

# Diversity of soil fungi in a tropical deciduous forest in Mudumalai, southern India

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**An analysis of the diversity of sporulating fungi monitored over two consecutive wet seasons in the Mudumalai forest reserve of the Western Ghats, southern India, revealed a total of 46 operational taxonomic units. A majority belonged to the class Deuteromycetes, followed by Ascomycetes. *Mortierella* was the dominant genus followed by *Fusarium* and *Penicillium*. We observed a mean of  $5.63 \pm 2.37$  and  $7.63 \pm 2.97$  genera per hectare and estimated a 50 ha-wide total of 43 and 69 genera during the two seasons respectively. We estimate the number of species in the 16 hectares sampled as 207 and 284 respectively. The mean fungal population density over the same two seasons was  $2.67 \pm 1.49 \times 10^4$  and  $2.14 \pm 0.69 \times 10^4$  cultivable clones per g of soil. Indices of diversity were 3.1 and 3.6 (Shannon–Wiener), 5.3 and 8.8 (Fisher’s alpha) and 0.79 and 0.86 (Simpson) respectively, all in terms of genus as OTU.**

**Keywords:** Abundance, biodiversity, diversity indices, fungi.

TROPICAL forests cover only 7% of the earth’s land surface, but are believed to contain at least 50% and perhaps as many as two-thirds of all species<sup>1,2</sup>. Fungal species are especially important components of biodiversity in tropical forests. As major contributors to the maintenance of the earth’s ecosystem, biosphere and biogeochemical cycles, fungi perform unique and indispensable activities on which larger organisms including humans depend<sup>3</sup>. From the late 1940s, there has been a growing interest in soil mycology and soil-borne fungal diseases of plants, and this too has motivated studies on soil fungi and their ecology<sup>4,5</sup>. Though numerous species of fungi have been reported from the Western Ghats<sup>6,7</sup>, there appears to have been no study related to the diversity or dynamics of fungal populations in its forest soils<sup>5</sup>. General ideas about species diversity suggest that habitat heterogeneity is a major factor controlling diversity<sup>8</sup>.

We present an account of the diversity of soil fungi at the genus level in a 50 ha plot within a tropical deciduous forest in southern India. We have examined the structure of the fungal community at a depth of 0–5 cm as defined by population density, relative abundance and various meas-

ures of diversity during two consecutive wet seasons. Species-level diversity and estimates of intraspecies genetic diversity within the genera *Penicillium* and *Aspergillus* will be presented subsequently.

## Methods

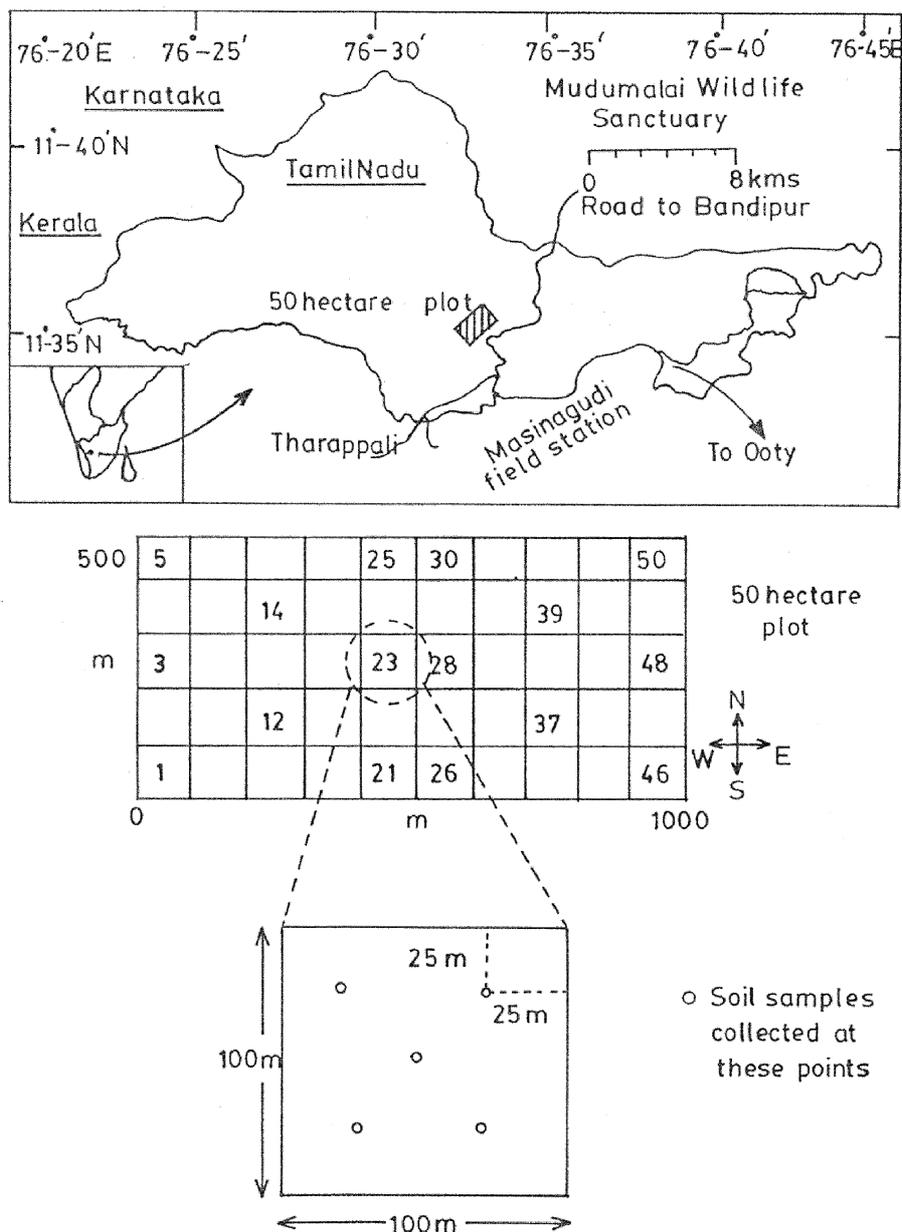
The 50 ha block of forest studied is situated in the 321 sq. km Mudumalai Sanctuary (within the Nilgiri Hills, Tamil Nadu, southern India; 11°32′E–11°43′N and 76°22′N–76°45′E at an altitude of 850–1250 m asl; Figure 1)<sup>9</sup>. The terrain is hilly and the soil has not been disturbed for about 30 yrs (logging was stopped in the 1960s). The vegetation in the plot consists of approximately 25,000 woody plants belonging to about 70 species<sup>10</sup>. The plot measures 1 km west to east and 0.5 km south to north, and is divided into 50 ha-sized units which are further sub-divided into 1250 quadrants of 20 m × 20 m (Figure 1). For sampling, 16 out of the total 50 ha were selected so as to represent roughly the entire range of the plot (Figure 1). Logistical problems associated with handling many samples prevented us from carrying out a more detailed survey.

