

SPATIAL DISTRIBUTION OF ARBUSCULAR MYCORRHIZAS ALONG AN ELEVATION AND EDAPHIC GRADIENT IN THE FOREST DYNAMICS PLOT AT SINHARAJA, SRI LANKA.

C. M. C. P. Chandrasekara, H. M. S. P. Madawala Weerasinghe¹, I. A. U. N. Gunatilleke & G. Seneviratne*

Department of Botany, Faculty of Science, University of Peradeniya.

*Institute of Fundamental Studies, Kandy.

ABSTRACT

To study how the endomycorrhizal spore density and diversity varies with soil parameters and elevation in the 25 ha forest dynamics plot in the Sinharaja forest, soil samples were collected from three transects, along the elevational gradient. Soil mycorrhizal spores were isolated using the wet – sieving and decanting method (Brundrett *et al.*, 1996). They were observed and counted under stereo zooming microscope with an external light source. Different morphotypes of spores were identified using external spore characteristics to find out spore diversity. Soil chemical and physical properties such as water potential, pH, total soil organic carbon and plant available phosphate were analyzed using standard methods.

There is a decreasing trend in spore density and spore diversity with increasing elevation ($R^2 = 21.6\%$, 40.6% respectively) but spore density does not vary with four main habitat types in the 25 ha FDP plot. The number of small spores of 45-125 μm contributed more to the total spore counts in all samples examined. Soil parameters except water potential, do not correlate with endomycorrhizal spore density and diversity. Spore diversity showed a significant positive correlation with soil water potential ($P = 0.041$), but not spore density.

INTRODUCTION

Arbuscular mycorrhizae (AM) establish mutualisms between plant roots and zygomycete fungi in the Glomales. The AM fungi (AMF) provide mineral nutrients to their plant hosts in exchange for carbohydrates. Although the fungi comprise only 150 – 170 asexual morphotypes, or species, this mutualism is found in the roots of 70 – 80 % of terrestrial plant species (Trappe, 1987). In nutrient-poor soils of the humid tropics, many late-successional trees are obligately dependent on AMF and only grow beyond seed reserves if infected. Therefore, studying the ecology of AMF would improve our understanding on the functioning of tropical forests (Chris, 2000).

Compared to documenting of losses in the diversity of plant, vertebrates and arthropods, microorganisms have been relatively ignored (Chris, 2000), though they have long been recognized as important symbioses in tropical forest interactions. Most plant species rely on mycorrhizae for uptake of nutrients and water;

these associations are obligate for many tropical plants (Janos, 1980a). Janos (1980b) hypothesized that disturbance of tropical forests reduces mycorrhizal fungal populations and inhibits forest regeneration. Species richness of mycorrhizal fungal communities has been correlated with the species richness of plant communities in temperate grasslands and tropical agroecosystems (Nancy & David, 1997).

These evidences prove that there is a strong relationship between vegetation in a particular area and mycorrhizal fungal communities. Brundrett (1991) suggested that plants with mycorrhizal associations are known to predominate in most natural ecosystems, but little is known about phenological variations in mycorrhizal activity in many habitats. In addition, Moersoen *et al.*, (1998) pointed out that knowledge on the local distribution of both ectomycorrhiza (ECM) and arbuscular mycorrhiza in tropical forests is sketchy.

¹Corresponding author E.mail:sumdilki@yahoo.com

Moyersoen *et al.*, (1998) suggested that in the Korup National Park, distribution of ECM fungi may be related to soil properties, as has been described in other tropical, subtropical, and temperate forests. He proposed that differences in ECM fungal species composition of forests of different ages (and consequently different soil properties) are related to the different abilities of fungal species to access organic nutrients. In the same way, it would be interesting to investigate if the recent reports of a trend of the spatial distribution of AM morphotypes could be related to soil properties (Brundrett & Abbott, 1994). In addition, mycorrhizal colonization of fine roots occupying a defined volume of soil will depend on a balance between root and fungal activity which is influenced by several factors including soil properties, root phenology, predation, local disturbance and spore dispersal (Brundrett, 1991).

Moyersoen *et al.*, (1998) pointed out that although propagules were not easily quantifiable, their presence could partly explain small-scale differences in mycorrhizal inoculum potential. Important propagules of vesicular-arbuscular mycorrhizae (VAM) fungi are spores, networks of hyphae in soil, dead root fragments and other organic material occupied by fungal structures. Spores have traditionally been considered to be the most important propagules of VAM fungi and mycorrhizal formation by germinated spores has been well documented (Brundrett & Abbott 1994).

Analysis of spore populations in soils is currently the only method to assess the species composition of AM fungal communities, but interpretation of these results remains conditional. Spores do not perform a mutualistic function. Furthermore, isolates of AM fungi vary greatly in spore production; some isolates produce spores copiously, while others rarely or never sporulate. Thus, while interspecific comparisons of spore populations are generally not useful, intraspecific comparisons (across treatments) may be meaningful. Such a comparison by Nancy & David, (1997) revealed that spore populations of only one species (*Glomus microcarpum*) were significantly affected by vegetation type. In addition, Read (1991) at a global scale and Janos (1980) for tropical rain forests both hypothesized that morphological and physiological differences between different soils that lead to distinctive patterns of symbiosis in different biomes.

Moyersoen *et al.*, 1998 studied the vertical and horizontal distribution of ECM and VAM at Korup National Park Rain Forest, Cameroon using fractional colonization of roots. They found both mycorrhizal types (ECM and VAM) varying widely in their degree of colonization throughout the litter and in the soil organic and mineral horizons, at least to a depth of 35 cm. Soil properties as well as other factors such as root distribution of tree species might influence the vertical distribution of both ECM and VAM mycorrhizal types. Further, they added that the more heterogeneous horizontal distribution of ECM at Korup National Park Forest was clearly related to the more scattered distribution of ECM trees.

Most tropical rainforest trees have strongly aggregated spatial distribution patterns (Condit *et al.*, 2000). This aggregated spatial distribution remains an unanswered question in tropical forest ecology so far. However, non-random distribution of species in an ecosystem may be due to the variations in the availability of light, nutrients, water or their interactions and the biotic environment (Condit *et al.*, 2000). Burslem *et al.* (2000) suggested that such non-random distribution or clumping could be due to restricted seed dispersal patterns or due to an association of species with habitat variables such as soil parameters etc. Such a distribution pattern of tree species is observed in the 25 ha forest dynamic plot at Sinharaja lowland rain forest, Sri Lanka. The floristic composition and structure within this plot varies along a small-scale altitudinal gradient. In addition, the spatial distribution patterns of tree species within the 25 ha Forest Dynamics Plot show an interesting correlation with topography (Gunatilleke *et al.*, 2004).

Clark *et al.* (1998) stated that an increasing number of studies demonstrate that species distributions are strongly aggregated with respect to variation in topography, soil water and soil nutrient status. In addition, Janos (1983) suggests that mycorrhizae in lowland tropical rain forests have a potential role in nutrient cycling, plant growth and a possible influence on their species composition.

At Sinharaja, soil physico-chemical and biological properties in the undisturbed primary forest have been compared with those from

disturbed or converted sites, which were abandoned after shifting cultivation and have either remained as *Dicranopteris* fern lands or been planted with *Pinus caribaea* (Hafeel, 1991; Maheswaran and Gunatilleke, 1987). In addition, Hafeel and Gunatilleke (1989) and Hafeel (1991) compared endomycorrhizae populations in the natural forest with those in a *Pinus* plantation and a *Dicranopteris linearis* fernland. But the soil physico chemical properties in relation to endomycorrhizal spore populations within the natural forest have not been studied. In this regard, knowledge about the variation of endomycorrhizal spore populations within the natural forest itself will be important in the exploration of reasons for this non-random distribution observed within the forest.

Therefore, this study compared how the endomycorrhizal spore density and diversity varies with soil parameters and elevation along this small-scale elevational gradient of 151 m in the 25 ha forest dynamics plot in the Sinharaja forest. In addition, the variation of soil parameters with elevation has also investigated.

