

**ECOLOGICAL STUDIES ON BIODEGRADATION OF LEAF LITTER OF ALDER  
(*Alnus nepalensis* D. Don) WITH SPECIAL REFERENCE  
TO THE ROLE OF MICROBES AND ISOPODS**

BY  
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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT  
FOR THE DEGREE OF  
**DOCTOR OF PHILOSOPHY**

TO



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## CERTIFICATE

We certify that the thesis entitled, *ECOLOGICAL STUDIES ON BIODEGRADATION OF LEAF LITTER OF ALDER (ALNUS NEPALENSIS D. DON.) WITH SPECIAL REFERENCE TO THE ROLE OF MICROBES AND ISOPODS*, submitted by **Mr. Highland Kayang** for the Degree of Philosophy of the North-Eastern Hill University, Shillong, Meghalaya, embodies the record of original investigation carried out by him under our supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the **Ph.D. Degree**. This work has not been submitted for any degree of any other University.

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**DEDICATED  
TO MY  
MOTHER  
AND  
FATHER**

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**GENERAL INTRODUCTION**

The colonization of leaf surfaces by fungi presents an interesting study with regard to substrate relationship (Pugh and Buckley, 1971). The fungal colonizers on leaf surface comes either from air or from soil. The occurrence of many phylloplane fungi may be directly related to air microflora, which in turn is related to the production of the deciduous propagules elsewhere (Dickinson, 1976). Relatively, little work has been done to understand the ecology and role of naturally occurring microbes on the leaf surface and their relationship with air microflora in general and of forest trees in particular.

The leaf surface releases fungal spores which largely contribute to the air spora of the locality. Cycling phenomenon between fungi spores of air, soil and plant surface may be maintained in the specialized environment (Mishra and Srivastava, 1971a). There are, however, few fungal species specific to either air or to phylloplane (Lamb and Brown, 1970; Sinha, 1971; Mishra and Tiwari, 1976a).

The seasonal and diurnal periodicities of microbes in the air is mainly dependent on climatic conditions especially temperature, relative humidity, rain and wind

speed. The spores present in the air may have a great impact on the leaf surface mycoflora where leaf surface acts as a landing site (Pugh and Buckley, 1971; Gregory, 1971). Relatively little work has been done to understand the effect of air fungal population over different fields in relation to the leaf surface fungi (Sinha, 1971; Mishra and Tiwari, 1976a; Kumar and Gupta, 1976, 1980; Dixit and Gupta, 1980).

Further, studies on leaf surface mycoflora are helpful in understanding the succession of decomposers responsible for active turnover of minerals from leaf litter (Gupta, 1982). The leaf surface supports a unique habitat to a definite microbial community by providing different ecological niches. The leaves of different plant species may therefore harbour a distinctive microbial community (Lamb and Brown, 1970). Host specificity is, however, controlled by the physical and chemical nature of the leaf (Kerling, 1964). Many workers (Gregory, 1950, 1961; Last, 1955a; Mishra, 1968; Sinha, 1971; Kumar and Singh, 1981; Pennycook and Newhook, 1981) have noticed that climatic factors like temperature, humidity, wind, light and rain influence the leaf surface microorganisms. Quantitative and qualitative composition of the microflora is affected by the weather conditions and the age of leaf

(Mishra and Srivastava, 1970a; Sinha, 1971; Sharma and Mukherjee, 1976; Hayes, 1982).

Decomposition of plant litter is governed by a complex interactions between biotic factors such as microorganisms, soil fauna, nutrient and carbon components of litter and abiotic factors i.e., temperature and moisture. Microorganisms and soil animals interact synergistically in the biodegradation of litter and both of them are strongly influenced by factor like soil, litter quality, temperature and moisture (Crossley, 1970 and Witkamp, 1971).

The litter consists of dead or decaying leaves, twigs, branches, flowers, fruits, bark and other debris; leaves however contributes towards the major litter component. The accumulation of organic matter on the forest floor, represents an energy and nutrient storage pool, a diverse habitat for many different types of heterotrophic organisms, and a substratum for plants. The amount and nature of litterfall has an important bearing on soil formation and the maintenance of its fertility. ✓

Swift et al (1979) distinguished among three different processes that contribute to complete the decomposition, catabolism, cominution and leaching. Catabolism

involves the biological conversion of organic compounds into inorganic forms such as carbon dioxide and mineral nutrients. It can be regarded as the final step in the decomposition of complex molecules whereas other two processes mainly have a modifying effects on the decomposition rate.

✓ Decomposition accomplishes a fundamental role in ecosystem cycling processes by keeping plants supplied with essential nutrients. A vital sets of energy flows ✓ and nutrient transfer results from litterfall and its subsequent biodegradation in forest ecosystem (Crossley, 1970 and Witkamp, 1971). ✓ Litterfall is the major pathway for the return of dead organic matter and many of its contained nutrient and non essential elements from the aerial parts of the plant community to the surface of the soil.

✓ The amount, composition and subsequent decomposition of litter in forest ecosystem is of major importance in studies of energy flow, nutrient cycling and primary production (Ovington, 1962). The quantity of litterfall over the entire year is equally an important parameter as its seasonal periodicity. Differences between the rates of litterfall and its breakdown result in the formation of a layer of dead and decomposing leaves, woody material,

reproductive structures and other organic materials on the surface of the soil known as the litter layer, forest floor or the O horizon. The amount of the litter accumulation is dependent on its input, organism successional time and the rate of its decomposition (Olson, 1963). The decomposition rate is, in turn is determined by the nature of the litter input, temperature, moisture, soil type, altitude etc.

The litter is decomposed in the soil litter subsystem by a variety of microorganisms which are involved in succession phases of colonization, exploitation and exhaustion of organic substrate and are responsible for the chemical transformations and degradation of complex organic molecules into simpler ones. Microorganisms predominate in litter decomposition, primarily because of their enzymes capable of biodegrading simple and complex carbohydrates like lignins and their products (Witkamp, 1971). Enzymes produced by microorganisms in the forest soils are mainly responsible for the decomposition of organic matter and mineral cycling.