The seasons at the study site include a dry season from January to mid-April, pre-monsoon season from mid-April to mid-June, and two wet seasons. Mid-June through the penultimate week of September is referred to as Wet-I (Southwest monsoon; ‘I’ because it comes earlier in the year) and September end to December as Wet-II (North-east monsoon; ‘II’ because it falls later in the year)<sup>9</sup>. Soil samples were collected thrice: 22–28 March 1995 (in the dry season, for a pilot survey), 23–28 September 1995 (during Wet-II) and 9–13 July 1996 (during Wet-I). The dry season samples were taken merely to standardize the methodology and not subjected to detailed analysis. The species isolated during Wet-I and Wet-II may include those found in the dry season as well as others, though this remains a conjecture at present.

From each selected hectare, the soil was collected (between 10:30 am and 4:30 pm each day) under sterile conditions with the help of 4 cm iron cores from four symmetrically situated locations near the corners of a square as well as from the centre to a depth of 5 cm (Figure 1). Fungi were isolated by the serial dilution method<sup>11</sup>, the level of dilution being selected to give 30–50 colonies on a single 10 cm Rose Bengal Agar (RBA)

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**Figure 1.** Location of Mudumalai Sanctuary within Nilgiri Biosphere Reserve, South India, with a schematic representation of the collection area. Individual hectares numbered as illustrated.

plate maintained at 25°C. Each colony was subcultured on RBA<sup>12</sup> prior to being maintained on potato dextrose agar (PDA) at 25°C. 50 µl of streptomycin (33 mg/ml stock) was added to 50 ml of the medium to avoid bacterial contamination<sup>13</sup>.

Standard procedures based on colony, spore and structural morphology were followed for identification at the generic level<sup>14-17</sup>. Colonies that obviously belonged to different types but could not be conclusively identified at the generic level were denoted by serial numbers (1-7) for the Wet-II batch (September 1995) and by letters (A-H) for the Wet-I batch (July 1996). Edaphic conditions were measured during all collections.

The following indices of diversity, with the genus as the operational taxonomic unit (OTU), were computed<sup>18</sup>. Ideally, the indices should be based on species-level identification. This problem was addressed (see later) by estimating the number of species corresponding to each genus in the survey. A colony obtained on the primary growth plate was identified with a single spore in that soil sample. The indices are:

Shannon-Wiener index: 
$$H' = -\sum_{i=1}^S p_i \log_2 p_i$$

where  $S$  is the number of OTUs and  $p_i$  the proportion of total samples belonging to the  $i$ th OTU.  $H'$  varies between 0

and  $\log_2 S$  and is the information content of the relevant sample (units, bits per OTU).  $H'$  close to 0 indicates low diversity, whereas a value close to  $\log_2 S$  indicates high diversity.

Brillouin's index:  $H = 1/N \log_2[N!/(n_1! n_2! \dots n_S!)]$ ,

where  $H$  is Brillouin's index (units, bits per OTU),  $n_i$  the number of individuals belonging to the  $i$ th OTU ( $i = 1, 2, \dots, S$ ) and  $N = n_1 + n_2 + \dots + n_S$ . Pielou<sup>19</sup> has pointed out that the Shannon–Wiener index is reliable only when random samples are drawn from a large community. With collections, when sampling is carried out without replacement as is done here, Brillouin's index  $H$  is a more appropriate measure.

Fisher's index ('alpha diversity'):  $S = \alpha \ln(1 + N/\alpha)$ ,

where  $S$  is the number of OTUs in the sample,  $N$  the number of individuals in the sample and  $\alpha$  Fisher's index of diversity. The assumption here is that the number of OTUs increases logarithmically with the number of individuals. If so,  $\alpha$  is a measure of the rate of increase of the number of OTUs with respect to increasing (logarithmic) population size when the size is large.