Objectives of this study are two fold.

- i. Compare the variation of endomycorrhizal spore population along a small-scale elevation gradient in relation to soil parameters, in the 25 ha forest dynamics plot at Sinharaja.
- ii. To determine the diversity of endomycorrhizae with the help of different spore morphotypes.

MATERIALS AND METHODS

Study site

The study was conducted in the 25-ha Forest Dynamics plot in Sinharaja, which represents a part of an undisturbed lowland rain forest locally classified as *Mesua-Doona* association (Fig. 1 A). On a regional scale it represents a mixed Dipterocarp forest (Gunatilleke *et al.*, 2004).

Over the 17 year period (1984-2000) of study on climatic data, Munidasa *et al.*, (2002) found that the mean annual rainfall at Sinharaja as 5016 mm and it varied from 4080 to 5907 mm. According to them, mean annual minimum and maximum temperatures for the study period were 22 and 28°C respectively. Highest monthly

temperatures were observed in April (25.3°C), May (25.9°C) and January (25.1°C) and it was least in December (23.8°C). In addition, Zoysa & Raheem, (1993) pointed out that the high annual temperature of the Sinharaja is typical of the tropics, recording little seasonal variation, but with marked daily ranges. The lowest mean monthly temperature has been observed during the wettest season and the highest during the driest season (Fig. 1B). Conventional temperature patterns however change during long periods of drought or excessive rainfall.

Topographically, the plot represents an elevational range of 151 m, rising from 424 m to 575 m above sea level. It has a valley, lying between two slopes, a steeper higher slope facing the southwest and a less steep slope facing the northeast. Seepage ways, spurs, small hillocks, two perennial streams and several seasonal streamlets cut across these slopes.

Three physical parameters, namely, elevation, slope, and/or convexity of each of the 20 m x 20 m quadrates were taken into consideration in the identification of different topographic habitats of the 25 ha plot at Sinharaja and subsequently, eight habitat categories were identified. These were named as (i) high steep spurs, (ii) high less steep spurs, (iii) high steep gullies, (iv) high less steep gullies, (v) low steep spurs, (vi) low less steep spurs, (vii) low steep gullies and (viii) low less steep gullies (Fig. 2).

Sampling

To compare endomycorrhizal populations at different elevations, soil samples were collected from three transects, 10m apart from each other, along the elevational gradient from the ridge to the valley (Fig. 2). Soil sampling was done in mid March 2004. In each transect, soil samples were collected at 15 m intervals from ridge to the valley using a soil corer (5 cm diameter and 15 cm depth). Exact elevations, at which soil samples were taken in all three transects, were obtained from the data base of 25 ha Forest Dynamics Plot at Sinharaja rain forest (Table 1) and the mean of elevations in three transects were taken for presenting data. At each sampling point five soil cores were collected randomly, mixed thoroughly and stored in polythene bags. These bags were tightly closed to avoid any evaporation. At the

laboratory, part of the soil samples was stored in a refrigerator at 4°C and the remaining was air dried, sieved using 2mm sieve and kept in polythene bags, until they were processed. A total of 41 samples were collected from the three transects

Quantifying the endomycorrhizal spore population in soil

Soil mycorrhizal spores were isolated using the wet – sieving and decanting method (Brundrett *et al.*, 1996). Spores were isolated on filter papers (which were already divided into 8 parts), and were carefully observed under stereo zooming microscope with an external light source and the number of spores in each portion was counted. Different morphotypes of spores were identified using external spore characteristics to find out the diversity of endomycorrhizal spore populations along the elevation in relation to soil parameters. Spore characteristics such as colour, size, markings on the spore wall etc. were used during the identification of morphotypes.

Soil Physical and chemical properties

The following soil chemical and physical properties were analyzed in the soil laboratories at the Institute of Fundamental studies (IFS), Sri Lanka. Soil Water potential using the filter paper method (Anderson & Ingram, 1993); soil pH by making a soil suspension in 4 mM CaCl₂ solution in 1:1.5 ratio; total soil organic Carbon by the wet oxidation of organic carbon with acidified dichromate, followed by a colorimetric method (Anderson & Ingram, 1993); plant available phosphate by extracting soil with Olsen solution (Sodium bicarbonate solution) at pH 8.5, and measuring the amount of P by the Phosphomolybdate method colorimetrically (Anderson & Ingram, 1993).

Statistical Analysis

Regression analyses were done to find out the variation of soil endomycorrhizal spore populations and soil parameters along the elevation gradient. Further more, correlation between soil parameters with endomycorrhizal spore density and diversity were determined with the help of correlation analysis. All these tests were performed using Minitab (version 13) software.

RESULTS

Distribution of VAM spores with elevation

The total number of endomycorrhizal spores was higher in upper slopes as well as in lower slopes, but less in mid-slopes. However, results showed a decreasing trend in spore density with increasing elevation (Fig. 3), and regression analysis revealed that $R^2 = 21.6\%$, although it is not significant ($P = 0.094$).

When the total number of spores was plotted against different elevational classes, it shows highest spore density in lowest elevation class compared to other elevation classes. Spore density seems to be slightly lower in middle elevation class and highest elevation class than in the lower elevation classes (Fig.4). However these differences are not statistically significant ($P=0.478$). In addition, there is no clear difference in spore density with convexity; i. e. spore density does not vary with four main habitat types in the 25 ha FDP plot (Fig. 5), and also it is not significant ($P=0.724$). It is also important to note that, the number of small spores of 45-125 µm contributed more to the total spore counts in all samples examined (Figs. 4 & 5).

Different morpho types along elevation

In all samples analyzed, about 15 different spore morphotypes were identified, although 9 morpho types were rarely present. In addition, Morphotype 1 and morphotype 2 (Table 2 for characteristic features and Figure 6) were very common in all the samples studied and morphotypes 3, 4, 5 and 6 were less common.

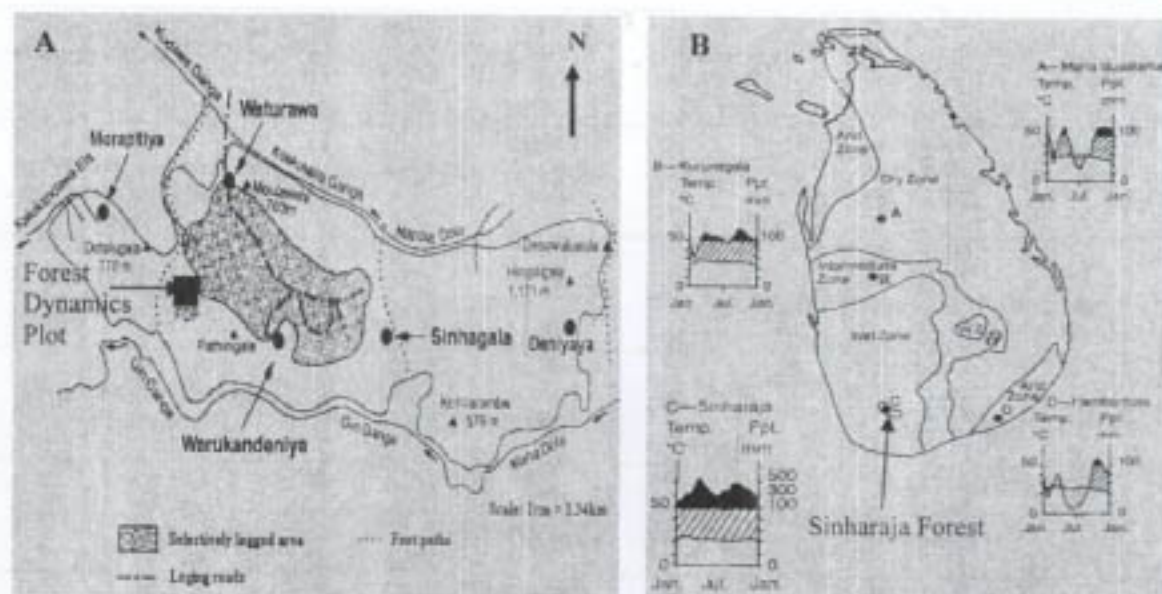


Figure 1 (A) Location of the Forest Dynamics Plot (■) in the Sinharaja rain forest (B) Location of Sinharaja forest in Sri Lanka with respect to the different climatic zones in the country (Source: Gunatilleke *et. al.*, 2004)

