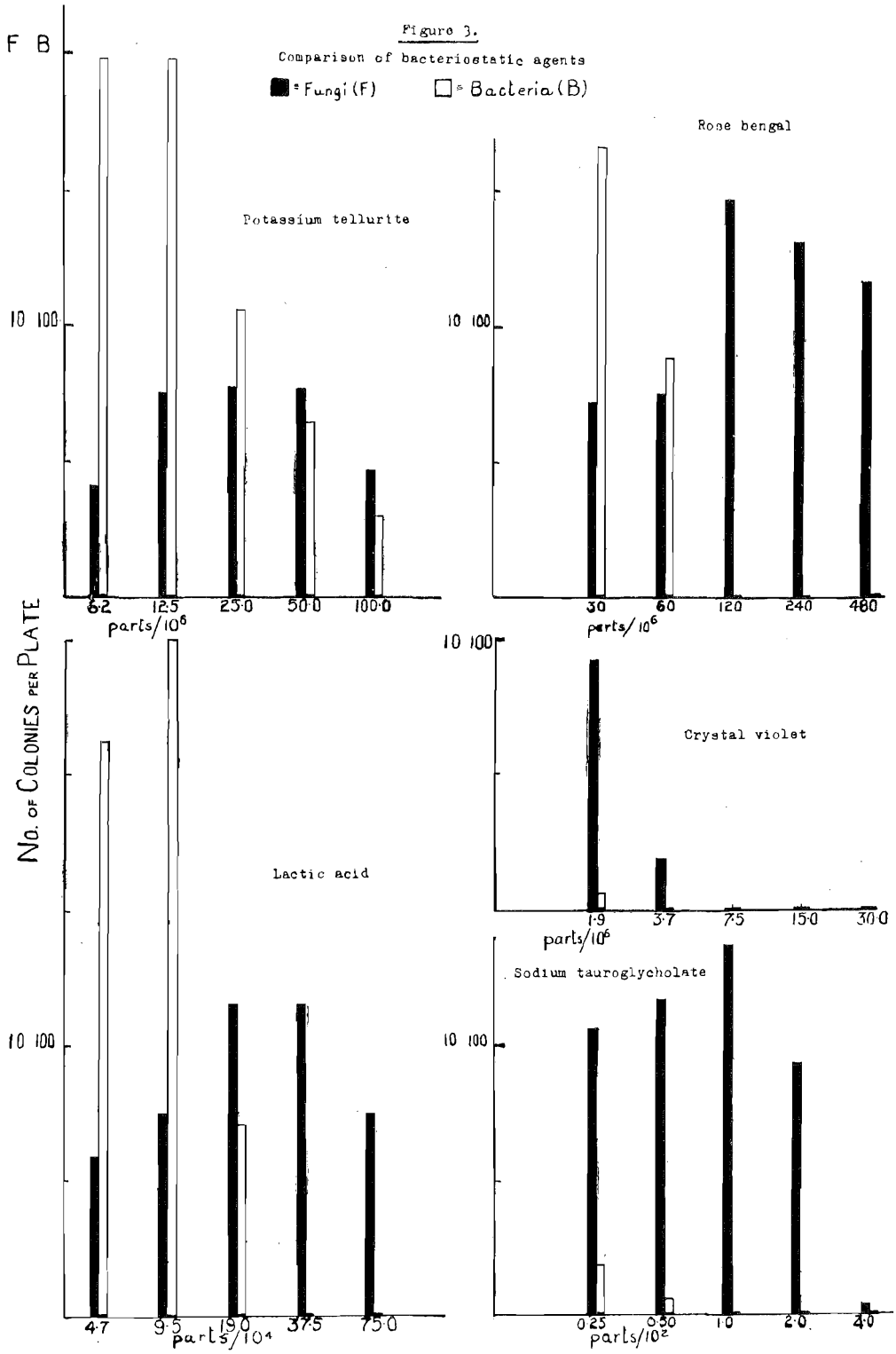


Figure 3.
Comparison of bacteriostatic agents
■ = Fungi (F) □ = Bacteria (B)
F B
Potassium tellurite
Rose bengal
Lactic acid
Crystal violet
Sodium tauroglycholate
10 100
No. of Colonies per Plate
6·2 12·5 25·0 50·0 100·0
parts/10⁶
30 60 120 240 480
parts/10⁶
4·7 9·5 19·0 37·5 75·0
parts/10⁴
1·9 3·7 7·5 15·0 30·0
parts/10⁶
0·25 0·50 1·0 2·0 4·0
parts/10²

Goodall (1952, p. 205) also mentions this point in discussing Rommell's Homogenetätsgrenze. The slopes of these two lines in their early stages could possibly be used as a basis for estimating the departure of the population from a random distribution.