Jaccard's index ('beta diversity'):

$$S_{ab} = S_{AB}/(S_A + S_B - S_{AB}),$$

where  $S_{AB}$  is the number of OTUs shared by two locations ( $A$  and  $B$ ),  $S_A$  the number of OTUs in location  $A$  and  $S_B$  the number of OTUs in location  $B$ .  $S_{ab}$  is a measure of the extent of overlap between the OTUs in locations  $A$  and  $B$ :  $S_{ab} = 0$  if  $S_{AB} = 0$  (no overlap) and  $S_{ab} = 1$  if the OTUs in  $A$  and  $B$  are identical ( $S_A = S_B = S_{AB}$ ).

Simpson's index (modified by Pielou):

$$1 - D = 1 - \sum_{i=1}^S n_i(n_i - 1)/[N(N - 1)],$$

where  $n_i$  is the number of individuals in the  $i$ th OTU,  $S$  the total number of OTUs and  $N$  the total number of individuals. The diversity is a minimum when only one OTU exists, i.e., if  $n_i = N$  for some  $i$  and  $n_i = 0$  otherwise,  $1 - D = 0$ . It is a maximum when all species are represented equally (each  $n_i = N/S$ ). Then  $1 - D = (1 - 1/S)$  approximately for large values of  $N$ .

Evenness index (1)  $E = H'/\ln(S)$ ,

where  $H'$  is the Shannon–Wiener index of diversity and  $S$  the number of OTUs.

Chao's index (1)  $S_1 = S_{\text{obs}} + (a^2/2b)$ ,

where  $S_1$  is Chao's OTUs richness estimate 1,  $S_{\text{obs}}$  the number of OTUs observed in the sample,  $a$  the number of

OTUs made up of a single individual and  $b$  the number of OTUs containing two individuals.

Chao's index (2)  $S_c = S_{\text{obs}} + (L^2/2M)$ ,

where  $S_c$  is Chao's OTUs richness estimate 2,  $S_{\text{obs}}$  the number of OTUs observed in the sample,  $L$  the number of OTUs which occur in only one quadrat and  $M$  the number of OTUs which occur in two quadrats.

## Results

There were marked differences in soil moisture content between the dry (~4.2%) and wet (~18%) seasons, and a slight alkalinity and high temperature (both in the soil and atmosphere) was characteristic of the dry season (mean pH 7.05 (wet, 6.60–6.92); mean soil temperature, 29.5°C (wet, 23.3–24.1°C)).

### Population density and OTUs

The mean fungal population density over the entire 50 ha plot ranged from  $1.13 \pm 0.31 \times 10^4/\text{g}$  (dry) to  $2.67 \pm 1.49 \times 10^4/\text{g}$  (Wet-II) and  $2.14 \pm 0.69 \times 10^4/\text{g}$  (Wet-I). The numbers, which refer to cultivable clones, may be taken as equivalent to the number of spores recovered per gram of soil. Population density was estimated as follows. After weighing the original soil sample, mixing it thoroughly in sterile water, carrying out serial dilutions under sterile conditions and finally plating about 0.25 ml on a 10 cm agar plate, the colonies obtained were counted<sup>11</sup>. The difference between the first value and the others is significant ( $t$  test,  $P < 0.05$ ), whereas that between the latter two is not. Over the entire 16 ha-sized areas surveyed, the number of distinct fungal genera (plus higher OTUs where genera could not be identified) was 25 for Wet-II and 37 for Wet-I. The number (mean  $\pm$  SD) of genera/g of soil, which in the present study implies per hectare, is between  $5.63 \pm 2.37$  (Wet-II) and  $7.63 \pm 2.97$  (Wet-I). The number of OTUs found after combining data from both collections was 46. In what follows, we use the terms 'OTU' and 'genus' interchangeably since each unidentified OTU appeared, on the basis of morphology, to belong to a distinct genus.

### Types

The Wet-II batch (September 1995) yielded 607 independent colonies after clonal plating. These fell into 25 genera (Table 1). Most of the isolates belonged to the class Deuteromycetes (Fungi Imperfecti) followed by Ascomycetes, with just one isolate from Basidiomycetes. The majority (40%) was from the genus *Mortierella*; the next two in order of dominance were *Fusarium* (13.4%) and *Penicillium* (12.8%).

Others followed these in descending order until there were four genera, which were found just once each (Table 2). Fourteen of the 25 OTUs were rare forms, which we define as those that occur in two or fewer hectares out of the 16; overall, they were found in 12 out of the 16 ha. Classes in the Wet-I collection (July 1996) were identical to those in the Wet-II and the abundant genera were the same

