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Ecology of soil microflora and mycorrhizal symbionts in degraded forests at two altitudes

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Summary. Microbial populations were estimated in four different forest stands at different regenerational stages, two each at higher and lower altitudes. The fungal and bacterial populations showed marked seasonal variations at both altitudes. Quantitatively, the bacterial population was higher than the fungal population. Although 25 fungal species were isolated at the lower altitude, only 15 were obtained at the higher altitude. *Penicillium chrysogenum* and *Trichoderma viride* were dominant at the lower and higher altitudes, respectively. In the more degraded forest stand at the lower altitude both the fungal and the bacterial population showed a significant positive correlation with organic C ($r = 0.658$ and 0.735, respectively), whereas in the less degraded forest stand there was a significant correlation only between the fungal population and organic C ($r = 0.835$). At the higher altitude, however, a highly significant correlation ($P < 0.05$) was observed between the fungal population, soil moisture and organic C in both the forest stands. Disturbance to the soil and vegetation adversely affected the microbial population, and also affected endogonaceous spores. At the lower altitude, plants in the more degraded forest stand were more mycotrophic compared to those in the less degraded stand. The level of mycorrhizal infection showed a highly positive correlation with soil moisture, organic C, total N, and available P. The spore population, however, was correlated negatively with these parameters. Three different endogonaceous genera, *Glomus, Gigaspora*, and *Acaulospora*, were identified during the course of investigation. *Glomus*, however, was dominant.

Key words: Altitudes – Disturbance – Mycotrophy – Microbial population – *Glomus* spp. – *Gigaspora* spp. – *Acaulospora* spp. – Vesicular-arbuscular mycorrhizae

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Soil is a complex living entity containing a diverse group of microorganisms. The microbial community is one of the most important components of soil. The population composition and the activity of microorganisms are largely regulated by soil physicochemical properties (Mishra 1966; Tiwari et al. 1987), and by climate and vegetation (Mishra and Sharma 1977; Kauri 1982). For example, the addition of organic matter to soil has a variable effect on the growth of microorganisms (Guidi et al. 1988), and each vegetational community tends to harbour a characteristic population of soil mycoflora (Wohlrab et al. 1963).

The ecology of microorganisms, however, cannot be considered solely in terms of their relationships with the abiotic environment, because their success in a given situation reflects their ability to co-exist with other microorganisms. The North-Eastern region of India, where ecological conditions tend to promote the development of forests, is a particularly interesting habitat.

Some mycobionts help in the establishment of tree seedlings in unfavourable conditions such as low nutrient availability. About 95% of the plant species are mycorrhizal, the great majority forming vesicular-arbuscular mycorrhizae (Trappe 1977; Malloch et al. 1980). Soil disturbance may reduce vesicular-arbuscular mycorrhizal infection, and thus the P-adsorption efficiency of the plant (Evans and Miller 1988; Fairchild and Miller 1988).

The incidence and extent of mycorrhizal development may vary with the season (Staffeldt and Vogt 1975) and with the nutrient status of the soil. Reeves et al. (1979) proposed that non-mycorrhizal plants are effective colonizers of disturbed habitats and exert a profound influence on species composition. The rate of recovery of each habitat was inversely proportional to the degree of disturbance.

The present work was designed to investigate the distribution and successional pattern of soil microorganisms and mycorrhizal fungi over different seasons of the year for two different stages of forest disturbance at higher and lower altitudes in the foothills of the North-Eastern Himalayan range.

Materials and methods

Selection, climate and description of site

The study was conducted at two altitudes, the lower one (100 m above mean sea-level) at Byrnihat and the higher (1500 m above mean sea-level) at Shillong. At each altitude, two forest stands on laterite sandy loam soil, showing different stages of regeneration, were selected. In each of the four stands an area of 1 ha was demarcated for detailed study. The study areas were located between 25°C34'N and 92°47'E at Shillong and between 26°N and 92°45'E at Byrnihat.

The climate of the study areas is subtropical monsoonic. At Byrnihat the annual rainfall varied from 10 to 480 mm, and the mean minimum and maximum temperatures ranged between 22°C and 37°C and 25°C and 27°C, respectively. The annual rainfall at Shillong varied from 7 to 1100 mm, and the mean minimum and maximum temperatures ranged from 0°C to 16.4°C and 14.9°C to 22.5°C (Fig. 1).