Experimental Results

Choice of bacteriostatic agent

A number of trials were run investigating bacteriostatic agents. Figure 3 shows the results of one trial and illustrates the general relationships between varying concentrations of a number of such agents and the counts of fungal and bacterial colonies. Four replications of each concentration were used and the plates all inoculated from the same soil suspension.

Rose bengal, sodium tauroglycolate and acidification showed the most promise. Rose bengal was finally selected for the general survey work as it restricted the average size of fungal colonies and allowed a greater ease in sterilisation and gave consistent results. Martin and Harding (1950) indicated that rose bengal allowed a wider range of genera to develop than acidification, but the results of the trials conducted by the author were not consistently in favour of any one treatment. In the trial shown in fig. 3, 120 p.p.m. of rose bengal gave the optimum fungal counts. In others the range of 60–120 p.p.m. showed no significant differences. The concentration of 60 p.p.m. was used in most of the work but this occasionally breaks down, as also noted by Martin (1950). Apparently certain groups of bacteria can tolerate this concentration of rose bengal under suitable conditions, when the 120 p.p.m. gives the optimum count. Above this concentration there is a distinctly fungitoxic effect. In the range 60–120 p.p.m. the optimum count of fungi is first reached at the concentration which reduces the bacterial count to a figure so low as not to inhibit fungal growth.

Choice of medium

Warcup's modification of Czapek's medium was compared with a number of other formulae, which use other sources of nitrogen such as casein hydrolysate, malt extract, and ammonium nitrate and also varying sugar concentrations. The final medium used was a sucrose-nitrate-yeast extract formula similar to Warcup's except that the sugar was reduced to 0.1 or 0.3 per cent. As there is little to choose between many of these media for general work preference was given to the simpler form. There is no sure guide to the selection of a medium for optimum results in a particular problem so each individual must solve this problem himself. McLennan and Ducker (1954) after comparison with Warcup's modification, gave preference to a more complex medium of dextrose-peptone-malt extract for their particular investigation. As Garret (1952) suggested the secret of soil work seems to be bound up with the use of selective methods of culture, adapted to the need in view.

Table 1 gives the results of one experiment comparing different sugar concentrations.

TABLE 1

Comparison of sugar concentrations for isolation media

Number of colonies per plate		Number of species per plate	
3% sucrose	0.1% sucrose	3% sucrose	0.1% sucrose
19.5	26.2	2.8	4.2

Both significant at P = 0.01

Results favoured the lower sugar concentrations for total number of colonies and total number of species.

Frequency percentages as a measure of density of fungal units

Relative Density: The first two methods of utilising frequency percentages to estimate relative densities, *i.e.*, direct frequency percentages and the logarithm of the absence proportion, are of course very approximate. The extent to which they can be used for a true comparison of densities is dependent on the quadrat being small relative to the colony size. However, they are still useful methods and in many cases are the only ones available. The results given in Table 2 show that consistent results can be obtained from a restricted volume of soil. The sampling problem involved in dealing with very large areas requires further investigation. Also shown are the corresponding values for minus *log a*, which have been shown to be approximately proportional to the densities. For investigating significant differences the standard deviation for each value can be obtained from the equation $SD = \sqrt{npq}$ where n is the number of quadrats used, p the proportion showing the species present, and $q = 1 - p$.

TABLE 2

Percentage frequencies of fungal species in a sample of garden soil (10 quadrats per estimate)

Species	Sample 1	Sample 2	Combined Sample	
			Frequency	— log a
Aspergillus nidulans ..	100	90	95	1.30
Trichoderma viride	40	40	40	0.22
Chaetomium sp.	50	70	60	0.40
Penicillium sp. 1	10	20	15	0.07
Cladosporium	30	50	40	0.22
Aspergillus niger	10	40	25	0.12
Penicillium sp. 2	20	30	25	0.12
Helminthosporium sp. ..	10	10	10	0.05

Absolute Densities: For this experiment a sample of sieved garden soil was divided into two parts, one being sterilised in the autoclave. Then the unsterilised soil was thoroughly mixed in varying proportions with sterilised soil. The mixtures were 80%, 20% and 5% unsterilised soil and were each passed twenty times through a soil divider, the fractions being mixed together before the next passage. Though the soil was damp the particles were free running and a thorough mix was obtained, but there was no grinding of fragments. The soil was packed into large petri dishes (15 cm.) and incubated at 25° C. The first samples were taken the day after mixing and plated out in the 0.3% sucrose-nitrate-yeast extract medium. The soil from the larger quadrats was suspended in dilute agar and distributed throughout a number of plates. Table 3 gives the results obtained with four fungi, showing frequency proportions for the different sized quadrats and the calculated densities assuming the fungal colonies to be of an average radius 0.0 and 0.1 mm. The densities were calculated after determining the tangent of the angle between the regression line and the x axis graphically (see figure 4).