**Table 1.** Fungal genera, or higher OTUs, isolated in this study (with the number of isolates of each)

| Code  | Genus                   | Wet-II<br>(September 1995) | Wet-I<br>(July 1996) |
|-------|-------------------------|----------------------------|----------------------|
| Acr   | <i>Acrostalagmus</i>    | 22                         | 39                   |
| Alt   | <i>Alternaria</i>       | –                          | 2                    |
| Amp   | <i>Ampellomyces</i>     | –                          | 11                   |
| Asc   | <i>Ascochyta</i>        | –                          | 8                    |
| Asp   | <i>Aspergillus</i>      | 23                         | 21                   |
| Ast   | <i>Asteromella</i>      | –                          | 1                    |
| Bis   | <i>Bispora</i>          | –                          | 1                    |
| Cer   | <i>Cercospora</i>       | 5                          | 23                   |
| Cha   | <i>Chaetophoma</i>      | –                          | 3                    |
| Cla   | <i>Cladosporium</i>     | 22                         | 20                   |
| Cyl   | <i>Cylindrocladium</i>  | 5                          | –                    |
| Did   | <i>Didymocladium</i>    | –                          | 2                    |
| Fus   | <i>Fusarium</i>         | 81                         | 75                   |
| Hel   | <i>Helminthosporium</i> | 2                          | –                    |
| Mor   | <i>Mortierella</i>      | 240                        | 173                  |
| Muc   | <i>Mucor</i>            | 5                          | 8                    |
| Myr   | <i>Myrothecium</i>      | –                          | 2                    |
| Nig   | <i>Nigrospora</i>       | 1                          | –                    |
| Phyt  | <i>Phytophthora</i>     | 1                          | –                    |
| Pac   | <i>Pacilomyces</i>      | 11                         | 15                   |
| Pen   | <i>Penicillium</i>      | 77                         | 96                   |
| Pes   | <i>Pestototia</i>       | 3                          | 4                    |
| Pey   | <i>Peyrenellaea</i>     | –                          | 1                    |
| Rhi   | <i>Rhizoctonia</i>      | 40                         | 16                   |
| Sca   | <i>Scapulariopsis</i>   | 2                          | 5                    |
| Sph   | <i>Sphaerosporium</i>   | –                          | 2                    |
| Spi   | <i>Spicaria</i>         | 1                          | 7                    |
| Spo   | <i>Sporothrix</i>       | –                          | 1                    |
| Sty   | <i>Stysanus</i>         | –                          | 3                    |
| Tri   | <i>Trichoderma</i>      | 21                         | 31                   |
| 1     | Monilliales (order)     | 7                          | –                    |
| 2     | <i>Chaetomium</i>       | 3                          | –                    |
| 3     | Monilliales (order)     | 2                          | –                    |
| 4     | Monilliales (order)     | 16                         | –                    |
| 5     | Monilliales (order)     | 11                         | 5                    |
| 6     | Monilliales (order)     | 1                          | –                    |
| 7     | Monilliales (order)     | 4                          | 1                    |
| A     | Monilliales (order)     | –                          | 1                    |
| B     | Monilliales (order)     | –                          | 2                    |
| C     | Monilliales (order)     | –                          | 2                    |
| D     | Monilliales (order)     | –                          | 2                    |
| E     | Monilliales (order)     | –                          | 1                    |
| F     | Monilliales (order)     | –                          | 2                    |
| G     | Not viable              | –                          | 1                    |
| H     | Not viable              | –                          | 2                    |
| UKAsc | Ascomycetes (class)     | –                          | 2                    |

Dashes (–) indicate that the particular genus was absent. Some isolates could not be identified at the generic level. Sixteen genera are common to both collections. G and H were lost during maintenance. The isolate initially labelled by code number 2 was later identified as *Chaetomium*.

too, with slight changes in the precise rank-order (Table 2). The fungal types found in Wet-II and Wet-I overlapped largely as far as the abundant forms were concerned, but little in terms of the rare forms. Rank orders between the two isolates were highly correlated both in terms of abundance and spread (Spearman’s rank correlation test for nonparametric data,  $P < 0.01$  and  $P < 0.001$  respectively)<sup>20</sup>. The test was carried out only with respect to the 16 OTUs that were common to both collections. A comparison of the two sets of Jaccard’s indices of similarity showed that the overlap between the two collections was significant ( $t$  test,  $P < 0.01$ ). No significant correlation was found between the tree and fungal diversities within hectares as estimated by either the Shannon–Wiener or Simpson’s index ( $t$  test,  $P > 0.05$ ).

*Population density and environmental factors*

For the Wet-II collection, the population density in different hectares was not correlated with either soil pH, soil temperature, atmospheric temperature or soil moisture content (Pearson’s test for parametric data,  $P > 0.05$ )<sup>21</sup>. With respect to the Wet-I (July 1996) sample, the population density was positively correlated with soil pH and negatively correlated with soil temperature (Pearson’s test for parametric data;  $P < 0.05$ ). When the mean values of the two batches were compared with each other, only soil and atmospheric temperature were significantly different ( $t$  test for differences,  $P < 0.05$ ), while other edaphic factors (pH and moisture) remained unchanged.

*Estimates of primary diversity: Numbers of genera and species*

Table 3 lists a number of indices of diversity for the entire 50 ha plot: the Shannon–Wiener index, Brillouin’s index, Simpson’s Index, Fisher’s alpha, Chao’s index and the Evenness index. Some of these are based on an estima-

**Table 2.** Total number of genera (observed) and species (estimated). Mean number of genera per hectare is given in parentheses. Hectare numbers are according to Figure 1.  $N_{max}$  is the total number of genera calculated to exist in the entire region and  $a^*$  is a measure of size of the area (in ha) that must be sampled to obtain 50% of all the genera that exist within the area (see text for details)

| Hectare-wise area     | Number of genera<br>observed (mean) |         | Number of species<br>estimated (mean) |           |
|-----------------------|-------------------------------------|---------|---------------------------------------|-----------|
|                       | Wet-II                              | Wet-I   | Wet-II                                | Wet-I     |
| 23 + 28               | 6(3)                                | 14(7)   | 18(9)                                 | 34(17)    |
| +25 + 30 + 21 + 26    | 14(2.3)                             | 24(4.0) | 97(16.2)                              | 155(18.8) |
| +12 + 14 + 37 + 39    | 19(1.9)                             | 27(2.7) | 133(13.3)                             | 169(16.7) |
| All 16 ha             | 25(1.6)                             | 37(2.3) | 207(12.9)                             | 284(17)   |
| $N_{max}$ (estimated) | 43                                  | 69      | –                                     | –         |
| $a^*$ (estimated)     | 12.47                               | 11.70   | –                                     | –         |