In the North-Eastern region of India, native forest is now restricted to small areas. A large tract of abondoned Jhum land is covered by secondary forest growth. The more degraded forest stand was dominated by weedy species like *Ageratum conizoides* and *Eupatorium odoratum*, with a few tree stumps of *Mallotus philippinensis*, and *Manihot esculenta*, while the less degraded forest stand was dominated by tree species like *Holarrhena antidysenterica* and *Vitex glabrata*. At the higher altitude, both forest stands were dominated by *Pinus kesiya* along with *Alnus nepalensis, Myrica esculenta, Elaeagnus latifolia, Rhus javonica,* and the herbaceous *Rubus ellipticus, Osbeckia crinata,* and *Lantana camara.*

Collection of soil samples

Soil samples were collected randomly, aseptically, from a depth of 0–10 cm from five points in each site at both altitudes. The soil samples from each site were bulked together.

Analysis of soil physicochemical properties

The soil moisture content was determined by drying 10 g fresh soil in a hot-air oven at 150°C for 24 h.

The pH was observed on an electric digital pH meter in a 1:5 (w:v) soil-water suspension. For N, P, K and organic C measurements, the samples were air-dried and sieved (0.2 mm). Walkley and Black's rapid titration method was adopted to determine organic C (Allen 1974). Total N was estimated by the Indophenol blue method described by Allen (1974). The molybdenum blue method of Jackson (1967) was followed to determine the available soil P. Exchangeable K was extracted from the soil in an ammonium acetate solution (pH = 7) and was measured with a digital flame photometer (Systronics-121, India).

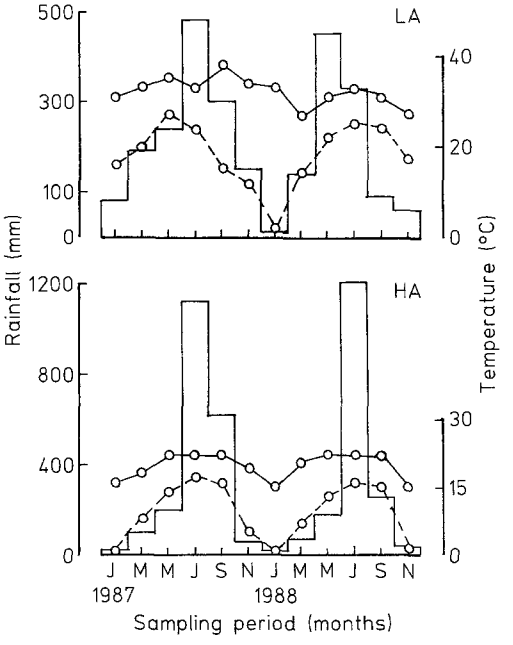

Fig. 1. Bimonthly variations in rainfall (*histogram*), and maximum (○———○) and minimum (○ – – – ○) temperatures at the study site. *LA*, lower altitude; *HA*, higher altitude

Isolation of soil fungi, bacteria and vesicular-arbuscular mycorrhizal fungi

Soil fungi were isolated by a dilution plate method (Johnson and Curl 1972) using a Rose Bengal agar medium (Martin 1950) and 10^3 dilution in water. The bacterial population was estimated by Waksman's (1922) method, using a nutrient agar medium (Difco Laboratories 1953) and a 10^5 dilution. The Petridishes were incubated at 25±1°C for 5 days and 30±1°C for 24 h for fungi and bacteria, respectively. Five replicates were taken in each case. The percentage relative abundance was calculated as:

$$\frac{\text{Total no. of individual fungi}}{\text{Total no. of all fungi}} \times 100.$$

Endogonaceous spores were isolated by the wet-sieving and decanting techniques of Gerdemann and Nicolson (1963); 10 g air-dry soil was suspended in 200 ml water and the suspension was shaken vigorously for 5 min, then passed through a series of sieves ranging from 500 to 63 µm. The sievings were collected on Whatman filter paper (no. 1) and scanned under a stereo-microscope for endogonaceous spores. The percentage mycorrhizal infection was determined by the root slide technique of Read et al. (1976).