For each of the lines drawn above it has been assumed that the fungal unit was of 0.1 mm. radius after mixing and that the line must go through that point.

The ratios of the calculated densities of the three mixtures tend to be higher than the known ratio of 4 : 1 if the fungal units are assumed to be of no appreciable diameter, *i.e.*, made up of spores and mycelial fragments only. However the ratios between densities on assuming an average radius of 0.1 mm. per fungal unit are closer to the known value. As mentioned before, the soil fragments were not ground up on mixing, so it is more likely that the fungi were largely present as units of appreciable diameter rather than fragments.

TABLE 3

Frequency values and calculated densities of fungal units for four species of soil fungi in three mixtures of known relative densities.

Fungal species	Soil mixture % unsterilised soil	Size of quadrant: Vol. cu. mm.	Size of quadrant: Equiv. radius mm.	Frequency (*a*)	Calc. densities (units per cu. mm.) (*b*)	Calc. densities (units per cu. mm.) (*c*)
Aspergillus nidulans	80	0.32	0.42	.27	1.3	.77
		1.4	0.69	.87		
	20	3.2	0.91	.40	.27	.21
		7.1	1.2	.93		
	5	7.1	1.2	.53	.12	.10
		22.0	1.7	.93		
Trichoderma viride	80	0.32	0.42	.47	2.0	1.23
		1.4	0.69	.93		
	20	3.2	0.91	.47	.24	.18
		7.1	1.2	.87		
	5	7.1	1.2	.40	.064	.053
		22.0	1.7	.87		
Mucor sp.	80	0.32	0.42	.47	1.1	.67
		1.4	0.69	.67		
	20	3.2	0.91	.60	.24	.19
		7.1	1.2	.80		
	5	7.1	1.2	.40	.070	.057
		22.0	1.7	.73		
Aspergillus niger	80	0.32	0.42	.07	1.1	.67
		1.4	0.69	.93		
	20	3.2	0.91	.47	.20	.15
		7.1	1.2	.80		
	5	7.1	1.2	.27	.031	.026
		22.0	1.7	.40		

(*a*) Fifteen quadrats of each size.

(*b*) Assuming the fungi were completely fragmented on mixing.

(*c*) Assuming an average radius of 0.1 mm. per fungal unit.

Frequency percentages as a measure of the total growth of a species

The soil mixtures mentioned in the previous sections were resampled after 10 days in the incubator and frequency percentages again estimated for two sized quadrats for the 80% mixture. The results obtained are listed in Table 4.

TABLE 4

Frequency percentages after 10 days incubation, with calculated total growth

Fungus	Frequency percentages for quadrat of radius 0.42 mm.	0.69 mm.	0.91 mm.	Calculated volume of soil occupied by fungus cu. mm. per cu. mm.
Aspergillus nidulans	60	80	—	0.29
Trichoderma viride	87	93	—	1.0
Mucor sp.	27	67	—	0.23
Aspergillus niger	33	40	—	0.20
*Chaetomium sp.**	10	—	29	0.018
W_6	14	—	14	0.16
*Penicillium sp.**	14	—	29	0.062

*21 quadrats of each size.