Table 1 Elevations at which samples were collected

T 1-Elevations(m)	T 2-Elevations (m)	T 3-Elevations (m)	Mean-Elevations (m)
430	440	434	435
440	452	461	451
468	465	462	465
477	477	465	473
480	485	477	481
494	494	486	491
498	498	489	495
508	508	509	508
519	519	522	520
531	531	536	533
557	545		551
565	552	552	556
570	570	570	570
572	572	572	572

Table 2. Some characteristic features of endomycorrhizal spore morphotypes observed in the study

Morphotype	Size Class (μm)	Colour	Shape	Surface	Others
1	Belongs to 45 – 125 125 -250 >250	Dark brown to light brown	Globular	Shiny	Some times having subtending hyphae at one or both ends
2	Belongs to 45 – 125 125 -250 >250	Black	Globular or Sickle shaped	Shiny	Some times white filament comes out, & found tiny dots on wall
3	Belongs to 45 – 125 125 -250 >250	Off white	Globular	Shiny	Sometimes transparent
4	Belongs to 45 – 125 125 -250 >250	White	Globular or Barrel shaped	Shiny	Extending hyphae starting from spore inside
5	Belongs to 45 – 125 125 -250 >250	Yellow to white	Globular	Transparent	-
6	Belongs to 45 – 125 125 -250 >250	Brown, yellowish or orange	Oval or lemon shaped	Shiny	-
7	Belongs to 45 – 125 125 -250 >250	Yellowish orange	Globular	Shiny	Has subtending hyphae, Splitted nature on wall
8	Belongs to 125 – 250	White	Oval, sickle or barrel shaped	Shiny	-
9	Belongs to 45 – 125 125 – 250	Reddish orange	Globular	Not shiny	-
10	Belongs to > 250	Blackish brown	Globular	Rough	It seems to be hard
11	Belongs to > 250 125 – 250	Brown	Oval	Not shiny	Swollen patches on wall
12	Belongs to 125 – 250	Black	Globular	Some times shiny	Hexagonal shaped arrangement on wall
13	Belongs to > 250	Brown	Elongated		Large, spiny projections coming out from spore
14	Belongs to > 250	Orange brown	Elongated	Warty walled	-
15	Belongs to > 250	White to ash	Oval		Sharp markings on wall

Habitat types in the Sinharaja Forest Dynamics Plot (FDP)

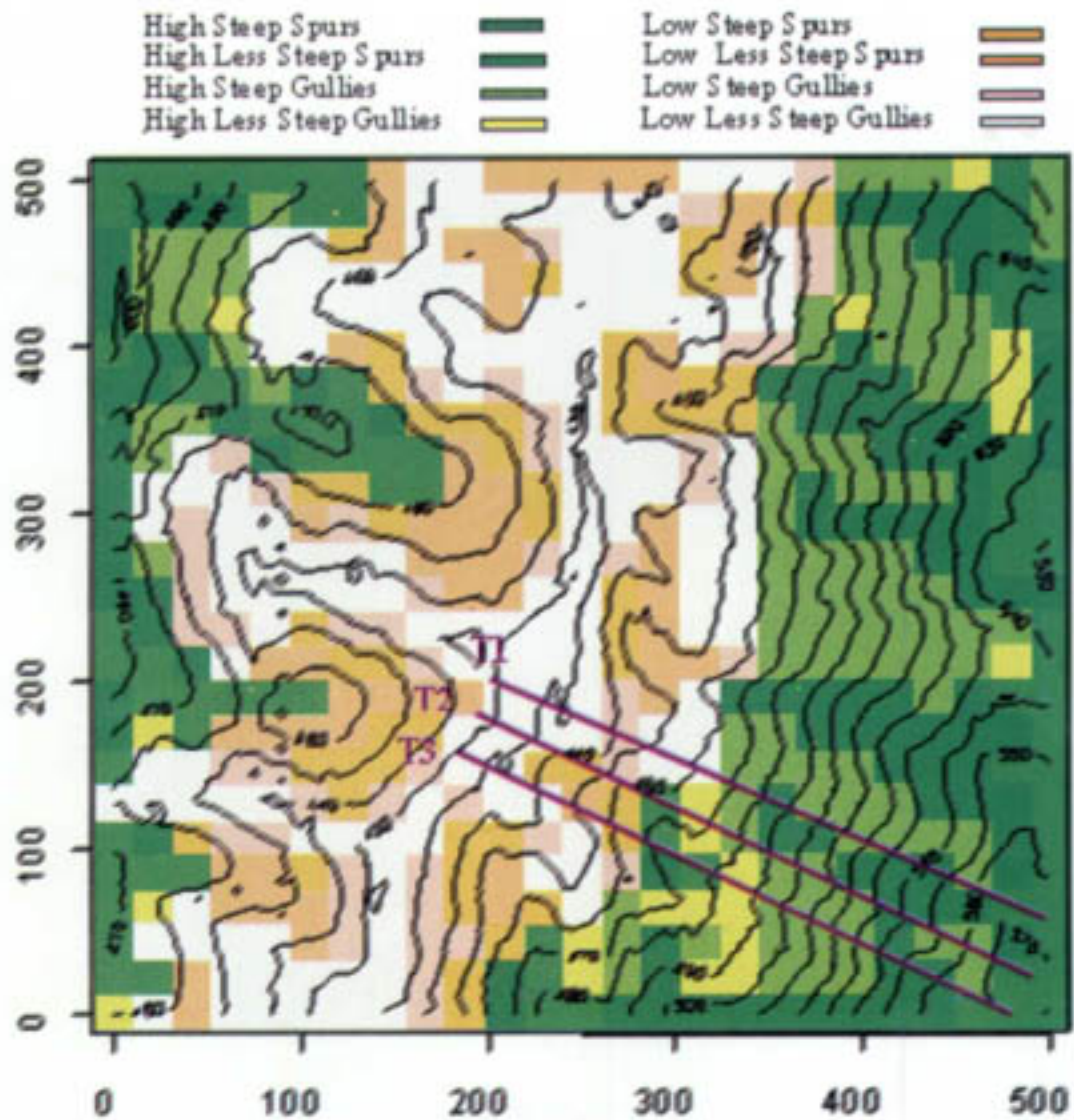


Figure 2. Eight habitats identified and the location of three transects (T1 – T3) within the FDP plot at Sinharaja (Source: Gunatilleke *et al.*, 2004).

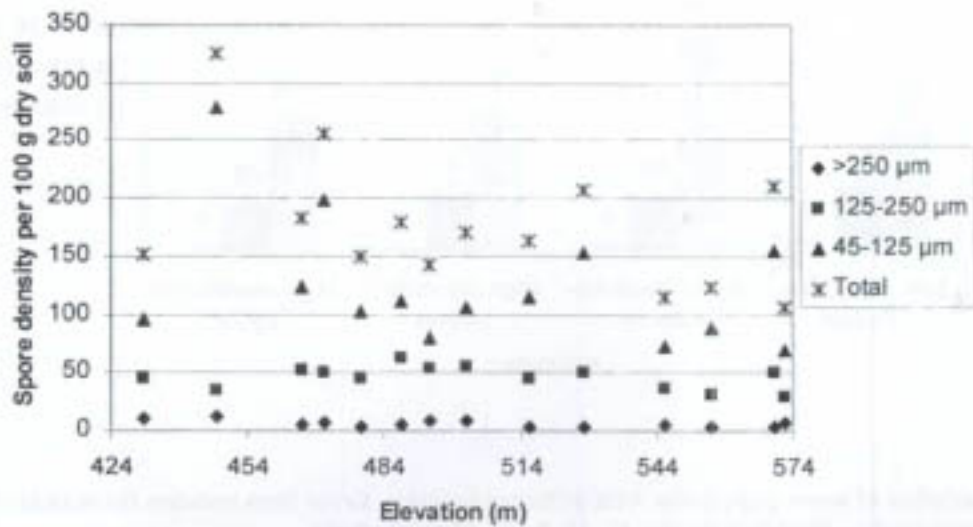


Figure 3. Variation in Endomycorrhizal spore population with elevation. Each point is a mean of three values.