Results

Soil physical properties

The soil was laterite, red to brown in colour and sandy loam in texture (sand 63.5%, silt 16.2%, clay 20.3%). It had a distinct upper horizon of organic C.

At the lower altitude, the soil moisture content varied from 9 to 30.5%, and at the higher altitude, from 17 to 34%. The moisture content was higher in the less degraded forest than in the more degraded one, at both altitudes. Values fell during winter but increased with the onset of rain.

At the lower altitude the soil was less acidic (pH 6.01–6.57) than at the higher altitude (pH 5.1–5.64). During the winter months the soil was more acidic than during the rainy season.

Soil nutrients

At both altitudes the less degraded forest stand contained more organic C than the more degraded stand. At the lower altitude, organic C varied from 0.3% to 1.9% in the more degraded and from 0.6% to 2.9% in the less degraded forest stand, while at the higher altitude it varied from 1.5% to 3%. The maximum organic C was estimated in May and July at the lower and higher altitudes, respectively.

Total soil N was also affected by the forest degradation. It was lower during dry winter months than in rainy months. At the lower altitude, total N ranged from 0.2% to 0.5%, and at the higher altitude, from 0.2% to 0.9%. Available P ranged from 2.5 to 7.3 mg 100^{-1} g and from

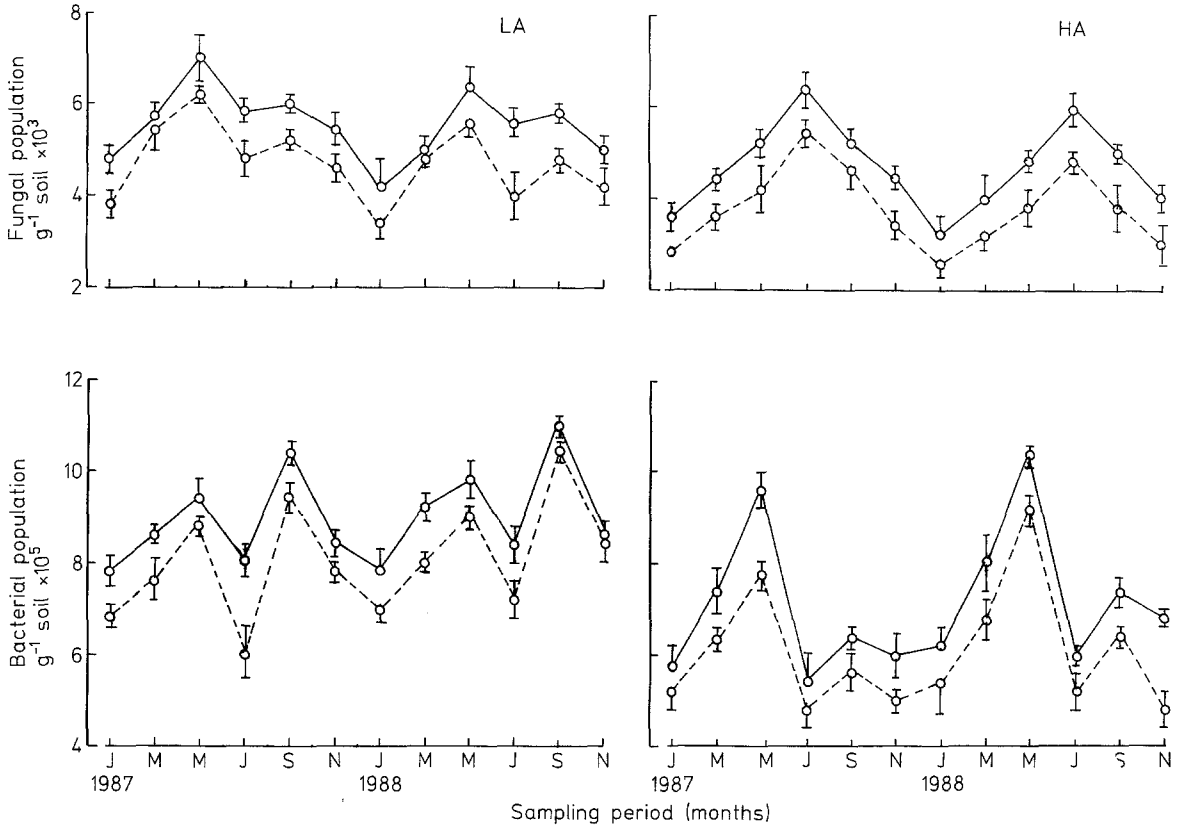

Fig. 2. Bimonthly variations in fungal and bacterial populations in more (○ – – – ○) and less (○———○) degraded forest stands at lower (*LA*) and higher (*HA*) altitudes. *Vertical lines* show limits of one SE on either side of mean

2 to 7.92 mg 100^{-1} g at the lower and higher altitudes, respectively. Exchangeable K was at a maximum in July at the lower altitude and in September at the higher altitude.

Population dynamics of microbes

Fungi. At the lower altitude the population of soil fungi showed very similar seasonal trends in both forest stands (Fig. 2). In general, fewer fungi were observed during the 2nd year of investigation. The more degraded forest soil harboured significantly smaller fungal populations (Fig. 2) than the less degraded soil. The maximum fungal populations were recorded in May in both cases, after