Decomposition of litter is basically carried out by microorganism, and fungi are known to be the chief colonizers and decomposers of plant litter (Hudson, 1968 and Hayes, 1979). Several workers have emphasized that

the early colonizers of leaf litter are bacteria, ascomycetes, fungi imperfects and some basidiomycetes which attack simple carbohydrates and cellulose and subsequently these organisms are followed by phycomycetes. Fungi and bacteria convert plant nutrient resource for soil animals. Soil invertebrates through grazing activity, litter communication and faeces production stimulate microfloral growth and make new substrates available for microbial exploitation. The breakdown of organic matter in the litter layers and soil is mediated through the synergistic activities of the fauna and flora combined with the leaching action of rainfall and throughfall which transports the breakdown products to the soil. The rates of activity of the soil and litter saprovores are directly affected by environmental factors, particularly the rainfall and temperature regimes.

Alexander (1961) stated that as the decomposition proceeds, hemicellulose and cellulose disappear whereas lignin like materials remain. He observed that during decomposition hemicellulose may have disappeared at a more rapid rate than the cellulose. He further stated that as the plant materials are decomposed by fungi, a variety of organic constituents are presented in turn to a succession of ecological groups of fungi, each orga-

nism or group of organisms altering the organic constituents until the process is complete. Later, he noticed that the fungi decomposing sugars and simple carbohydrates belonged predominantly to phycomycetes, cellulose decomposing ones to the ascomycetes, basidiomycetes and deuteromycetes and the lignin decomposers were restricted to the basidiomycetes only.

In plant litter, lignin is the component which is the last to start degradation (Berg et al, 1982) and it has been observed to be degraded comparatively slowly (Waksman, 1938) and being a slowly decomposed component, it will be rate determining for decomposition of a large part of the litter (Berg and Staaf, 1980). The release of nutrients from litter is dependent on the decomposition of the organic substances which acts as a carrier and has been observed to be proportional to weight loss (Staaf and Berg, 1977, 1982). With litter decomposition rate being critical for nutrient release it appears important to find factors which regulate the mass loss rate.

The role of fauna and microflora in the litter decomposition has been discussed and the possible effect of fauna on the growth and activity of specific components of the microflora has been documented.

Whilst generalizations may be made from litter fauna studies which include field observations, gut content

analysis and feeding preference experiments, it is difficult to make real conclusions on the effects of microbe-feeding litter invertebrates on the growth of specific microorganisms and on the capacity of such microorganisms for competitive colonization and exploitation of available substrates (Parkinson et al., 1979).

Forest ecosystems are inhabited by large numbers of soil invertebrates which depend primarily on a microflora food source. The invertebrates that inhabit soils are extremely diverse in form, differ greatly in size, occur in extremely large numbers, are more or less aggregated in distribution, both vertically and horizontally and often live deep with soil particles.

Soil invertebrates influence litter decomposition in both direct and indirect ways. The direct influence involves passage through the gut and subsequent digestion and assimilation. It may be of relatively little consequence since only 5-10% of total soil metabolism can be attributed to fauna (Macfadyen, 1963; Butcher et al., 1971). The more important indirect role of invertebrates involves factors such as inoculation of litter with microflora and alteration of microfloral activity by grazing and fragmentation of litter by feeding activity (Macfadyen, 1963; Burges, 1967; Ghilarov, 1970; Crossley, 1977). The

progress of litter decomposition encompasses interactions among different populations of microbes and microorganisms and environmental variables (Gunnarsson et al. 1988) each one affecting the quality of the litter as a substrate for other decomposers (Richard, 1987 and Choudhury, 1988). The size of the particles left by litter consumers determines which organisms are likely to attack the material subsequently (Richards, 1987 and Hagvar, 1988). Resource quality influences the types and rate of microfloral growth, faunal grazing and palatability to the saprophytic fauna, hence rate of litter decomposition. In spite of immense importance of fauna in microbial degradation, studies are meagre on isopod-microbe interaction during litter biodegradation.

The present investigation was therefore, carried out to understand the role of microbes-isopods during forest tree litter, as outlined below.

- Seasonal study of air fungal population at two forest stands of Alnus nepalensis D. Don in relation to the leaf surface fungi.
- Seasonal changes in microbial and isopod population during litter decomposition of alder (A. nepalensis).
- Mass loss and nutrient mineralization from alder

- litter in relation to the microbes and isopods.
- Microbial enzyme activity during alder leaf litter decomposition.
  - Influence of isopods on microbial population and nutrient release during the decomposition of leaf litter of alder.
-

**REVIEW OF LITERATURE**

## **Air Spora**

Cunningham (1873) reported the changes in atmospheric spore content of Calcutta prison.

Grainger (1954) accorded higher concentration of Helminthosporium avenae in air at lower level of infected oat crop.

Last (1955 b) studied the air spora within and above the mildew infected cereal crops and recorded higher population near the ground.

Gregory (1950, 1957, 1961) observed a direct correlation between air spora and the microflora of leaf surface.

Rishbeth (1959) estimated the spore trapping capacity of conifers. The number decreased only by death and washing off of the spores by rains which renewed subsequently by arrival of fresh spores.

Pady et al (1967) suggested that many of the airborne fungal hyphae are conidiospores which were probably released by the wind current from dead leaves of crops. He further (1971) stated that the leaf acts as an admirable

site for both saprophytic and parasitic fungi and under favourable conditions spores are produced in tremendous number which are released into the air.

Gregory (1971) suggested three main ways for the arrivals of spores (i) dry wind borne route, (ii) in rain drop, and (iii) in rain splash droplets.

Mishra and Tewari (1976 a) stated that an understanding of the nature, periodicity and density of the fungal propagules in the air is much helpful in making a forecast regarding the occurrence of fungal diseases and the quantum of viable pathogenic propagules likely to cause infection.

Bovallius et al (1978), Dixit and Gupta (1980) and Kumar and Gupta (1980) observed that the number of spores deposited on the leaf surface is nearly proportional to the number of spores in the air.

### **Microbial Population from the Phylloplane Region**

The leaf surface forms a unique habitat since it supports a definite microbial community by providing a complex of niches to various organisms viz., bacteria, actinomycetes, yeasts and fungi.

Debarry (1866) recognised the presence of fungi

on the surface of plants. He described Dematium pullulans as a fungus commonly occurring under such situation.

Brown (1922) in his experiment on leaf surface elegantly demonstrated that microbes could benefit from nutrients available on the surface of leaves. A range of materials including inorganic ions, free sugars, amino acids and growth regulating gibberellins have been detected in leachates.

Keener (1951) observed that the leaf soon after it unfolds from the bud is relatively a clear sheet which later becomes the seat of various microbial activities.