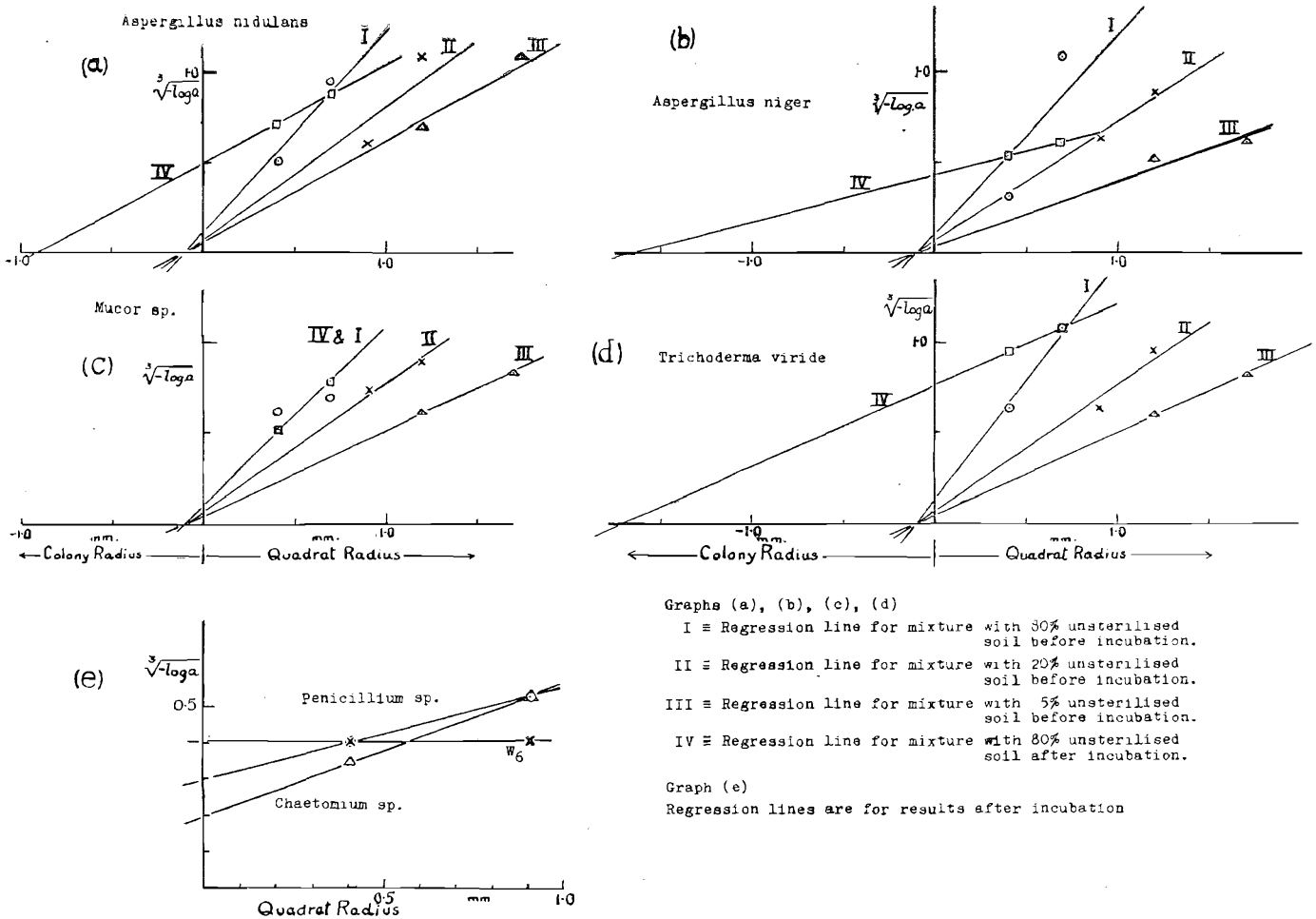


Fig. 4—Graph showing relationships between $\sqrt[3]{-\log a}$ and radius of quadrat for a number of species.

These results were graphed as previously and also shown in figure 4. *Trichoderma viride, Aspergillus nidulans,* and *Mucor sp.* show an increase in the average radius of the fungal colony, with a decrease in angle α, *i.e.*, a reduced number of colonies per cu. mm. This is as expected for in a random distribution a proportion of the original centres of growth are relatively close to each other and will grow together as colony size increases. The figures for *Aspergillus niger* are too variable for any reliable conclusions, but are mentioned because of the unexpected low frequency figure after incubation for the quadrat with 0.69 mm. radius. Such a result is explained on the basis that a number of the original fungal units have been rendered non-viable by some adverse factor, but that the remaining ones have later made considerable growth.