which the population decreased until July (Fig. 2). There was a slight increase in September, but a decrease in the following months. In the less degraded forest stand, the fungal population was significantly and positively correlated only with organic C, while in the more degraded stand, a significant correlation was established not only with organic C but also with total N and available P ($P<0.05$; Fig. 3).

At the higher altitude, however, the highest number of fungal colonies was detected in July. Therafter, the population decreased until January, and then increased again until the peak in July. The two forest stands at this altitude showed almost identical seasonal variations. The fungal population was high in the less degraded and low in the more degraded forest stand (Fig. 2). Highly significant ($P<0.01$) correlations were obtained with soil moisture content and organic C (Fig. 3).

Bacteria. There were more bacteria than fungi in all forest stands. At lower altitude, the number of bacteria was high in September in both stands, decreasing thereafter until January and increasing again in March (Fig. 2). At both altitudes, the less degraded forest soil contained more bacteria than the more degraded soil. At the higher altitude, the total number of bacteria showed a steady increase until May, followed by a sharp decline in July. In September, there was a sudden increase in the population.

At the lower altitude, in the more degraded forest, a significant correlation ($P<0.05$) was established between the bacterial population and organic C, but not in the less degraded forest. The reverse was observed at the higher altitude (Fig. 4).

Altogether, 25 fungal species were isolated at the lower altitude, mostly deuteromycetes. In general, almost all the fungal species isolated were in both forest stands. Among the few species found only in the less degraded stand were *Trichoderma harzianum, Verticillium* sp., *Bromella* sp., and *Penicillium citrinum.*

On the basis of relative abundance, *Penicillium chrysogenum* was dominant.

At the higher altitude, however, only 15 fungal species were isolated. Both forest stands showed a close similarity in fungal composition, with *Trichoderma viride* dominant.