tion of species numbers, which can be carried out from the figures pertaining to genera in two ways. *Penicillium* isolates are abundant in both collections. A detailed study (in preparation) shows that this genus is represented by 30 species. Let us suppose that every genus in our collection has members from 30 distinct species. Then we end up with an upper limit of 750 species (48/ha) during Wet-II and 1100 species (69/ha) during Wet-I. A more conservative estimate results from the alternative assumption that the greater the success of a genus in speciation, the wider its geographical spread. As a first approximation, let us consider the relationship to be linear: environmental variables and broad niche requirements being the same, we assume that a genus that consists of 100 species will be found in roughly twice as many locations as one consisting of 50 species. Overall, the 30 species of *Penicillium* were found in 13 of the 16 ha sampled. On this basis, during Wet-II, the number of species of *Mortierella* (found in 12 of the 16 ha) should be 28; of *Fusarium* (7 of the 16 ha) should be 16, and so on until *Helminthosporium* with two species and *Phytophthora* with one species, making 207 species in all (13/ha). Similarly, in Wet-I our estimate of the total number of species is 284 (18/ha).

A quantitative model enables us to go further. With its help we can do somewhat better than estimating the total number of fungal OTUs likely to exist in the 50 ha plot. In fact, we can ask how many OTUs could exist over the entire contiguous range of forest soils that – by assumption – constitutes an environment comparable to the 50 ha plot. The model depends on the hypothesis, supported by casual observation, of an approximately uniform spatial distribution of OTUs. A significant degree of non-uniformity, as might be caused by a tendency of the members of different species to clump (for example, because they are interdependent), or because of a high degree of environmental heterogeneity would invalidate the approach (which is described in Appendix 1). As will be seen later, there is in fact a small tendency for clumping at the generic level. The simplest version of the model leads to the following functional dependence of the number

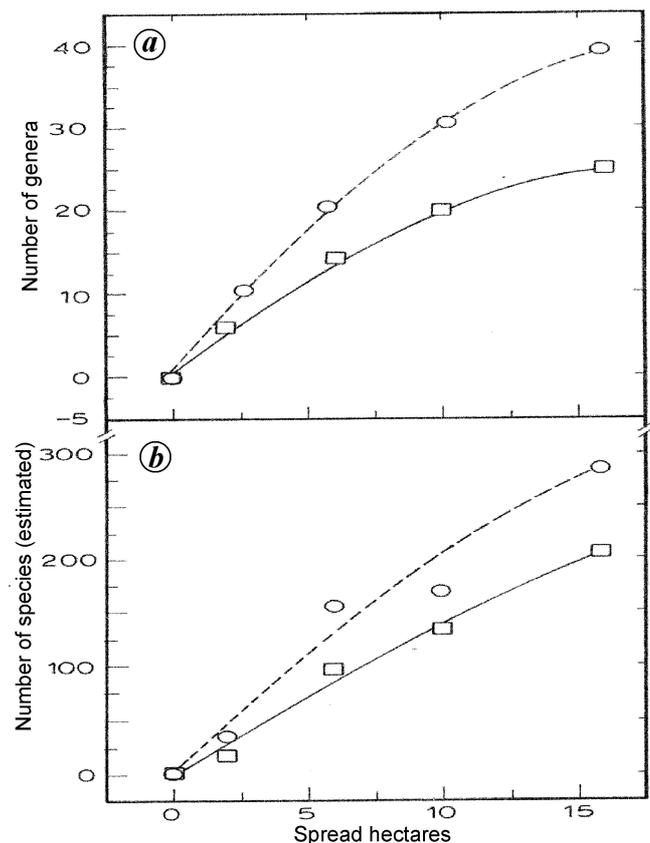
**Table 3.** Various measures of diversity over two seasons. Species diversity is measured on the basis of estimated number of species (see text)

| Diversity indices    | Wet-II (September 1995) | Wet-I (July 1996) |
|----------------------|-------------------------|-------------------|
|                      | Genera/species          | Genera/species    |
| Brillouin's index    | 3.02/–                  | 3.49/–            |
| Shannon–Wiener index | 3.13/7.25               | 3.56/7.51         |
| Fisher's index       | 5.25/111.68             | 8.75/215.73       |
| Simpson's index      | 0.79/0.98               | 0.86/0.99         |
| Evenness index (1)   | 0.97/–                  | 0.99/–            |
| Chao's index (1)     | 27.28 ± 0.73/–          | 41.73 ± 2.53/–    |
| Chao's index (2)     | 32.90 ± 2.37/–          | 59.88 ± 7.92/–    |

–, Denotes that a particular parameter was not calculated. The difference in the values of Fisher's alpha between the two collections is significant ( $P < 0.05$ ).

of OTUs ( $N$ ) on sampled area ( $a$ ):  $N = N_{\max}[a/(a^* + a)]$ , where  $a^*$  and  $N_{\max}$  are parameters whose significance will be explained later (Appendix 1).