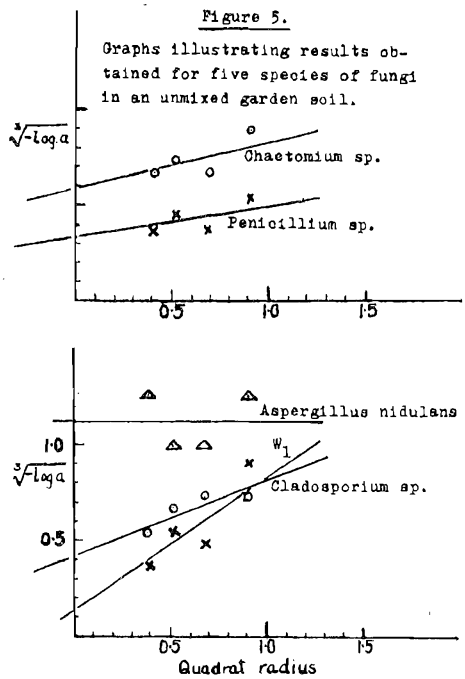


Figure 5.
Graphs illustrating results obtained for five species of fungi in an unmixed garden soil.

As has been shown above the volume of the soil occupied by a particular species is proportional to y_0^3 where y_0 = intercept on the y axis cut off by the regression line. Calculating the volumes from the previous graphs it is seen that all of the moulds considered have made considerable growth. Several other species, *Penicillium sp.*, *Chaetomium sp.*, and W_6 (unidentified) which had not been considered in the analysis made immediately after the mixing of these samples were examined in the later analysis and the picture was generally the same. One species gave the same frequency for two quadrats, the second quadrat being ten times the volume of the first, twenty-one quadrats being taken in each case. In this case the fungus is interpreted as being in the form of one or very few large colonies. The calculated proportion of the soil occupied by these fungi is also shown in Table 4 and the graphs in figure 4*e*. The estimate for *Trichoderma viride*, viz., 1.0 cu. mm., occupied per 1.0 cu. mm. of soil, yet the frequency proportion was less than one, is accounted for by the fact that the overlapping of colonies must be considerable when high frequencies and relatively large colonies are present.

Prior to the above technique being evolved, several trials had been conducted on a garden soil which had not been subject to mixing or any other artificial conditions in order to determine the optimum size of quadrat for investigating that particular soil. Four sizes of

quadrats had been employed and ten samples of undisturbed soil had been taken for each quadrat size. The results of these trials were reinvestigated and graphed for five species. The results are variable due to the low number of quadrats for each estimate of frequency but are sufficient to indicate the relative growth of the different species (figure 5). The frequency proportions for *Aspergillus nidulans* for the smallest and largest quadrats was 1.00. This has been graphed as 0.99. Here again due to overlapping of colonies the volume occupied would be much greater than 1.0 cu. mm. per cu. mm. of soil.

The Species-volume curve and the Estimation of Index of Diversity and Aggregation

This was investigated for a garden soil. Quadrats of four different sizes were used, the number of species for each size being averaged from ten quadrats. In addition it was possible to build up a curve by lumping the results of the ten small quadrats. The general relationship was similar to that for higher plant associations, *i.e.*, the curve built up by

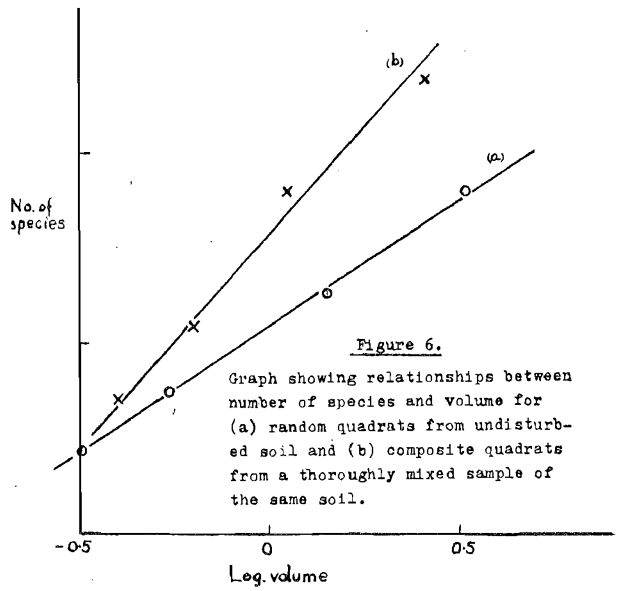


Figure 6.

Graph showing relationships between number of species and volume for (a) random quadrats from undisturbed soil and (b) composite quadrats from a thoroughly mixed sample of the same soil.

lumping small quadrats to give a value for the larger quadrats rises more steeply. On the log. volume transformation, though a straight line could have been readily fitted in both cases there was the suggestion of a curve in the case of the true species-volume curve.