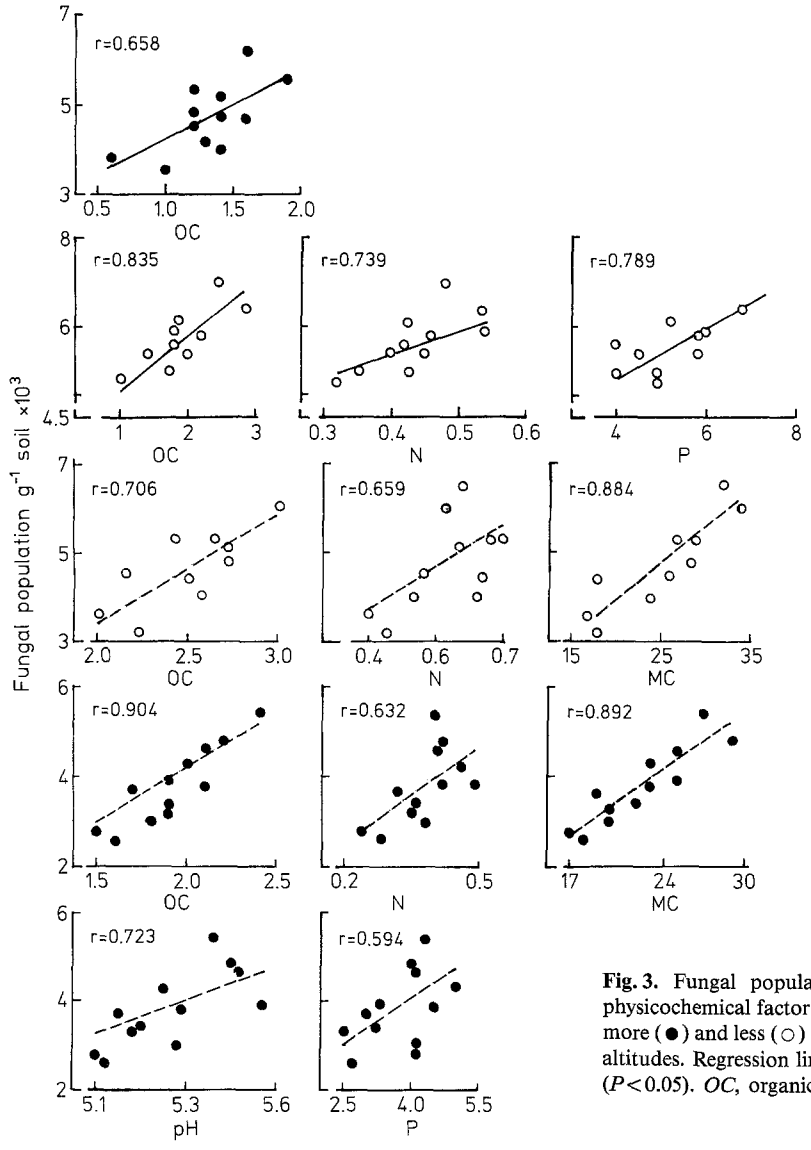

Fig. 3. Fungal populations (y axis) expressed as functions of different soil physicochemical factors (organic C, available P, total N, moisture content, pH) for more (●) and less (○) degraded forest stands at lower (———) and higher (– – –) altitudes. Regression lines are drawn only for statistically significant relationships ($P<0.05$). *OC*, organic C; *MC*, moisture content

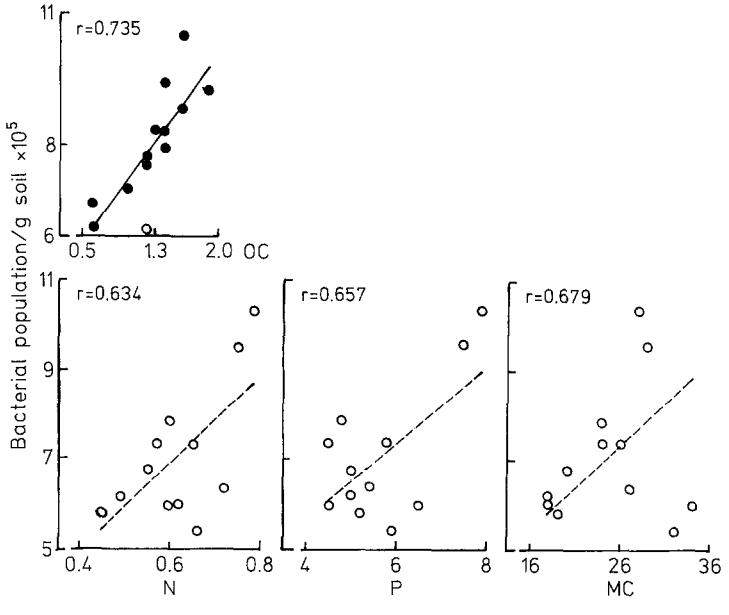

Fig. 4. Bacterial populations expressed as functions of different soil physicochemical factors. For other explanations, see Fig. 3