Last (1955a) studied seasonal incidence of Sporobolomyces on leaves of three crops viz., winter and spring sown wheat and spring sown barley.

Ruinen (1961) on the basis of his studies of the phylloplane flora of rain forest plants concluded that bacteria were the first colonizers of young leaves, and buds were the active sites of colonization. Ageing of the leaves results in a gradual increase of microbial cells. He also indicated that bacterial number usually increased significantly before appreciable numbers of yeast and filamentous fungi colonized green leaves. This view is generally accepted although it lacks confirmation.

Ruinen (1961) while studying the microbiology of phyllosphere of the humid tropic vegetation, observed divergent microflora including algae, fungi and bacteria, and recorded maximum population on senescent foliage. She noticed oligonitro-philic and nitrogen fixing bacteria as first colonizers of the leaf surface.

Hudson (1962) pointed out that the microorganisms which are present on the leaf surface in active state are known as colonizers and he classified the latter into two types (i) common primary saprophytes which are also present in the air so referred as field fungi, viz., Aspergillus spp., Penicillium spp., (ii) restricted primary saprophytes which are specific to the host plant.

Kerling (1964) studied leaf surface fungi on rye and strawberry and observed that the population of Botrytis cinerea increased rapidly as the leaves of strawberry approached to senescence.

Kendrick and Burges (1962); Hayes (1965) and Macaulay and Thrower (1966) have shown that the organisms present on leaves at senescence are important in determining the subsequent pattern of decomposition.

Last and Deighton (1965) discussed the possibility that epiphytic microorganisms on the leaf surface play

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an important part in controlling the incidence of plant diseases by producing antibiotics or by utilizing energy sources present on the leaf surface which might otherwise stimulate the growth of pathogens. They also indicated that the saprophytic microflora on leaves might act as initial colonizers in the succession of microorganisms which ultimately decompose plant tissues.

Last and Deighton (1965) pointed out that bacteria and yeast like fungi were more abundant on the leaves than the hyphomycetes. Members of sporobolomycetaceae dominated the surface of diseased leaves infected by fungi, nematodes and mites.

Dickinson (1965) distinguished three groups of leaf surface fungi viz., the transient fungi present on the leaf surface including yeasts and other fungal propagules which are capable of sporulating on leaf surface but are not isolated from washed discs. The second group of fungi dominated by Cladosporium herbarium were recorded from both leaf surface washings and washed discs. The third consisted of the forms growing vegetatively on leaf surface by pycnidia and were found only on moribound leaves.

Leben (1965) distinguishes two groups of epiphytes (i) residents, which multiply on the surface of healthy

plants or on debris on these surfaces, without noticeably affecting the host, (ii) casuals, which are on the plant surfaces, but may grow saprophytically on foreign debris.

Hogg and Hudson (1966) also recognised three distinct pattern of fungal distribution on the leaves of birch.

Hogg and Hudson (1966) examined young living leaves of Fagus sylvatica and found that the first colonizers, Discula quercina (conidial state of Gnomonia errubunda) appeared within 3 months of unfolding of the leaves.

Macauley and Thrower (1966) studied in the southern hemisphere the parasitic mycoflora within freshly fallen leaves of Eucalyptus regnans.

Dickinson (1967) pointed out that forms like Cladosporium, Stemphyllium and Alternaria were of frequent occurrence on the leaves of Pisum sativum. He further worked on fungal colonization of Pisum leaves and found that not all the fungi recorded by moist chamber technique were phylloplane saprophytes as several species viz., Penicillium spp. and Aspergillus fumigatus constitute a group of casual inhabitants of the phylloplane and their presence may merely reflect the relative abundance of their spores in the atmosphere.

Holoman (1967) investigated leaf surface mycoflora of three potato varieties where Aureobasidium pullulans and Cladosporium hebarium were the usual inhabitants. He placed the isolates obtained from washing technique in the four groups i.e., (i) Aureobasidium pullulans, (ii) Cladosporium herbarium, (iii) Alternaria tenuis, Botrytis cinerea and Fusarium spp., (iv) Miscellaneous.

Hudson (1968) has emphasized the value of the studies of leaf surface fungi to investigate the mycoflora of living leaves on the tree.

Hislop and Cox (1969) examined expanding apple buds as part of their study of phylloplane fungi and yeasts.

Paddy et al (1969) observed that many dead leaves of wheat due to rust infection (Puccinia recondida) favoured the growth of Cladosporium which sporulated heavily on dead leaves.

Lamb and Brown (1970) confirmed the microflora present of leaf surfaces which may be divided into two groups (i) the residents which are the actively growing saprophytic forms, (ii) the transient species which are the inactive forms and are deposited on the surfaces of the leaves as wind borne propagules. The residents are the epiphytic organisms which grow and reproduce as sapro-

phytes merely by chance on the leaf surface and active growth and reproduction in such forms do not occur.

Mckenzie (1971) investigated the seasonal incidence and possible interrelation of parasite and saprophytic fungi in Rye grass and white clover pasture in New Zealand.

Pugh and Buckley (1971) surveyed colonization of leaf surface by bacteria, yeast and fungi.

Mishra and Srivastava (1971a, b) investigated the leaf surface microflora of various crop plants but no such work on forest trees is available in India.

Collins and Hayes (1976) studied the seasonal incidence of microbes on the surface of first year needles of Pinus abies (Norway spruce) in castle over forest Dim-frieshire. The saprophytic microflora isolated from bud and needles during first year season of growth was characterized by a rapid increase in numbers to a peak value shortly after needle flushing.

Lindsey and Pugh (1976) studied the pattern of succession and distribution of microfungi on attached leaves of Hippophae rhamnoides. They noted the complex nature of the leaf surface with its dense covering of trichomes and the non-random distribution of fungi.

Dickinson and O'neil (1977) studied the behaviour of phylloplane fungi on Phaseolus leaves. Their results support the suggestion that humidity is a prime factor in determining the extent of leaf colonization.

Latch et al (1977) studied the fungal flora of Rye grass swards in Wale and found that Phoma spp. were the most commonly isolated fungi, followed by Acremonium spp., Cladosporium spp. and Tricellula aquatica.

Mishra and Dickinson (1981) investigated the phyllosphere and litter fungi of Illex aquifolium using leaf impression peals, scanning microscopy and cultural techniques. They reported a marked seasonal fluctuations of microbial population in older green leaves than on newly formed leaves.