A second trial with a sample of a different soil was used to check the true species-area curve with the one built up from a combination of numerous small quadrats after thoroughly mixing the soil and breaking up the soil aggregates as much as possible. The results were very similar to the previous case and are shown in figure 6. Thus any soil fungal community (or higher plant community) is capable of giving a range of values for the index of diversity from that which has been defined as the "true" index of diversity to that built up from the randomised plant units, which might be termed the "basic" index of diversity. The coefficient of aggregation could then be defined as $1 - \frac{\text{"true index of diversity}}{\text{"basic" index of diversity}}$. In the case illustrated in figure 6 this would be 0.42 approximately. From this definition a thoroughly randomised association would have a coefficient of aggregation of zero.

Correlation between Colony Counts and Frequency Percentages

For the quantitative estimation of soil fungi by colony counts several conditions must be fulfilled—(1) the soil fragments must be thoroughly broken up to ensure that all mycelium

is released; (2) equal lengths of mycelium of different species must on average give equal numbers of fragments; (3) none of the species must be sporing profusely. It is doubtful, however, whether all types of mycelium will fragment with equal facility and one can never be sure whether spores are present or not. Consequently there is always an element of doubt. McLennan and Ducker (1954) give evidence to indicate that such colony counts are correlated to some extent with total mycelium counts and Tresener *et al* (*ibid*) state that in their opinion the sporing of soil fungi is rather meagre.

The evidence from this work supports the above broad conclusion, as the species which gave the highest colony counts usually gave the highest frequency percentages. On the other hand, it is not difficult to find exceptions, *e.g.*, in one experiment *Aspergillus niger* was present in fourteen out of twenty-one quadrats with a total number of colonies of seventeen. Two other fungi present in only one quadrat each, showed colony counts of fourty-one and one hundred and forty colonies for their respective quadrats.

Discussion

In considering the above methods certain limitations should be pointed out. There is little doubt that some fungi form compact colonies and others sparse colonies, *i.e.*, the density of mycelium within the volume occupied by the fungus varies and the above methods do not differentiate such cases. A combination of colony counts with frequency percentages would help to some extent but does not eliminate the possible influence of spores. Also it is easy to picture a fungus whose mycelium is widespread but the unoccupied volumes between the strands are so large that it is possible to take quadrat samples and miss the species in an appreciable number of cases.

In all discussion random distribution of the units has been assumed, yet aggregation has been demonstrated on the other hand. The basic assumption is really that the fungus is in the form of aggregates (presumably in most cases as colonies) which are distributed at random. If colonies themselves tend to be grouped together than the method compensates to some extent by demonstrating a larger average radius and fewer colonies.

Some of the results from undisturbed soil samples indicate that the colony radius is extremely small. The interpretation must be cautious but if sufficient quadrats are used to give reliable results such a result indicates that the species is present as some small discreet form, *e.g.*, a chlamydospore, or some other compact resting body.

The index of diversity is a useful measure of the richness of the flora. It hardly seems wise, however, to assume a straight line relationship of species against log. volume and extrapolate beyond the experimental results when aggregation of a species into a colony and antibiotic displacement of species is very likely present. The curve built up from small samples taken from a thoroughly mixed soil might be amenable to a basic mathematical formula but one which is first affected by interaction of species and possibly at a later stage approaches a random distribution when quadrats are large enough to eliminate interactions and unit size is better treated in an empirical manner until considerably more data is available.

A suggested basis for estimation of the index is to fix the lower sized quadrat in the population to be considered so that approximately 3–5 species are present on an average per quadrat and then to estimate the number of species present in a large quadrat of constant relative size, *e.g.*, tenfold the original quadrat.

The extent to which a statistical basis can be developed in this work is not clear at present. Though there is a reasonable means for comparing frequencies, *e.g.*, for one species in different communities, different species in the same communities, or the comparison of communities when a series of frequency percentages for a number of common species is known. One obvious problem is that of sampling for larger volumes of soil. The approach suggested by Goodall (1952b) for analysis of point quadrat results might be applicable. Soil microbiology, however, has its own difficulties in this line.

The problem of a suitable quadrat size for plant ecological studies is often solved for each community by means of the species-area curve. Considering the work above this does not seem to be a suitable method for soil work. The problem is more akin to that of a grassland as the life forms of the various species tend to be uniform and the common species have a high coverage. So the use of a large number of small quadrats is favoured for any preliminary investigation of a soil.

Also these results could be used to investigate fidelity and indicator values in the same way as outlined by Goodall (1953).

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