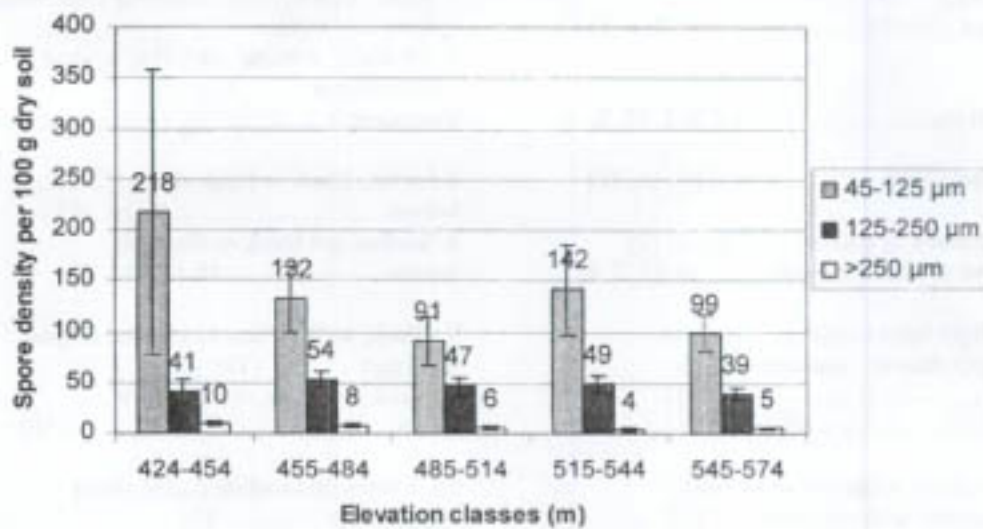


Figure 4. Variation of spore population with elevation. Error bars indicate the standard error (SE) of the mean value. Sample sizes are 5, 10, 10, 6, and 11 respectively.

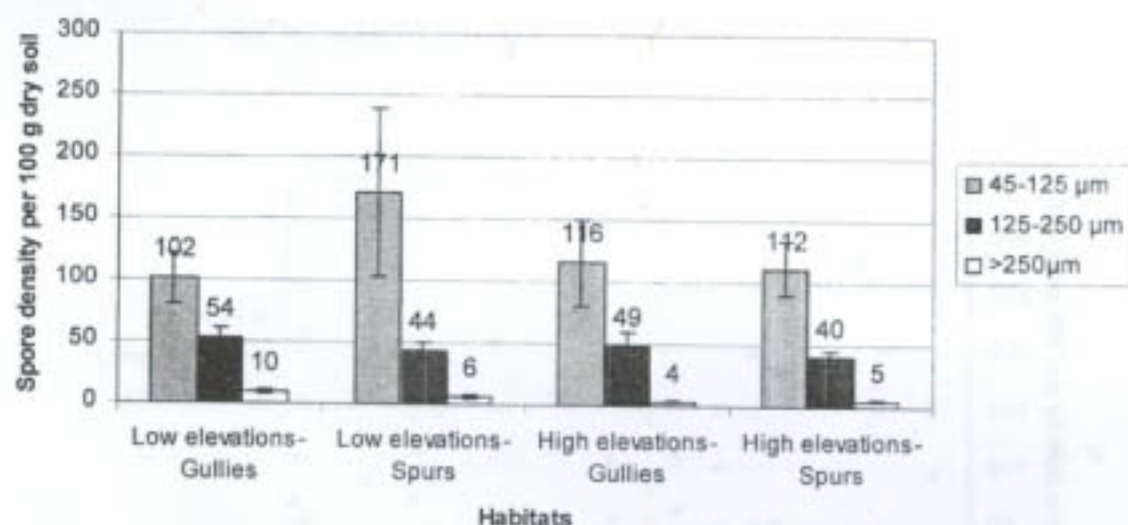


Figure 5. Variation of spore population with different habitats. Error bars indicate the standard error (SE) of the mean value. Sample sizes are, 11, 11, 6 and 14 respectively.

Key to the morphotypes of endomycorrhizal spores in the Sinharaja 25 ha Forest Dynamics Plot.

1. Spores globular.....2 {1, 3, 4, 5, 7, 2, 9, 10, 12,}

1. Spores not globular..... 11 {2, 4, 8, 6, 11, 15}

2. Surface shiny.....3 {1, 3, 7, 12, 2, 4}

2. Surface not shiny.....8 {5, 9, 10, 12}

3. Brown to black in colour.....4 {1, 2, 12}

3. Not brown or black; coloured.....6 {3, 7, 4}

4. Dark to light brown, with or without subtending hyphae at one end or both ends..... (1)

4. Black.....5 {2, 12}

5 With or without white filament; wall surface marked with tiny dots.....{2}

5 Wall with hexagonal shaped markings.... {12}

6 Yellowish orange, with subtending hyphae and wall surface with cracks..... {7}

6 White of off white.....7

7 White, with hyphae extending from within spore..... {4}

7 Off white, without extending hyphae, (Sometimes transparent)..... {3}

8 Surface black or blackish brown..... 9 {10, 12}

8. Surface not black or blackish brown..... 10 {5, 9}

9. Black, wall surface hexagonal shaped markings..... {12}

9. Blackish brown, wall surface rough..... {10}

10. Yellowish to white (some times transparent)..... {5}

10. Reddish orange.....{9}

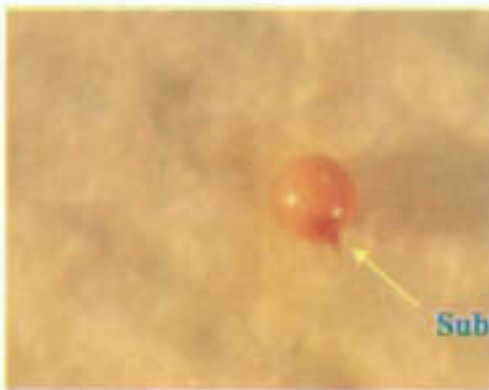


Plate 6. Some spore morphotypes observed in the present study

11. Sickle shaped.....12 {2, 8}
 11. Not sickle shaped3 {4, 6, 11, 15, 8}
12. Black, with or without white filaments wall surface marked with tiny dots.....{2}
 12. White; not so..... {8}
13. Oval shaped.....14 {6, 8, 11, 15}
 13. Not oval; Barrel shaped or elongated..... 17 {4, 8, 13, 14}
14. Surface shiny.....15 {6, 8}
 14. Surface not shiny..... 16 {11, 15}
15. White coloured..... {8}
 15. Brown, yellowish or orange..... {6}
16. Brown coloured; wall surface with swellings..... {11}
 16. Not brown, white to ash..... {15}
17. Barrel shaped, shiny and white 18 {4, 8}
 17. Elongate, not shiny and brown to orange brown.....19 {13, 14}
18. With hyphae extending from within spore.....{4}
 18 Not so.....{8}
19. Large spiny projections extend from spore..... {13}
 19. Not so; wall surface warty.....{14}

Total number of morphotypes varies with elevation (Fig. 6), and there is a decreasing trend in spore diversity with increasing elevation ($R^2 = 40.6\%$). Regression analysis revealed that this trend of decreasing spore diversity with elevation is significant at 95 % significant level (P value = 0.014).