Daniel (1985) investigated the saprophytic fungal communities of the phyllosphere of Eucalyptus viminalis by serial washing, superficial sterilization and direct observation. According to him, the fungi form two differential groups phylloplane species and endophytes on leaving leaves. The phylloplane community was similar to that found on other plants but endophyte exhibited host specificity. Both groups showed variation in frequency but phylloplane species showed distinct seasonal pattern with

maximum in autumn - winter and minimum in summer with positive and negative correlation with humidity and temperature.

Kehri and Chandra (1987) observed the variation of both bacterial as well as fungal population of phyllosphere and phylloplane with season and climate conditions in Eichhornia spp. having Aspergillus, Cladosporium and Fusarium spp. as the dominant form.

Vardavakis (1988) studied the phyllosphere mycoflora of Cistus incanus, Arbutus unedo and Quercus coccifera by leaf impression, culture method and reported seasonal variation of dominant and resident fungal species. He also observed variation in abundance of each fungal species.

Adhikari (1990) observed that the mycoflora of green and dried stored leaves of three grasses (Heteropogon contortus, Themadas anathera and Setaria glauca) were colonized by almost similar species of fungi. He further stated that the mycoflora were governed by environmental factors and the biochemical nature of leaves.

### **Microbial Population of Litter**

Fungal succession on deciduous tree leaf litter was investigated by Saito (1956).

Ecological succession of fungi on naturally occurring plant debris, decomposing on the surface of the soil has been subject of intensive study by many workers (Webster, 1956, Hudson and Webster, 1958).

Pugh (1958) observed a definite change in the fungal flora of Carex paniculata L. leaf litter which he attributed to temperature and moisture content of the litter.

The study of fungal succession in decomposing coniferous litter was summarized by Kendrick and Burges (1962).

Caldwell (1963) observed fungal succession on the decomposing beech leaf litter. Most of the fungi were common in early and late stages of decomposition.

Garrett (1963) in his investigation on fungal colonization of dead plant remains in or upon the soil noted that sugar fungi were primary colonizers and compared the fungal succession with autogenic plant succession.

William (1963) reported that even under an apparently uniform vegetation cover considerable variation occurs in the microbial populations.

Witkamp (1963) studied the seasonal change in

fungus and bacterial counts from five leaf species decomposing in coniferous and deciduous stands. He observed that numbers of microbes was positively correlated to the temperature, moisture and stage of litter decay.

Carre (1964) stated that fungal succession was more influenced by weather rather than accumulation and availability of food substrates during the first two years, and there occurs a definite successional pattern in natural conditions.

Hayes (1965) in his studies of scot pine litter decomposition reported that the microclimate influenced the development of dominant species of microflora. He further (1965b) studied the microfungi associated with the leaf litter of Abies, Picea and Pinus sylvestris and reported those factors which influenced the dominant species of litter microflora.

Hering (1965) while studying the fungal succession on plant litter observed a specific pattern of fungal species. The primary fungal flora was dominated by Trichoderma and Penicillium species.

Leigh (1965) forwarded the view that increase in species richness enhances the stability of the community during the process of litter decomposition.

Witkamp (1966) studied the bacterial and fungal population during decomposition of Mulberry, red-bud, white oak, loblolly pine litter in Tennessee and found that the microbial densities and annual weight losses of litter were significantly positively correlated.

Hogg and Hudson (1966) investigated the succession of micro fungi on Fagus sylvatica leaves from the time of unfolding upto eighteen months after leaf fall.

Macauley and Thrower (1966) observed the change in the succession of five different groups of fungi on the decomposing leaf litter Eucalyptus regnans. They observed moniliales as the primary colonizers followed by mucorales at later stage. The disappearance of certain fungal species during the course of investigation even when the nutrients were sufficient was attributed to the antagonistic effect of some fungal species.

Chang and Hudson (1967) observed a regular pattern of fungal succession of wheat straw compost. /

Hudson (1968) reviewed the ecology of fungi on above ground remains and presented a general scheme for the colonization of decaying leaves where the succession begins with parasites followed by primary saprophytes. Later on, the primary saprophytes are over-ridden by secon-

dary saprophytes such as ascomycetes and deuteromycetes. Phycomycetes are the last to colonize the litter.

Brandsberg (1969) conducted a qualitative study of the microflora associated with the degradation of different coniferous litters. Altogether, he isolated 128 fungal species without any pronounced difference in the microfloral composition on the duff of different tree species. He also observed a definite successional pattern on the decomposing leaf litters.

Harrison (1971) reported that tannins were inhibiting to the growth of fungi during oak litter decomposition. He concluded that tannins have an influence on the nature of early fungal succession, and may be the cause of the relatively low level of microbial activity in decomposing oak litter.

Tubaki et al (1971) examined fungal succession on sterilized leaves of Castanopsis and Quercus in natural condition and observed a regular pattern of succession.

Holm and Jensen (1972) carried out an examination on Fagus litter and they found a bacterial flora very similar in composition to that on mature leaves in the canopy.

Sharma and Dwivedi (1972) examined the fungal succession on decaying grass (Setaria glauca) and observed differences in the total number of fungi at different time intervals on different plant parts i.e., stem, sheath and blade. They attributed the fluctuation in fungal population to various factors like the moisture content of the substrate, temperature, humidity and competition between the colonizers.

Rai (1973) while studying the fungal succession on decaying leaves of Saccharum munja noticed various kinds of fungi which appeared at different stages of decay and fungus flora was found to vary as the leaf tissues decomposed and disorganised. He recognised three groups of fungi for their distinct patterns of distribution.

Eicker (1973) studied the microflora of Eucalyptus maculata leaf litter using three different isolation techniques. He observed a total of 45 species representing 22 genera from various litter horizons.

Watson et al (1974) studied the fungal succession on loblolly pine litter and foliage in North Mississippi. They observed dominance of deuteromycetes, phycomycetes, ascomycetes and basidiomycetes in F-layer while, only basidiomycetes in H layer.

Dadalaury (1975) pointed out that the distribution of fungi is not determined by a single environmental factor, but by a combined environmental factors like moisture, temperature and organic carbon.

Vittal (1976) in his studies on fungal colonization of litter grouped the fungi as dominant, common, frequent, occasional and rare depending on their percentage frequency.