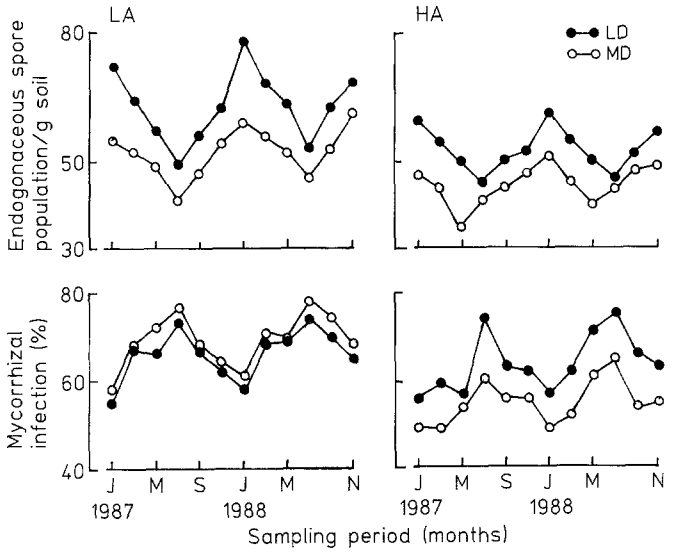

Fig. 5. Bimonthly variation in mycorrhizal infection (%) and endogonaceous spore population in more (●——●) and less (○——○) degraded forest stands at lower (*LA*) and higher (*HA*) altitudes

Vesicular-arbuscular mycorrhizal symbionts

At the lower altitude, all the plant species in the more degraded stand were more mycotrophic than those in the less degraded one. In most cases, heavy infection was observed during the spring and rainy season and a low level of infection in winter (Fig. 5).

At the higher altitude, however, apart from endomycorrhizal plants, some species had ectomycorrhizal infections. The plants occupying the more degraded site were less mycotrophic than those on the less degraded one. Heavy infection was encountered during May-July (Fig. 5).

The total number of endogonaceous spores varied from rhizospheric soil to soil and from season to season. At the lower altitude the less degraded forest soil harboured more spores than the more degraded one. In most cases the maximum spores were obtained in winter and the minimum during the rainy season (Fig. 5). At the higher altitude a similar trend was observed. At the lower altitude, a maximum number of 85 spores per g air-dry soil was obtained, and at higher altitude, a maximum of 70 spores per g air-dry soil was obtained.

Three genera of endogonaceous spores were identified during the entire investigation, *Glomus, Gigaspora* and *Acaulospora*. Among these genera *Glomus* was dominant. The spore population was heterogenous at the higher altitude and contained all three genera, but was more or less homogenous at the lower altitude and included only *Glomus*, except in warmer months when *Gigaspora* was isolated. The level of mycorrhizal infection was correlated positively, and generally significantly, with soil moisture, organic C, and pH (Fig. 6).

Discussion

A marked seasonal variation was observed in the soil microbial population, and was significantly correlated ($P<0.05$) with soil organic C and soil moisture. Mishra (1966) and Tiwari et al. (1987) attributed the seasonal microbial variation to the soil organic C content, soil moisture, and pH. The low microbial counts during the winter months in the present study may be related to the low