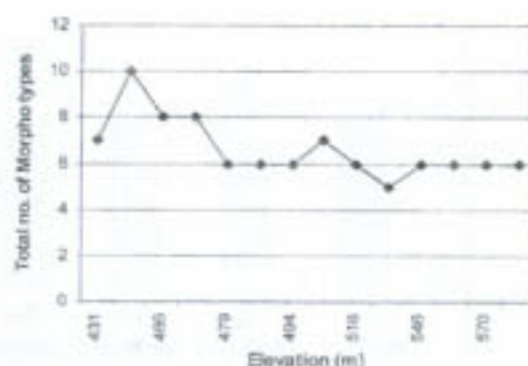


Figure 7. The distribution of morphotypes with elevation. Presence and absence were considered in counting morphotypes at different elevations

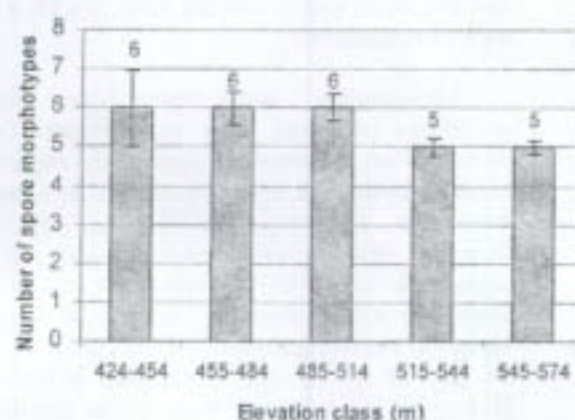


Figure 8. Spore diversity in different elevation classes. Error bars indicate the standard error (SE) of the mean value. Sample sizes are 5, 10, 10, 6, and 11 respectively.

There is no significant difference in spore diversity among different elevation classes ($P=0.443$) but, number of spore morphotypes is higher in lower elevation classes than upper elevation classes (Fig. 8). Like spore density, spore diversity is also higher in low elevation spurs compared to other three habitats (Fig.9), but this variation of spore diversity among habitats is not statistically significant ($P=0.09$).

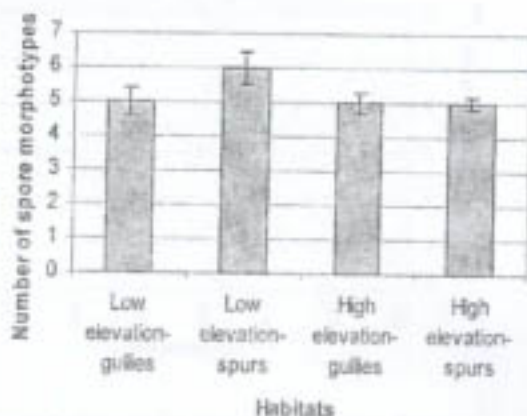


Figure 9. Spore diversity in different habitats. Error bars indicate the standard error (SE) of the the mean value. Sample sizes are 11, 11, 6, and 14 respectively.

Morphotypes 1 and 2 have a higher relative abundance compared other morphotypes. These two types are the more dominant in the study site. Relative abundance of morphotype 3, 4, 5, and 6 were less, indicating that they are the less dominant spore types in the study site and the abundance of other morphotypes could be negligible when comparing with these 6 spore types. So the morphotypes 7, 8, 9, 10, 11, 12, 13, 14, and 15 are the rare spore types in this area (Fig. 11a). In addition, there is no significant difference in the abundance of dominant morphotypes between high elevations and low elevations ($P > 0.05$). But among less dominant spores, morphotype 5 has significantly higher abundance in higher elevations compared to lower elevations ($P = 0.002$), even though the total number of spores is always higher than in lower slopes.

Morphotypes 1, 2, 3, 5, and 6 with highest frequencies compared to other morphotypes (Fig. 10b). Out of 14 sampling levels along elevation those 5 morphotypes can be seen in all elevational levels and therefore, they are not confined to specific elevation. In addition morphotypes 4, 7 and 9 were appeared in approximately half of the samples, as their frequencies are much lower. So these spore types have an uneven distribution along elevation, because they are present in only some samples. Further more, the occurrence of

other spore types is very low, since they have very low frequency values, indicating that they are the restricted to some areas in the site.

There is no distinct distribution pattern in either spore morpho type, but only morphotype 2 showed an interesting pattern (saw tooth like) with elevation (Fig. 11). In addition, morphotype 5 has higher spore density in mid elevations compared to lower and upper elevations (Fig. 11).

Correlation of Endomycorrhizal Spore density and diversity with Soil physico-chemical parameters

Endomycorrhizal spore density and spore diversity correlates differently with soil parameters. Both spore density and diversity did not show any significant correlation with the soil parameters tested (Pearson correlation coefficients are close to 0 & P values are > 0.05).

Correlation analysis revealed that Soil pH does not correlates with endomycorrhizal spore density and diversity (Pearson correlation coefficient values are -0.269 and 0.068 respectively). Soil pH values indicate that soil is acidic at all elevations with small differences with elevation (Fig. 12) and there is no significant variation of soil pH with elevation ($R^2 = 13.0\%$ & $P = 0.206$).

Endomycorrhizal spore density and number of morphotypes (spore diversity) do not show correlation with % organic Carbon (Pearson correlation coefficient values are -0.188 and -0.337 respectively). But, % organic Carbon seems to be increasing with increasing elevations (Fig. 12) and regression analysis revealed that this variation is significant at 95% significant level ($R^2 = 40.7\%$ & $P = 0.014$).

Bicarbonate extractable phosphate concentration varies with elevation but did not show a clear pattern (Fig. 12). It does not show a significant correlation with number of spore morphotypes (Pearson correlation coefficient = -0.389) and Endomycorrhizal spore density (Pearson correlation coefficient = 0.073).

Variation of spore density and diversity with spore size

Spore density is higher in smallest spores (in between 125 - 250 μm) compared to larger ones

and in contrast spore diversity is higher in largest spore size class ($>250 \mu\text{m}$). Seven morphotypes were common in all three size classes; morphotype 1, 2, 3, 4, 5, 6 and 7. Some morphotypes were restricted to only one size class; morphotype 8 and 12 were found only in the $125 - 250 \mu\text{m}$ size class

and morphotype 10, 13, 14 and 15 were found only in the $> 250 \mu\text{m}$ class. In addition, morphotype 9 was common in both small and medium size classes and morphotype 11 was common in medium and large size classes (Table 3).