McKenzie et al. (1976) pointed out the importance of the primary fungal colonizers during decay of leaves of poplar and plum and stem of wheat. They observed that the rust infected plant material supported quantitatively different fungal succession from that on non-infected ones.

Wani and Shinde (1977) studied the biological decomposition of wheat straw. They found that Aspergillus species were the most rapid wheat straw decomposes under laboratory conditions.

Mehrotra and Aneja (1979) studied the succession of fungi while investigating the microbial decomposition of Chenopodium album litter. They observed the dominance of deuteromycetes during the initial phase of decomposition while the final stage was dominated by ascomycetes and deuteromycetes.

Sandhu and Sidhu (1980) studied the fungal succession on decomposing bagasse for twenty weeks. They isolated nine fungal species in which Aspergillus fumigatus and A. torreus were common.

Das (1980) studied the fungal succession and microbial population of Pinus kesiya litter of different plantations. He observed Trichoderma viride and Penicillium chrysogenum as dominant species during decomposition.

Mishra and Dickinson (1981) studied the phylloplane and litter fungi of Ilex aquifolia. They obtained a marked seasonal variation in the mycoflora.

Kjoller et al. (1985) studied the bacterial dynamics during the decomposition of alder litter. They observed the number of aerobic, amylolytic and proteolytic bacteria was high after leaf fall and decreased towards the end of decomposition. They further suggested that the fluctuation in the organisms of anaerobic group was mainly influenced by moisture.

Struwe and Kjoller (1985) enumerated the number of aerobic and anaerobic bacteria present on the litter at monthly interval and observed higher bacterial population on decomposing litter.

Kuter (1986) isolated different fungal species from both green and senescent decomposing sugar maple leaves. A quantitative variation was observed in the fungal population which followed a consistent pattern.

Tiwari (1988) studied the fungal and bacterial population of pineapple litter of different plantations. He recorded low bacterial and fungal population in the beginning which increased as decomposition progressed and declined towards the final stage. Altogether, 19 fungal species from decomposing leaf and roof litters were recorded.

### Isopod Population

Edney (1954) and Cloudsley-Thompson (1956) <sup>have</sup> shows that all woodlouse species require saturated air or a moist substrate in their permanent habitats and differ only as regard tolerance to sub-optimal conditions during wandering.

Kevan (1962) reported that moisture content of the soil is of vital importance to the soil fauna and temperature is probably less important to soil animals than it is to surface dwelling species.

Drift (1963) reported the forces outside the soil community which influence population densities are the climatic factors.

Paris (1963) showed that there was heavy mortality among A. vulgare recruits in summer due to (dissiccation) and adults in winter due to drowning. He suggested that these causes of mortality could regulate the population through interactions with shelter sites.

Edney (1966) concluded that the behavioural adaptation of many arthropods are more important than any physiological adaptation. Even so, it is remarkable that a woodlouse Hemilopstus reaumuri should be able to exist in a desert.

Many soil invertebrates tend to aggregate (Hughes 1972) and therefore huge sampling programmes are necessary for the detection and surveillance of slow changes in population densities.

Sutton (1972) indicated that A. vulgare abundance crashed from  $5000 \text{ m}^{-2}$  to  $150 \text{ m}^{-2}$  between late 1968 and the following spring. This was due to the action of a pathogenic fungus and not to either of the density dependent processes involving weather.

McQueen (1976a) modelled the affects of temperature on the growth, reproduction and survival of Porcellio spinicornis say and from stimulation using field temperatures measured at Toronto, Canada concluded that the effects

of temperature alone were sufficient to maintain a stable population (McQueen and Carina, 1974).

Chotko (1977) stated that several species of Diplopods also play an important role in the leaf litter decomposition. He concluded that animals are more active during the summer months than in the autumn.

Davis and Sutton (1978), however, there is a surprising consistency in the biomass data for isopoda and diplopoda in deciduous woodlands and grassland suggesting that homeostatic mechanisms may be involved.

Santos et al. (1978) found that the spatial differences in population density and numbers of taxa of soil micro-arthropods were directly related to surface litter accumulates and soil organic matter across a desert watershed.

Franco et al. (1979) found a similar relationship between population density of most taxa and organic matter following the winter rainy season.

Al-Dabbagh and Block (1981) stated the lack of a clear role of weather in the population dynamics is in contrast to the belief commonly stated in studies of isopods that temperatures and relative humidity strongly affect their population density.

Santos ~~and~~ Whitford (1983) examined the spatial and seasonal variation in soil microarthropods population on the gypsum dunes at white sands National Monument in Southern New Mexico and reported a direct relationship between organic matter and density and taxonomic diversity of soil microarthropods.

Warburg et al. (1984) reported that the population dynamics is primarily controlled by climatic factors.

Ditlhogo et al. (1992) have shown that the abundance of many invertebrates is negatively affected by the presence of water.

### **Litter Decomposition**

Melin (1928) observed that the percentage of nitrogen in leaf litter was strongly affected by the rate of mass loss and by other chemical factor.

Mikola (1960) found that decomposition of fine needle litter in pine and spruce stands was proportional to the mean summer temperature.

Edwards and Heath (1963) reported that when soil animals are excluded from a decomposing litter, fragmentation is insufficient and this leads to reduce consumption by microorganisms.

Witkamp (1963) established a positive correlation between microbial population and the rate of litter breakdown. He also noted that temperature, moisture and quality of litter controlled the microbial population and rate of decomposition.

Heath et al. (1966) were of the opinion that polyphenols and similar constituent of litter were responsible for the different rates of decay of different tree species.

Heal et al. (1967) studied the litter decomposition in antarctic soil and related the slow rates to low temperatures and a restricted growing season.

Minderman (1968) noted that loss of various chemical components occurs at an exponential rate during decomposition of litter.

Hudson (1968) stressed the possible importance of relative humidity and substrate moisture content as main factors influencing rate of litter decomposition.

Harrison (1971) suggested that tannin affected the rate of mass loss in oak leaves.

Ivarson (1973) observed that the rate of decomposition increased with the increase in temperature.

Heal and French (1974) suggested that high decomposition rate in the first year after litter accumulation were due to leaching effects and not to biological activity

Howard and Howard (1974) and Jensen (1974) and much attention to devise the technique for studying the decomposition of litters in different climate zones.