We note first that the number of genera keeps increasing with the number of hectares that are sampled, but at an ever-decreasing rate (Table 2). For example, with reference to Wet-II and Figure 1, the number of genera in the central two hectares, numbers 23 and 28, was 6; when we add the four nearest 'outward' hectares sampled, numbers 21, 26, 25 and 30, this becomes 14; after adding the four next nearest hectares, numbers 12, 14, 37 and 39, it increases to 19; finally, if we add the counts from the six 'outermost' hectares, numbers 1, 3, 5, 46, 48 and 50, we end up with 25 genera (Table 2). In other words, the incremental increase in genus richness per unit area falls steadily as the sampled area increases outwards from the centre of the 50 ha plot towards the periphery (Figure 2a). Now we proceed to fit the observations to a genus number vs area relation of the form  $N = N_{\max}[a/(a^* + a)]$ . Here  $N$  is the number of distinct genera found within a sampling area  $a$ ,  $N_{\max}$  the total number of genera that exist in the entire region (within which the sampled area



**Figure 2.** Representation of incremental increase in genus and species richness per hectare. The rate of increase in the number of genera falls steadily as the sample area increases outwards from the centre of the 50 ha plot towards the periphery (a). This is not true for the estimated number of species, which grows at an ever-increasing rate (b). Solid and dotted lines represent Wet-II and Wet-I respectively.

has been assumed to constitute a typical portion) and  $a^*$  is a measure of the size of the area required to be sampled to pick up 50% of the total number of genera. Thus,  $N = 0.5N_{\max}$  at  $a = a^*$ . The upshot of this analysis is that over the entire contiguous range of the 50 ha plot,  $N_{\max}$ , the total number of fungal genera that one might expect to isolate, given the conditions used in this study, is 43 in Wet-II and 69 in Wet-I (Table 2).

A similar exercise could not be carried out with respect to species numbers: the more abundant genera in our collection began to appear in the more outlying hectares, and the estimated number of species kept increasing in proportion to the sampled area. Alpha diversity and the Shannon–Wiener and Simpson’s indices are usually measured in terms of species richness. A simpler measure of richness (from the centre outward) helps us to predict the maximum number of genera within the study area. This was found to be 43 (Wet-II) and 69 (Wet-I) as mentioned earlier. We have corrected for possible artefacts caused by rarefaction (Chao’s calculation) by selecting hectares randomly<sup>22</sup>. Chao’s estimate makes use of the formula  $S_{\text{est}} = S_{\text{obs}} + a^2/b$  to revise the observed number of OTUs,  $S_{\text{obs}}$ , and yield an estimated number of OTUs,  $S_{\text{est}}$ . Here  $a$  is the number of singletons (OTUs represented exactly once) and  $b$  is the number of doubletons (OTUs represented exactly twice). Analysing from 16 randomly chosen hectares (in ten simulations) in this manner we get, for the estimated number of genera,  $32.90 \pm 2.37$  (Wet-II) and  $59.88 \pm 7.92$  (Wet-I), where the numbers represent mean values with standard deviations. These estimates are not all that different from the earlier ones of 43 and 69 respectively. But both means are lower, indicating a higher number of doubletons relative to singletons, and therefore pointing to a weak tendency for genera to cluster.

## Discussion

### Methodological issues

Reported values of soil fungal diversity and population density are often a reflection of the methods used to recover the fungi, with optimal sampling methods differing from organism to organism<sup>23,24</sup>. Identification is complicated by the fact that fungal life cycles in the soil and in the laboratory can be quite different<sup>25</sup>. Therefore, instead of attempting species identification, many researchers classify individuals at the generic level<sup>26</sup>. Fungi are so nutritionally diverse that there is no one medium that can isolate all of them. The spatial distribution of microorganisms in soils<sup>27</sup> and the need to overcome the wide range of microorganism–soil particle interactions<sup>28</sup> are the main limitations to sampling soil microorganisms quantitatively and representatively. The technique of direct isolation from particles of soil would have yielded more counts, but the faster growing fungi would still be favoured<sup>29</sup>.

However, an advantage of using the dilution plate count technique is that all the developing colonies can easily be picked off from the plates for further sub-culturing.

### Reliability

The technique used in the present study is a standard one<sup>30</sup>. Going by our observations, the media used in our study appear to favour Deuteromycetes the most, followed by Phycomycetes. *Chaetomium*, *Chaetophoma* and *Rhizoctonia* were the only genera found belonging to the class Ascomycetes and Basidiomycetes. *Aspergillus* and *Penicillium* usually appeared abundantly in collections because of their prolific sporulating capability. Their antibiotics may suppress the growth of other fungi in the wild. If true, this would once again suggest that both population density and diversity are higher than what our observations indicate. The substantial degree of turnover (= non-overlap) that we find with respect to the less abundant forms, between two collections separated by ten months (Table 2), is also an indicator that the 50 ha plot likely supports many other fungal types. Viable spores which are non-culturable in the laboratory would have escaped our screen<sup>31</sup>. Taken together, all of these reinforce the point that what we report, both in terms of number and variety, must be an underestimate. How much of an underestimate, is hard to say. Similar studies in the literature are meagre, but quantitative data that exist compare favourably with our findings<sup>5,29,30,32,33</sup>.