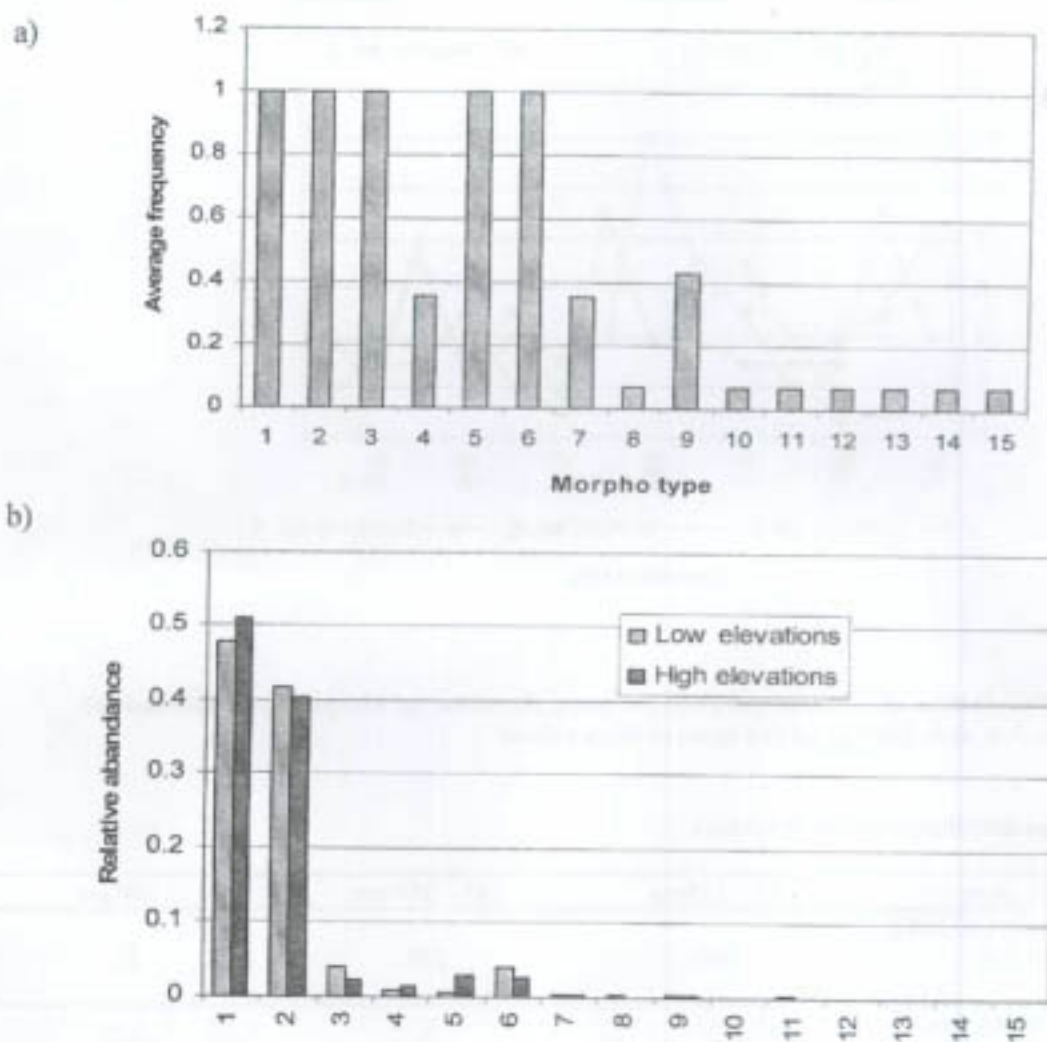


Figure 10. (a) Relative abundance (Elevations $< 400 \text{ m}$ are considered as low elevations and $> 400 \text{ m}$ as high elevations); and (b) average frequencies of morphotypes

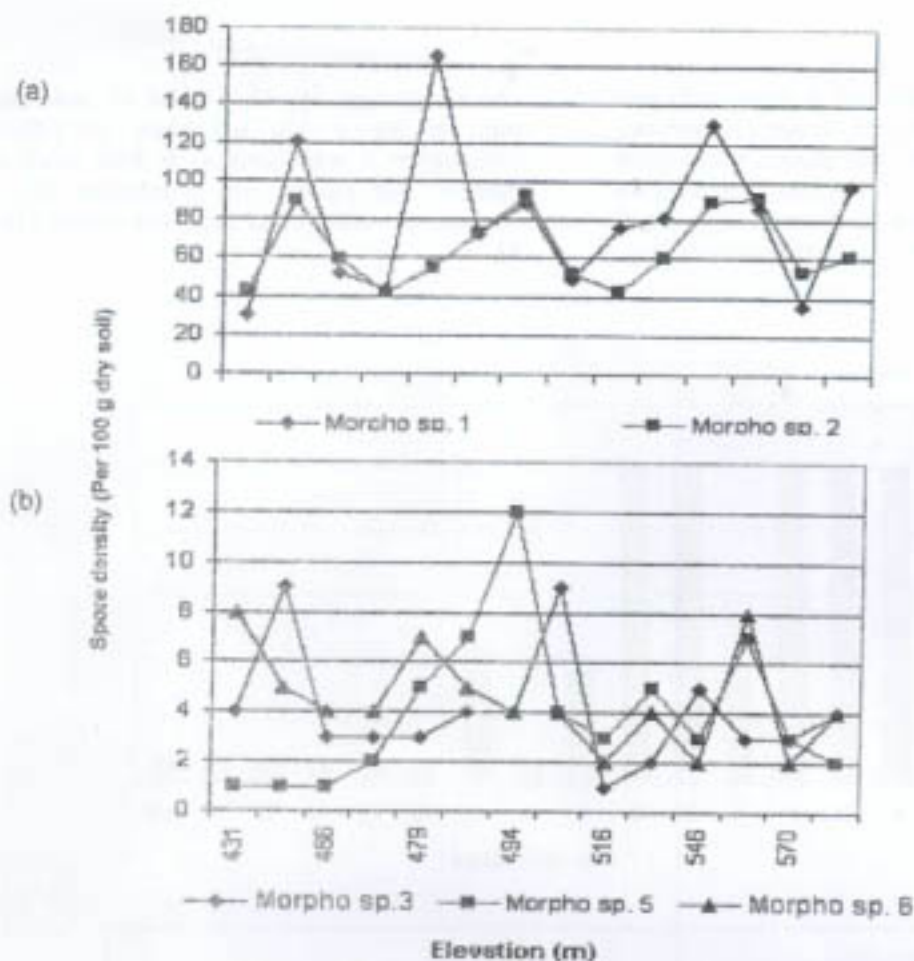


Figure 11. Distribution of common morphotypes along elevation; (a) Morphotype 1 & 2 and (b) Morphotype 3, 4, & 5. Each point is a mean of three values.

Table 3. Size distribution of Morphotypes

Size class	45 – 125 μ m	125 – 250 μ m	> 250 μ m
Spore density per 100 g dry soil	682	230	33
Spore diversity (no. of Morphotypes)	8	11	12
Morphotypes	1, 2, 3, 4, 5, 6, 7, 9	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12	1, 2, 3, 4, 5, 6, 7, 10, 11, 13, 14, 15