Van Cleve (1974) concluded that P+Ca concentration, ligning + tanning and carbohydrates including cellulose were main components to determine the rate of litter decomposition.

Williams and Gray (1974) observed that during periods of droughts, litter decomposition rates are drastically retarded and the number of saprophagous animals in the litter reduced.

Edward (1975) was of the opinion that the decomposition is primarily a biological process and the rate at which it occurs is dependent upon temperature. Activity of decomposers in litter was initiated by the moisture.

Lamb (1976) studied the decomposition of organic matter on the forest floor of Pinus radiata plantations and correlated it to the weight loss and microbial respiration.

Lousier and Parkinson (1976) noted the return of inorganic nutrients to the soil via tree leaf litter fall. The total weight of these nutrients returned to the soil was 116 kg ha<sup>-1</sup>.

Martyniuk and Myskow (1976) studied the microbial decomposition of plant material in soil as influenced by some environmental factors.

Fogel and Cromack (1977) found the lignin level to be more-rate-determining than the C/N quotient or total nitrogen level.

Lawrey (1977) stated that degree of exposure of barren soil was another factor which may influence early stage of decomposition. He observed that an exposed soil surface affords low number of microorganisms than shaded ones.

Brinson (1977) and Edmonds (1979) have suggested that temperature and moisture were the most important abiotic factors controlling the rate of decomposition.

Blanchette and Shaw (1978) found that population of yeast and bacteria increased significantly as the decomposition of wood was brought about by basidiomycetes.

Maclean and Wein (1978) were of the opinion that the decomposition rate is controlled by factors like

temperature, moisture regimes, substrate quality, microflora and micro-fauna. They further stated that decomposition occurs at a faster rate in the younger forest stands than in the older ones.

Edmond (1979a) observed that sludge initially decomposed more rapidly in the clear cut areas than in the forest but the rate was similar after 400 days as determined by changes in specific gravity. He further stated that the temperature and moisture were the two important factors which regulate the decomposition rate (Edmond, 1979b).

Berg and Staaf (1980) observed that the decomposition of chemically complex organic part of the plant litter takes place in several steps. The soluble and some of the solid carbohydrates are degraded first and the remainder polymer carbohydrates and lignin decomposes later at a slower rate.

Howard and Howard (1980) studied the effect of species, site, soil type and climatic variation on leaf litter decomposition. They concluded that the site on which the tree grew and the soil on which the litter decomposed markedly influenced the weight loss, moisture content, respiration and pH. They further added that the moisture

content of litter affected the composition of microbial population.

Panwar and Sharma (1981) have attributed the slow rate of decomposition to high lignin, tannin and relatively poor nitrogen content of Scirpus tuberosus litter beside, the abundance of sclerenchymatous tissues and unfavourable climatic conditions.

Berg et al. (1982) observed that extractive substances from the needles of scot-pine were lost rapidly, while structural constituents, such as non-cellulose polysaccharides (hemicelluloses) celluloses and lignins decomposed slowly.

Nagy and Macauley (1982) studied the effect of relative humidity and substrate moisture on the rate of decomposition of Eucalyptus leaf litter and pointed out that biological decomposition is not significant until relative humidity and moisture content values are above 75% and 13% respectively.

Rai and Srivastava (1982) while studying the decomposition of leaf litter in a tropical dry mixed deciduous forest observed that the rate of decomposition was high during rainy months and low during winter.

Schinner (1982) investigated the altitudinal effect on litter decomposition. The rate of decomposition decreased with increase in altitude. ✓

Wood et al. (1982) reported that the role of microfauna in N mineralization was more likely through indirect excretion by the grazers, rather than through direct excretion by the grazers.

Anderson et al. (1983a) stated that soil fauna play a critical role in the biological turnover and nutrient release of plant residues by fragmenting the plant residues, resulting in enhanced microorganism activities and grazing of microflora by fauna.

Cuenca et al. (1983) worked out the rate of coffee litter decomposition under shade. Litter bags of 0.03 mm size showed slow decomposition rate than of .5 mm mesh size bags. Nitrogen and phosphorus exhibited an increase in concentrations as decomposition progressed while potassium, calcium and magnesium followed a decrease.

Santos and Whitford (1983) considered that the most important groups of microarthropods with highest densities probably make greatest contribution to litter decomposition and regulate decomposition rate.

Woods and Raison (1983) assessed the rate of decomposition of summer leaf fall (abscised leaves), winter leaf fall containing some green leaves and mature green (picked) leaves in sub-Alpine forests. They observed a positive linear relationship between initial concentration of nitrogen and phosphorus and rate of weight loss.

Schwintzer (1984) studied the decomposition and nitrogen dynamics of Myrica gale litter. He observed that only 40% of the initial biomass and 10% of initial nitrogen were lost during first five years of decomposition, which was correlated to the high initial lignin content of the litter.

Yi et al. (1984) found pronounced differences in bacterial and fungal numbers between the barren soil and the forested areas as well as among different forest types.

Jansson and Berg (1985) studied the decomposition of Scots pine litter in central Sweden. They analysed the climatic influence on litter decomposition.

Christensen (1986) observed no difference in weight loss pattern of straw buried at 5, 10 and 15 cm depths. Straw exposed to soil showed an initial rapid weight loss but thereafter, the rate of loss became almost constant.

Straw on the soil surface on the other hand, followed somewhat a <sup>very</sup> irregular pattern of weight loss.

Moore (1986) investigated the effects of temperature, and moisture on decomposition of hardwood and coniferous leaf litter. He observed that the soil horizon, litter type, temperature and moisture significantly affected the rates of decomposition.

Parmelee and Alston (1986) were of the opinion that soil animals are significant regulators of decomposition and nutrient release through their interaction with the soil microflora.

Berendse et al. (1987) stated that plant residue decomposition and nutrient release are known to be affected by their chemical composition and the nature of soil organisms.

O'Connell (1987) studied the Eucalyptus litter decomposition at three contrasting forest sites of southwestern Australia. He observed similar rate of weight loss and nutrient mobility at all the sites. He further, suggested that the changes in the amount of less mobile elements in litter differed significantly between the sites.

Saini (1987) noticed high decomposition rate of Sesbania aculeata litter in beginning and slow in later part under the field condition. The weight loss after 92 days was 71%. 96% and 85% for stem, leaves and roots respectively.