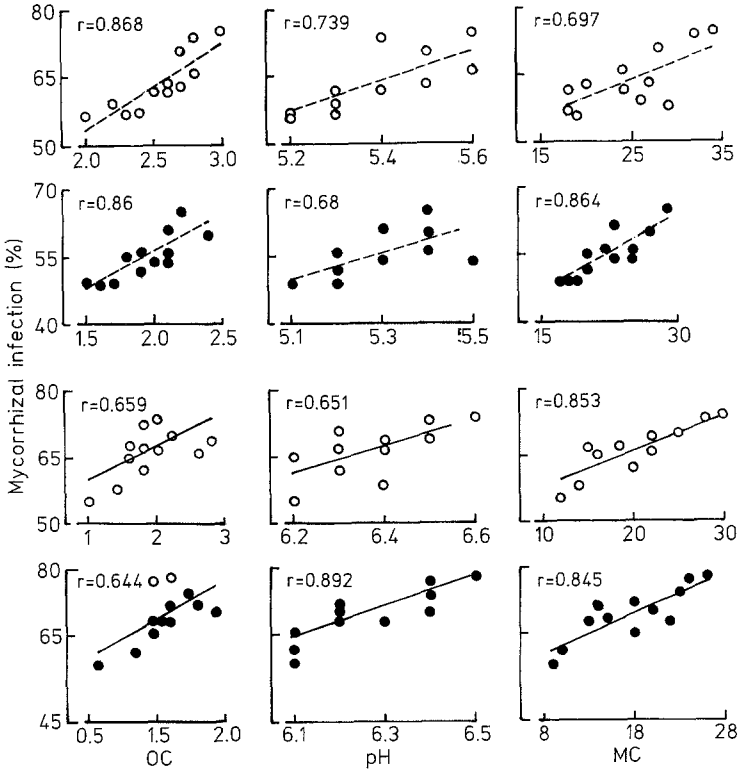

Fig. 6. Mycorrhizal infection (%) expressed as a function of different soil physicochemical factors. For other explanations, see Fig. 3

temperature and low moisture conditions, which may not have been conducive to microbial growth, while the decrease in population during July may be attributed to the high rainfall, which might have leached the available nutrients. Mishra and Sharma (1977) attributed a July decrease to fungal propagules being washed away with plant materials from the hill slope due to heavy rain. The two peaks observed in the bacterial population are in accord with Kauri (1982), who reported one bacterial peak in autumn and another in spring. The peak obtained in April in the present study may have been a result of favourable moisture and temperature conditions facilitating the transfer of soluble organic matter to soil.

A quantitatively similar species composition was obtained in both forest stands at the higher altitude, but at the lower altitude slight variations were observed between stands. This can be attributed to the change in vegetational cover at the sites and to variations in the nutrient status of the soils (Wohlrab et al. 1963). The dominance of *P. chrysogenum* and *T. viride* is in accord with observations by Bissett and Parkinson (1979), who pointed out that for a given community one or a few species are numerically dominant.

In the more degraded site at the lower altitude, nutrient stress and intense competition between plants appear to explain the occurrence of a heavy mycorrhizal infection in the plants. The fibrous root systems of the early-colonizing herbaceous species that occupied the site may also have contributed to the high level of infection, allowing better contact between the roots of different plant species and thus helping to spread the infection. Other possible causes of a high level of mycorrhizal infection include dominance of the disturbed site by mycorrhizal weeds (Miller 1979), plant age (Martin 1971), and deficiencies of N and P in the soil (Hayman 1975). Miller (1979) concluded that the occurrence of vesicular-arbuscular mycorrhizae is controlled by the degree of disturbance and harshness of site. These mycorhizae can be considered an essential feature of stress-tolerant strategy.

Scanty populations in the more degraded forest stand are in accord with results reported by Reeves et al. (1979). They concluded that soil disturbances lead to a reduction and possibly the elimination of vesicular-arbuscular mycorrhizal propagules. Spore populations may also be influenced by factors like organic matter and soil moisture. The inoculum potential of vesicular-arbuscular mycorrhizal fungi in soil has been shown to vary spatially in plant communities, both vertically and horizontally.

The low soil moisture content of the more degraded sites at both altitudes is likely to be a result of the sparse canopy and the dominance of weeds, which would cause a high rate of water loss from the soil. The duration and availability of water, therefore, may be limited on these sites, ultimately having an adverse effect on the population of microorganisms and symbionts.

The high-altitude soil was more acidic than the low-altitude soil; this feature may have been related to the dominance of pine litter in the soil. However, no significant difference in pH was observed between forest stands at the same altitude.

The increase in organic C in May may have been due to the addition of organic matter during February-March, caused by litterfall in the deciduous forest at the lower altitude and the increased activity of microorganisms in late autumn. A low organic C content in soil during winter has been attributed to losses due to soil respiration and mineralization (Tiwari et al. 1987).

The high nutrient concentrations in the comparatively undegraded forest stand may be attributed to the high concentration of organic matter and dense microbial population, conditions in which nutrients remain immobilized (Singh et al. 1989). The maximum value of available P and total N was obtained in May-June. Singh et al. (1989) also found maximum levels of P and N during summer months. They attributed this to microbial activity and to reduced plant growth curtailing the demand for nutrients. In the present study, the drop in P content during the winter corresponded to a lower microbial population and low activity levels. Thus, it may be concluded that apart from the effects on nutrient status, soil disturbance has a profound effect on microbial and symbiont populations. This will ultimately lead to a slow regeneration of forests.

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