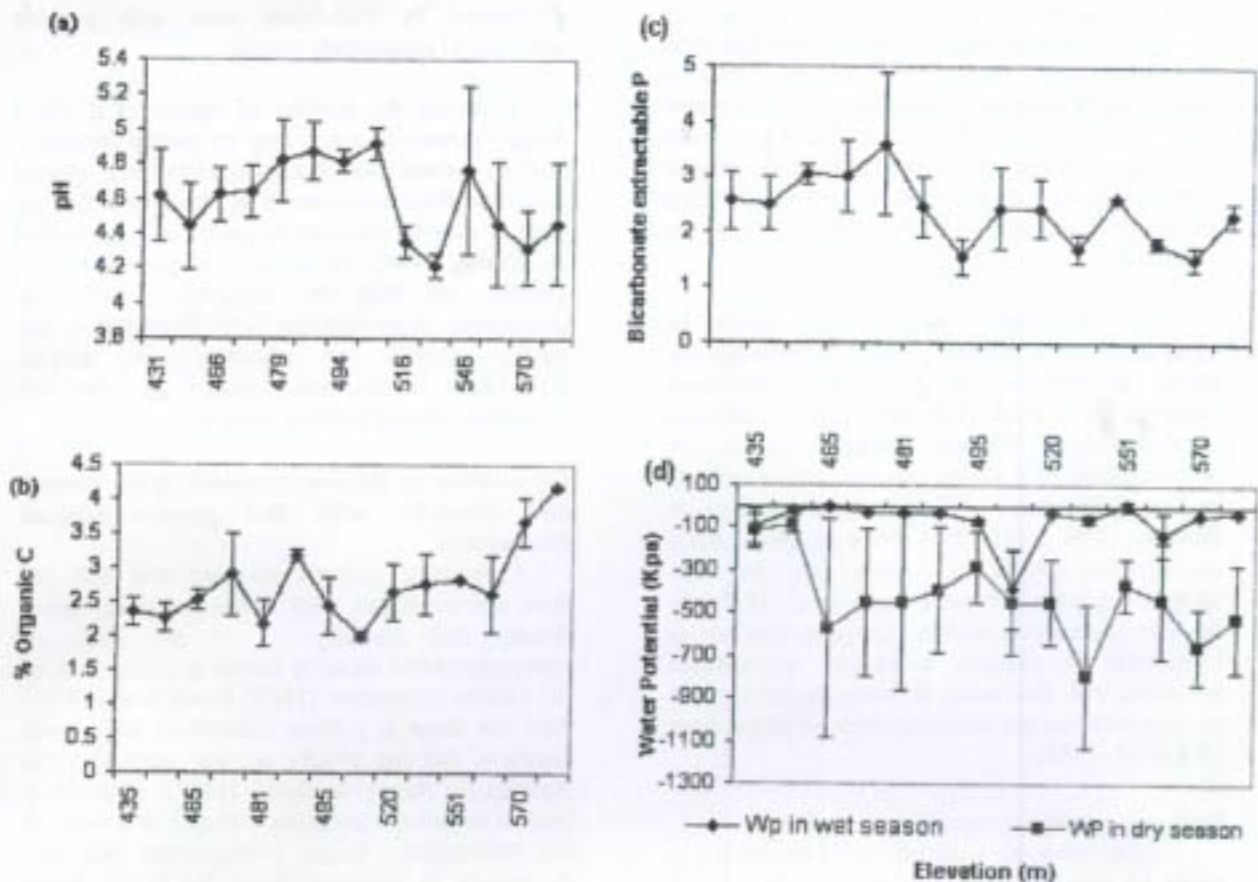


Figure 12. Variation of soil parameters (a) pH, (b) available phosphate, (c) organic Carbon, and (d) water potential, with elevation. Each point is a mean of three values and error bars indicate the standard error of the mean values.

Results showed that the water potential significantly decreased with increasing elevation, during the drier periods at Sinharaja ($P=0.023$ & $R^2 = 36.0\%$), but not during the wet season ($P=0.897$ & $R^2=0.1\%$) and it is more or less similar in ridge as well as in valleys (Fig. 11). Spore diversity (Number of spore morphotypes) showed a moderate positive correlation with soil water potential (Pearson correlation coefficient =0.552) and the correlation was significant at 95 % confidence level ($P = 0.041$). In contrast, the spore density does not show a significant correlation with water potential (Pearson correlation coefficient =0.155 & $P= 0.597$). All morphotypes do not show a significant correlation with soil pH, organic C, water potential and bicarbonate extractable phosphate (Pearson correlation coefficients are < 0.5 & P values are > 0.05).

DISCUSSION

Distribution of VAM spores with elevation

Higher density of spores at lower elevations could be explained by the accumulation of spores, which are coming down with rainwater. Because of the absence of specific hosts for the inoculation in lower slopes spores remain as it is without germinating might be another reason for the high density of spores at lower elevations. In support of this, Janos, (1980) pointed out that there might be many factors, which could be attributed to the variation in spore populations in a given site. Since they are obligate fungi, endomycorrhizal fungal spores always need live root contacts for germination. In the absence of suitable hosts they may persist as spores. Therefore, findings of this research would support the opinion of Janos (1980).

The spores in the sporocarps are loosely arranged, thus they might be easily detached from the sporocarps during wet sieving, thereby increasing the number of small spores. As pointed out by Janos (1980), the predation and parasitism of the large spores, which are rich in lipids, may be common in these ecosystems. Therefore, a decline in number of larger spores could always be expected.

The sporulation pattern also could be considered as a possible factor influencing the spore populations of this site. Laboratory experiments have shown that different combinations of VA mycorrhizal fungal species with different host plants showed different trends in root colonization and sporulation (Bevage & Bowen, 1975). While some VA mycorrhizal fungi are rapidly sporulating, others may be non-sporulating in some conditions (Warcup, 1975). At any rate, spores with smaller size range may not be significant in causing successful mycorrhizal infections with host roots, for their longevity and nutrient reserves are less than those of larger ones (Zak *et al.*, 1982).

Different morphotypes along elevation

Higher diversity of spores at lower elevations could be due to the low diversity of host plant species at those areas. Because of this spores do not have an opportunity to germinate and inoculate the potential host and therefore they may remain in the soil for a long time. This supports the findings of Moyersoen *et al.* (1998), where they found a more heterogeneous horizontal distribution of ectomycorrhizas in Korup National Park Forest, south-west Cameroon, and suggested that it may be due to the scattered distribution of ECM trees. An important feature of this park is that smaller number of ECM trees contributes a disproportionately high percentage of the total basal area. They studied root mycorrhizal fractional colonization and soil parameters in transect across areas of Low ECM and high ECM abundance of potentially ECM trees.

In contrast to his ideas, Brundrett & Abbott (1994) reported that ECM and VAM inoculum potential in upper soil horizons of a Jarrah forest, located in the Mediterranean climatic zone of Western Australia, was heterogeneous but could not be related to differences in plant cover. This forest is a sclerophyllous plant community

dominated by *Eucalyptus* trees with a wide diversity of understorey shrubs.

However the number of spores of a VAM fungus present in a soil may be poorly correlated with its current level of activity since some species do not produce many spores and the spores that are present may be dormant or parasitized (Brundrett & Abbott, 1994). In addition, Brundrett, (1991) pointed out that the capacity of different propagules of mycorrhizal fungi to persist in soil during periods of inactivity, to tolerate disturbance, or to resist predation by other soil organisms are not well understood.

Correlation of Endomycorrhizal Spore density and diversity with Soil physico-chemical Parameters

Correlation analysis revealed that Soil pH does not correlate with endomycorrhizal spore density and diversity. In the study of endomycorrhizal status in rubber growing soils of Sri Lanka, Jayarathne (1983) found that soil pH does not show any direct correlation with spore numbers. But my results are not agreed to the findings by Nancy & David (1997), where they found a negative correlation with pH. In a study of the mycorrhizal fungal communities and soil parameters in transects along the forest, forest edge and grassland within the Lomas Barbudal Biological Reserve in Costa Rica.

Endomycorrhizal spore density and number of morphotypes (spore diversity) do not show correlation with % organic Carbon. Present findings do not support the findings of Nancy & David (1997) where they found that some *Glomus* species were positively correlate with Carbon. In addition, Hafeel (1991) showed a positive correlation of endomycorrhizal spore population with organic matter in a study of comparing endomycorrhizae populations in the natural forest with those in a *Pinus* plantation and a *Dicranopteris linearis* fernland.

Bicarbonate extractable phosphate concentration does not show a significant correlation with number of spore morphotypes and Endomycorrhizal spore density. In contrast to the findings of Hafeel (1991) where he found that VAM spore populations were negatively correlated with available Phosphate, VAM spores

do not show any significant relationship with available phosphate at the level in the study.

At lower elevations, where the amount of water is high, the water potential was significantly high compared with that at the upper elevation. The spore density and diversity is also higher in lower elevations (Figs. 3 & 7). It is clear that when the availability of water is high endomycorrhizal spore population is also high. This supports the findings of Jayarathne, (1983) where he observed positive correlation of spore populations with soil moisture in Rubber growing soils of Sri Lanka. So, water availability might be a possible reason for the aggregated spatial distribution pattern of plant species in the 25 ha Forest Dynamic Plot at Sinharaja, where the plants that need more water might be aggregated in the lower elevations whereas plants that need less water may be aggregated in upper elevations.

Spore diversity (Number of spore morphotypes) significantly correlates with soil water potential. This is contradictory to the findings of Nancy & David (1997) where they found that species richness of VAM spores was not correlated with soil properties. Deacon (1997) described an important function of mycorrhizal fungi as that it benefits plants by tapping soil water reserves, because mycelial cords can transport water from deeper soils. The lower spore density and diversity at higher elevations may be because they may have inoculated plant species to facilitate water absorption, as the water potential at higher elevations is low. Mycorrhizal colonization of plant species aggregated in the upper elevations may be higher as the availability of water and spore population is low.

Variation of spore density and diversity with spore size

The spores in the sporocarps may have been detached to form a larger number of small spores, and since they are from one or two sporocarps, all spores are more or less same. This might be a reason for low diversity of small spores.