Laishram and Yadava (1988) observed an inverse linear relationship between the percentage of the original mass remaining and the nitrogen content. Significant positive correlation was also established between initial nitrogen and lignin content and remaining biomass.

Taylor and Parkinson (1988) explored the relative influence of substrate type, temperature and moisture on decomposition rate of Aspen and Pine litter in laboratory microcosm. They observed that temperature exerted more influence than watering rate for both the species.

Upadhyay et al. (1989) studied the decomposition of ten central Himalayan forest litter in terms of temporal changes in nutrient concentration, weight loss and its spatial pattern. They observed a rapid loss of labile nutrients and concluded that the placement of the bags and the rainy season have maximum influence on litter breakdown.

Reddy (1989) noticed the greater mass loss during

the initial months, particularly during the post monsoon period, was most probably due to the leaching of water soluble substances from the litter followed by higher microbial and faunal activity.

Kalburtzi et al. (1990) studied the decomposition of Fababean and wheat straw and release of N, P, K, Mg and Ca under field conditions. They observed an increase in the concentration of N and P in the beginning of decomposing material which were decreased at the end of the experiment. The weight loss was neither affected by the straw species nor the container type.

Tian et al. (1992) observed that the presence of soil fauna effects on decomposition and nutrient release implies that decomposition and nutrient release of plant residues can be improved by enhancing soil faunal activities.

#### **Microbial Enzyme Activity During Litter Decomposition**

Ross (1966) reported that the composition of pastures influences the activity of carbohydrates enzymes in soil which suggested that changes might occur due to changes in vegetation succession.

Selby (1968) studied the mechanism of biodegradation of cellulose and showed that the insoluble cellulose

substrate is first degraded to an intermediate soluble form and then to sugar.

Benoit and Starkey (1968) reported that tannins inhibited cellulolysis by blocking cellulolytic enzymes.

Ghose (1969) studied the heterogeneity of cellulose and concluded that it was a multi enzyme system which involves the concerted action of different enzymes.

Khan (1970) found that invertase activity in plots with a 5 year rotation of grains and legumes was almost double that of a wheat fallow sequence.

Stutzenberger et al. (1970) observed a significant relationship between extractable cellulolytic activity and cellulose decomposition in municipal soil waste compost.

Lignin monomer analogs have been shown to inhibit cellulase and xylanase synthesis by cellulolytic fungi (Varadi, 1971).

Pancholy and Rice (1973) determined amylase, cellulase, invertase, dehydrogenase and urease activities every other months for a year in soils of 2 old fields, successional stages and a climax stand in each of 3 vegetation types, the tall grass prairie, the post oak black jack

oak forests and the oak pine forest. The activities of amylase, cellulase and invertase were higher generally in the first successional stage, intermediate in the second stage and lowest in the climax stand. On the other hand, dehydrogenase and urease activity were generally lowest in the first successional stage, intermediate in the second stage and highest in the climax. This trend was observed in all 3 vegetation types throughout the year.

Berg (1975) examined the cellulase releasing systems in fungi and bacteria and observed a similar functional cellulase system in both the organisms.

Forbes and Dickinson (1977) determined the cellulolytic activity of three Fusarium isolates under a range of physical and nutrient conditions. They concluded that nitrogen source and concentration were major influencing factors for cellulolytic activities. They observed maximum cellulase activity at an initial or neutral pH.

Spalding (1977) observed a significant correlation between CO<sub>2</sub> evolution and amylase; cellulase and xylanase (except invertase) activities in decomposing coniferous leaf litter.

Ross and Speir (1979) investigated cellulase and hemicellulase activities of top soils and tussock plant

materials. Neither cellulase nor hemicellulase activity was detected in the green leaves. They observed much higher enzyme activity in standing dead sheaths and blades than top soil.

Singh and Kunene (1980) studied cellulose decomposition by four isolates of Pyricularia oryzae. They observed a suppression in the cellulolytic activity by glucose.

Duxburry and Tate (1981) measured invertase, cellulase, amylase, xylanase, acid phosphatase, peroxidase and polyphenol oxidase in a Pahokee muck soil. They observed a significant variation in these enzymes due to variation in soil depth and crop cover.

Ross (1981) determined invertase and amylase activities in Kauri plant material and in a moroid, strongly acid litter cone profile of Waipoua clay. He detected highest invertase activity in fresh leaves and twigs and lowest in mineral soil horizons, while amylase activity was highest in litter horizons.

Speir and Ross (1981) studied the enzymes activities of tussock litter exposed around the base of tussock grass. They observed a significant correlation between urease, phosphatase, sulphatase, invertase, amylase, cellulose and hemicellulose activities and litter moisture

contents, total nitrogen and C/N ratio. Except phosphatase all the enzymes were present in higher amount in exposed litter than unexposed ones.

D'Souza and Volfova (1982) observed that pH played an important role in the biosynthesis of cellulase.

Singh (1982) while investigating the effect of carbon and nitrogen sources on the production of cellulolytic enzymes in Acrocylindrium oryzae observed that these nutrients had a considerable influence on growth, sporulation and production of cellulolytic enzymes.

Aneja (1983) studied the litter colonizing ascomycetes for their cellulolytic, pectolytic and lignolytic abilities and observed maximum cellulase activity in Chaetomium erectum while Thielavia minor did not show any activity.

Sinsabaugh and Linkins (1987) measured the inhibitory potential of several leaf litter extracts towards Trichoderma viride cellulase components and found differences among both enzymes and litter types. However, the pattern of inhibition did not appear to be consistent with interspecific differences in cellulose dynamics over decomposition.

Sinsabaugh and Linkins (1988) in their investigation using absorption isotherms showed that litter with low initial lignocellulose indices (LCI, the ratio of lignin of lignocellulose) such as dogwood had the most affinity for exocellulase, which is known to bind to crystalline cellulose. In contrast litters with high LCI values, such as oak had the greatest affinity for endocellulase suggesting that it was immobilized primarily by non-cellulose litter component. Although enzyme immobilization on naturally decomposing litters is more complex, the absorption data were consistent with earlier observations that (i) ratio of endo to exocellulase activities tends to increase with decomposition, (ii) deciduous litters with higher initial LCI have greater endo to exo cellulase ratios.

Humification tends to reduce enzyme extract ability and increase activation energies (McClaugherty and Linkins, 1988, 1990) leaching to lower activities at low temperatures.