### Population densities

The fungal population density obtained by us falls within  $1.13 \pm 0.31 \times 10^4$  to  $2.67 \pm 1.49 \times 10^4$ /g of soil. The population density is uncorrelated with most edaphic factors, the exceptions being soil pH (positively correlated) and soil temperature (negatively correlated) – and that too just for the Wet-I (July 1996) collection. This leaves open the question whether the dry data too would have shown indifference to soil moisture; a referee has pointed out that the presence of a stream that runs through the interior of the 50 ha plot could have led to significant micro-environmental differences between otherwise dry and wet periods. It is not clear why the correlation with soil pH is different in the case of the Wet-I (positively correlated) and Wet-II (no correlation) isolates, even though the pH itself remained unchanged. As suggested by the same referee, it would be interesting to examine whether this has anything to do with the fact that September, which falls between Wet-I and -II, corresponds to a temporal minimum in precipitation. The mean population density is not significantly different between the two wet seasons. When compared with the wet seasons, however, the number of colonies per gram of soil shows a decline by about 50% in the dry season. Rainfall is the most important

predictor for plant diversity<sup>14</sup>, and this could be the reason for the difference. The low count in the March 1995 (dry) collection is also likely to have been a consequence of the annual fires that occur between January and March<sup>34</sup>. In all cases, the abundance of a type (the number of different hectares on which a given genus occurred) was strongly correlated with its population size.

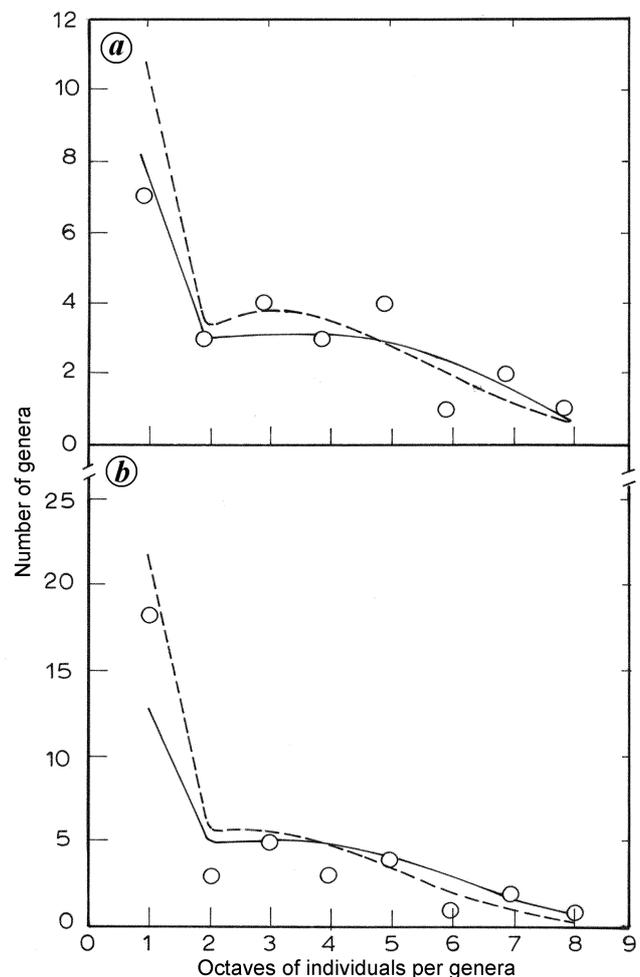
### Diversity

When we compare our results with those reported in earlier studies on soil fungal diversity, we find fair agreement as far as population counts go; but the extent of diversity we see is significantly higher than that reported previously<sup>29,32</sup>. *Mortierella*, a member of the class Phycmycetes, dominated our collections, followed by *Penicillium* and *Fusarium*. Earlier reports have indicated that *Aspergillus* may be dominant in disturbed soils<sup>29,33</sup>; note that the soil studied by us has been undisturbed for about 30 years. In our case the pattern of distribution of the more common forms, though not of the rarer forms, repeats itself over the two wet seasons. *Cylindrocladium*, *Nigrospora*, *Helminthosporium*, *Phytophthora* and *Chaetomium* were present in the Wet-II (September 1995) batch but absent in the Wet-I (July 1996) batch. Similarly, *Ascochyta*, *Asteromella*, *Bispora*, *Didymocladium*, *Myrothecium*, *Peyronellaea*, *Sporothrix*, *Stysanus* and *Sphaerosporium* were found in Wet-I (July 1996) but absent in Wet-II (September 1995). *Penicillium* was the most abundant form we observed during Wet-II and it was found in 11 of the 16 ha; *Mortierella* was the most abundant in Wet-I and occurred in 13 of the 16 ha.

Between the two wet seasons, changes in soil and atmospheric temperature – but not in moisture or pH – were significant (data not shown). The former is, therefore, a possible explanation for changes in primary diversity at the level of genera (Table 1) and for changes in Fisher's alpha (Table 3). As can be seen, the Shannon–Wiener index of diversity,  $H'$ , and the Brillouin index,  $H$ , are almost the same, indicating that sampling with and without replacement were equivalent. The absolute values of  $H'$  (computed on the basis of genera) in both wet seasons are about 67% of their theoretical maxima, implying that the distribution of individuals over the different genera tends more in the direction of being uniform than being skewed. All our estimates of Simpson's index are close to 1, meaning that the probability is very low that any two individuals chosen at random belong to the same genus (and even less so, to the same species). Within the 50 ha plot, the hectare-to-hectare index of diversity (Jaccard's index) is not correlated with distance: two hectares that are close to each other may be as different from one another as two hectares that are far from one another (data not shown). Further, a number of indices of fungal diversity – number of genera, Fisher's alpha, Shannon–Wiener

index and Simpson's index – are uncorrelated with indices of tree diversity (Pearson's test,  $P > 0.05$  in all cases). This may be a reflection of the fact that we have monitored only free-living soil fungi and not fungi associated with plants or trees.