Finally, this study concludes that the endomycorrhizal spore diversity and density were both higher at the lower elevations compared to the corresponding values at the upper elevations. In addition, spatial distribution of endomycorrhizal spores within this area could not be explained only

by soil parameters, because VAM spore density and diversity did not correlate with soil parameters tested. But spore diversity was positively correlated with water potential. As Moyersoen (1993), showed the mechanisms responsible for the distribution of both ECM and VAM mycorrhizal types are complex and include factors other than soil properties. Competition of mycorrhizal species for common resources may influence their distribution. Therefore, to get an overall idea about the distribution of VAM populations in Sinharaja, other factors such as predation, distribution of host tree species, local disturbances and spore dispersal that might influence the distribution of VAM spore population, along with soil properties must be taken into consideration.

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REFERENCES

- Anderson, J. M. & Ingram, J. S. I. (1993). *Tropical Soil Biology and fertility. A hand book of methods.* (2nd ed.) CABI publishing Wallingford, UK.
- Ashton, P. S. (1977) Dipterocarpaceae. *A revised handbook to the flora of Ceylon.* Vol. 1 (Eds. M. D. Dassanayake & F. R. Fosberg). Pp. 364-423. Smithsonian Institution, Washington D. C.
- Bevege, D. I. & Bowen, G. D. (1975) *Endogone* strain and host plant differences in development of vesicular-arbuscular mycorrhizas. In: *Endomycorrhizas* (Eds. F. E. Sanders, B. Mosse and P. B. Tinker), Acad. Press, London. Pp. 77-86.
- Brundrett, M., Bongher, N., Dell, B., Grae, T. & Malajezuk, N. (1996) Australian center for international agricultural research. Canberra.
- Brundrett, M. C. (1991) Mycorrhizas in natural ecosystems. In: Macfayden A, Begon M, Fitter A. H., eds. *Advances in Ecological Research*, Vol.21 London: Academic press, 171 - 313.

- Brundrett, Mark C. & Abbott, L. K. (1994) Mycorrhizal fungus propagulus in the Jarrah forest I. Seasonal study of inoculum levels. *New Phytologist*, 127, 539-546.
- Burslem, D. F. R. P., Grubb, P. J. & Turner, I. M. (1995) Responses to nutrient addition among shade-tolerant tree seedlings of lowland tropical rain forest in Singapore. *Journal of Tropical Ecology*, 83, 113-122.
- Clark, D. B., Clark, D. A. & Read, J. M. (1998) Edaphic variation and the mesoscale distribution of tree species in a neotropical rain forest. *Journal of Ecology*, 86, 101-112.
- Condit, R., Ashton, P. S., Baker, P., Bunyavejehewan, S., Gunatilleke, S., Gunatilleke, N., Hubbell, S. P., Foster, R. B., Itoh, A., LaFrankie, J. V., Lee, H. S., Losos, E., Manokaran, N., Sukumar, R. & Yamakura, T. (2000) Spatial patterns in the distribution of tropical tree species. *Science*, 288, 1414-1418.
- Connell, J. H. (1978) Diversity in tropical rain forests and coral reefs. *Science*, 199, 1302-1309.
- Chris Picone (2000) Diversity and abundance of Arbuscular - Mycorrhizal Fungus spores in Tropical Forest Pasture. *Biotropica* 32 (4a): 734-750.
- Deacon, C. W. (1997) Modern mycology (3rd ed.) University of Edinburgh, Great Britain.
- Greig-smith, P. (1952) Ecological observations on degraded and secondary forest in trinidad, British West Indies, 11. Structure of the communities. *Journal of ecology*, 40, 316-330.
- Gunatilleke, C. V. S., Gunatilleke, I. A. U. N., Eihugala, A. U. K. & Esufali, S. (2004) Ecology of Sinharaja Rain Forest and the Forest Dynamics Plot in Sri Lanka's Natural World Heritage Site. WHT Publications (Pvt.) Ltd.
- Gunatilleke, C. V. S., Gunatilleke, I. A. U. N., Perera, G. A. D., Burslem, D. F. R. P., Ashton, P. M. S., & Ashton, P. S. (1997) Responses to nutrient addition among seedlings of eight closely related species of *Shorea* in Sri Lanka. *Journal of Ecology*, 85, 301-311.
- Hafeel, M (1991) Soil physico-chemical and endomycorrhizal studies in natural and modified sites of Sinharaja rain forest Sri Lanka. M. Phil. Thesis, University of Peradeniya, Sri Lanka.
- Hafeel, K. M. & Gunatilleke, I. A. U. N. (1989) Distribution of endomycorrhizal spores in disturbed sites of a lowland rain forest of Sri Lanka. Mycorrhizae for Green Asia (eds. A. Mahadevan, N. Raman, & K. Nadarajan), pp. 37-45. University of Madras, Madras.
- Harms, K. E., Condit, R., Stephen, P., Hubbell & Foster, R. B. (2001) Habitat associations of trees and shrubs in a 50-ha neotropical forest plot. *Journal of Ecology*, 89, 947-959.
- Janos, D. P. (1980a) Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology* 61: 151-162.
- Janos, D. P. (1980) Mycorrhizae influence Tropical succession. *Tropical succession (suppl.)* 12: 2. pg. 56-64.
- Janos, D. P. (1980b) Mycorrhizae influence. *Tropical succession. Biotropical* 2: 56-64.
- Janos, D. P. (1983) Tropical mycorrhizas, nutrient cycles and plant growth. In: Hafeel, K. M. (1991) soil physico- chemical and endomycorrhizal studies, in natural and modified sites of Sinharaja rain forests of Sri Lanka, M. Phil. Thesis. University of Peradeniya, Sri Lanka.
- Jayarathne, A. H. R. (1983) The endomycorrhizas of rubber growing soils of Sri Lanka and their effect on plant growth. M. Sc. Thesis, University of Sri Jayawardanapura, Sri Lanka.
- Maheswaran, J. & Gunatilleke, I. A. U. N. (1987) Soil physico-chemical properties of Sinharaja Forest, Sri Lanka and the effect of deforestation. Proceedings of the third round table conference on Dipterocarps. (ed. A. J. G. H. Kostermans), pp. 121-134 UNESCO, Regional Office for Science and technology for south east Asia, Jakarta, Indonesia.
- Moorman, F. R. & Panabokke, C. R. (1961) Soils of Ceylon. *Tropical Agriculture*, 117, 4-65.

- Moyersoen, B. fitter, A. H. and Alexander, I. J. (1998) Spatial distribution of ectomycorrhizas and arbuscular mycorrhizas in Korup national Park rain forest, Cameroon, in relation to edaphic parameters. *New phytologists* 139, 311-320.
- Munidas, B. K. H. C. Gunatilleke, C. V. S. & Gunatilleke, I. A. U. N. (2002) Climate of Sinharaja rain forest, Sri Lanka: An attempt to understand the el-nino and la-nina events. *Ceylon Journal of Science (Bio. Sci.)* Vol. 30. Pp. 37-54.
- Nancy C. J. & David A. W. (1997) Soil Carbon, nutrients, and mycorrhizae during conversion of dry tropical forest to grassland. *Ecological Applications*, 7 (1). Pp. 171-182
- Premadasa, M. A. & Gunatilleke, C. V. S. ((1981) Pattern in a rain forest in Sri Lanka. *Journal of ecology*. 69. 117-124.
- Read, D. J. (1997) Mycorrhizas in ecosystems – Nature's response to the "Low land of Minimum". CAB International.
- Read (1991), Mycorrhizas in ecosystems. *Experientia* 47 376-391.
- Taiz, L. & Zeiger, E. (1998) Plant Physiology (2nd edition) Sinauer Associates, Inc., Publishers. Pp. 120-122.
- Trappe, J. T. (1987) Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint, In G. R. Safar (Ed.). *Ecophysiology of VA mycorrhizal plants*, pp. 5-25. CRC Press, Boca Raton, Florida.
- Whitmore, T. C. (1984) Tropical rain forests of the far east (2nd ed.). Oxford university press, Oxford, UK.
- Whitmore, T. C. (1990) An introduction to Tropical rain forests. Oxford University Press, Oxford, UK.
- Zoysa, N. D. & Raheem, R. (1993). Sinharaja – A rain forest in Sri Lanka. March for conservation Colombo, Sri Lanka.