The number of fungal genera increases with the area monitored and does so in the manner characteristic of a curve that flattens out asymptotically (Table 2, Figure 2a). It is this feature that enables us to estimate the total number of genera (observed + unobserved) within the area of study. About 12 ha is a sufficient area in which to find 50% of the total number of genera; thus, over 50 ha, one would expect to find  $50/(50 + 12)$ , or about 80%, of the total number of genera. However, this approach does not work when we try to predict species numbers. In fact, if we are justified in assuming that the extent of spatial spread of a genus is proportional to the number of species belonging to that genus, our data do not permit us to assign an upper



**Figure 3.** Logarithmic (solid line) and log normal (dotted line) fits to the observed data (stars) during (a) Wet-II (September 1995) and (b) Wet-I (July 1996) seasons. Abundance of genera plotted in octaves. The sharp peak shows that in the first octave rare forms are relatively more abundant than common ones (for details see Table 3).

limit to the total number of soil fungal species in the Mudumalai forest range (Figure 2b). Within the sampled (16 ha) area, we estimate the number of species as somewhere between 207 and 284 (Table 3). This should be compared with the number of woody plant (1 cm dbh) species found in the entire 50 ha plot, which is reported<sup>10,34</sup> to be 71. In terms of diversity too, the 50 ha plot is richer in fungi than trees: the Shannon–Wiener index for tree species 3.62 (J. Robert, personal communication) indicates an approximately two-fold difference (see Table 3).

The frequency distribution of the OTUs found by us is characteristic in that a small number of types is very abundant, while most are present in low abundance (Figure 3a and b). A quantitative analysis shows that both the logarithmic and log normal distributions<sup>35</sup> fit our observations equally well (chi-squared test,  $P < 0.01$ ). Mathematical model-based analyses of fungal communities are meagre<sup>36</sup>, but one report in the literature that we are aware of points in the same direction as ours. Thomas and Shattock<sup>37</sup> also found that the logarithmic and log normal distributions were best suited for a description of the abundance of 33 genera of filamentous fungi on the grass, *Lolium perenne*. The logarithmic and geometric distributions can be expected to be appropriate in situations wherein the ecology of the community is dominated by one or a small number of factors<sup>18</sup>; a point which, along with many others touched on in this discussion, forms a subject for future investigations.

## Appendix 1

There are at least two ways in which a model can be developed that leads to the relationship between the number of OTUs and area that is given in the text. Both are offered ad hoc, without any attempt at theoretical justification.

1. (a) The number of OTUs increases steadily as a function of the area sampled until finally all OTUs are counted. Therefore, the curve relating the number of OTUs with sampled area should increase monotonically; the simplest monotonic dependence would be a linear one. (b) As the sampled area increases, the number of OTUs keeps increasing – as in (a). However, the rate of increase falls steadily, because as the sampled area increases, previously observed taxa tend to recur. In this case the total number of OTUs in the ecosystem of interest is a quasi-asymptotic limit. Therefore, the number of OTUs vs area curve should increase at first and then saturate. As will be seen below, both the expected outcomes (i.e. expected on the basis of (a) or (b)) can be accommodated within a single functional form of the OTU vs area curve.

2. (a) The 50 ha plot constitutes a ‘fair sample’ of a much larger soil ecosystem. Similarly, the individual hectares that are actually sampled by us are ‘fair’ (sub-) samples of the 50 ha plot. Here the word ‘fair’ implies that when one samples a region of a certain size any-

where within the ecosystem (or, in our case, within the 50 ha plot), even though the OTUs that are found might vary from region to region, their expected number depends on the size of the sampled region alone. The justification behind this assumption will not be spelt out here. (b) The larger the number of OTUs found in a small sampled area, it is more likely that the overall biodiversity will be high, and therefore: the larger the number of OTUs remaining to be found in the rest of the ecosystem. (c) The larger the area that is sampled, the smaller the number of OTUs remaining to be found in the rest of the ecosystem.

In symbols, if  $a$  stands for the sampled area,  $N$  stands for a measure of diversity and  $N_{\max}$  for the total diversity, assumption 2(a) implies that the only variable on which  $N$  depends is  $a$ . Assumption 2(b) implies that  $N_{\max} - N$  is a monotonically increasing function of  $N$ ; 2(c) implies that  $N_{\max} - N$  is a monotonically decreasing function of  $a$ . The simplest relationship between  $N$  and  $a$  that satisfies these requirements is  $N = N_{\max}[a/(a^* + a)]$ . As it happens, this is also the simplest relationship that fits requirements 1(a) and 1(b). Here  $N_{\max}$  and  $a^*$  are parameters.  $a^*$  is a measure of the spatial scale which it is necessary to cover in order to get a reasonable idea of  $N_{\max}$ , the total number of OTUs. If  $a^*$  is extremely high, it may well be that  $N_{\max}$  (for the ecosystem as a whole) cannot be estimated at all with the available data, because  $N$  keeps increasing with  $a$  at a constant rate (within the 50 ha plot – the region that is sampled). If the  $N$  vs  $a$  relation holds good over an area that is restricted but is significantly larger than the 50 ha plot, it can be tested by starting from a convenient location and adding sampled regions. Purely for convenience, the test has been carried out choosing a starting location from about the centre of the 50 ha plot.

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