Linkin et al. (1990) found that an index of cellulose interaction the product of exocellulase and endocellulase activities was correlated with the rate of disappearance of cellulose from decomposing leaf litter both within and between species. This relationship suggested

that interspecific differences in cellulase activity might be functionally related to differences in decomposition rates.

Kshattriya et al. (1992) observed that the production of microbial enzymes is influenced by the chemical nature of the litter, the environmental conditions and the microbial populations which utilise different labile and recalcitrant compounds for their metabolism.

#### **Influence of Isopods on Microbes Population in Litter**

Dudich, Balogh and Loksa (1952) calculated that all the annual litter fall in woodlands is eaten eventually by the soil fauna. More than 60% and usually about 90% of this food litter is returned to the soil as faeces (Drift 1951; Gere 1956; Bocock 1963).

Plant material ingestion has been observed by Dunger (1956) who found that an adult Folsomia fimetaria consumed  $0.049 \text{ mm}^3$  of decomposing leaf material per day at  $18^\circ\text{C}$ . The leaves were not sterilized and the collembola may have mainly assimilated microorganisms.

Drift and Witkamp (1959) stated that the activities of microorganisms can be stimulated by the action of the soil fauna and therefore the exclusion of certain invertebrates from samples of litter may itself produce changed

conditions in the litter. They further found out that the faecal pellets of a soil arthropods were a more favourable habitat for litter microorganisms than whole leaf litter.

Drift and Witkamp (1960) and Nicholson et al. (1966) suggested from a comparison of the decomposition rate of arthropod faeces, mechanically ground litter and intact leaves, that the main contribution of soil animals to decomposition processes was a short term enhancement of microbial activity by litter communication. A criticism of such studies is that they exclude the dynamic effects of coprophagy and direct grazing on microbial populations.

Macfadyen (1963) showed, however, that the animals may enhance energy flow through bacterial and fungal populations by the indirect effects of their feeding activities. He also concluded that the overall rate of decomposition of plant debris is increased when the material is converted to faeces. Contrary to this condition there was no indication of a higher overall rate of decomposition in the faeces than in litter (Bocock 1964; Hering, 1965).

Nicholson et al. (1966) showed no significant differences in weight losses from millipede faeces and intact leaves enclosed in fine mesh litter bags for a year in the field.

Macfadyen (1968) have argued that the indirect effects of small arthropods in litter and soil may be very important.

Bodvarsson (1970) found that soil <sup>or in the laboratory</sup> (inhibiting) collembolan species had a lower percentage of hyphae in their guts than surface living species.

Coleman and McGinnis (1970) studied the effects of grazing generally by measuring rates of ingestion by different animals taxa in the field.

Gray and Williams (1971) stated that the microbial growth of plant material and faeces has two characteristic growth phases, an initial burst of activity as the resource is colonized during the first few weeks followed by a lower level of activity as the readily utilizable materials exhausted, toxic metabolites accumulates and the culture begins to senesce.

Anderson and Healey (1972) reported that some collembola species show significant variation in the proportion of fungal material found in their guts, but they were not able to relate this to availability of fungal hyphae in the field.

Peschken and Beecher (1973) and Harris (1973)

have pointed that the effect of relatively inconspicuous herbivory by invertebrates may have profound effects on the distribution and abundance of plant debris.

Reyes and Tiedje (1976) demonstrated that the gut of isopods is a favourable environment for bacterial growth and although some digestion of bacterial cells occurred. The net effect was an increase in counts on faeces with respect to the food.

Addison (1977) in his studies on two collembolans species, Folsomia regularis and Hypogastrura tullbergi showed that although both species consumed both fungal hyphae and organic material (plant residue), F. regularis was by preference as organic matter feeder and H. tullbergi preferred fungi. Nevertheless the survival of F. regularis was greatest when fed with leaves or humus of *Dryas*. For H. tullbergi feeding on a number of dematiaceous fungi (e.g., P. herbarium, Cladosporium cladosporoides; sterile dark forms) allowed at least three times the survival<sup>e.</sup> achieved on *Dryas* leaves and humus. However, the growth rate of H. tullbergi (at 10°C in the laboratory) was approximately three times greater on P. herbarum than on a sterile dark fungus. When fed on *Dryas* leaves collembolans lost weight over the study period.

Visser and Whittaker (1977) showed that Onychiurus subtenuis selectively fed on sterile dark mycelium in the laboratory and that animals collected in the field at the snow edge had sterile dark hyphae in their guts.

Hanlon and Anderson (1979) demonstrated that grazing by small numbers of microarthropods (collembola) stimulated microbial respiration but more intense grazing pressures decreased C mineralization rates and exerted a strong differential effect on microorganisms, the standing crop of bacteria increased while the growth of fungi was inhibited.

Booth and Anderson (1979) pointed out, quoting Levi and Cowling (1969) and Dowding (1976) that the nutritional quality of an individual species of fungus will vary, depending on the nutrient content of the substrate on which it is growing.

Hanlon and Anderson (1979) showed that a litter decaying Basidiomycetes, Coriolus vessicolor (L ex Fr) was extremely sensitive to grazing by Collembola but it is evident that the fast growing microfungi, such as Penicillium, Mucor and Trichoderma are also susceptible. Bacteria were stimulated by macroarthropod feeding activities and increased upto 10 times control levels.

Hanlon and Anderson (1980) reported marked decrease in fungal biomass and greater increase in the bacterial standing crop of decomposing oak litter inoculated with macroarthropods. This has been attributed to grazing from microsites influencing microbial activity (Anderson et al. 1983) which in turn could significantly alter nutrient dynamics.

Hanlon (1981) showed that abundance of grazing collembola in combination with low nutrient quality of the substrate had a dramatic effect in reducing the fungal growth and metabolism.

Santos and Whitford (1981) reported that the soil microflora uncoupled decomposition from abiotic constraint, thereby resulting in a higher rate of decomposition.

Studies by Silva et al. (1985), Mackay et al. (1986) and Whitford et al. (1986) have shown that although surface litter accumulation are colonized by a diverse group of microarthropods, the soil fauna have no apparent effect on rates of decomposition.

Hassal et al. (1986a) showed that many more animals migrated into litter colonized by Cladosporium (a fungus common in L-layer litter) than into litter colonized by Basidiomycetes 290 (another L-layer inhibitor, but known to be toxic to the animals).

Visser et al. (1987) have shown that a wide variety of fungi are carried by Collembolans as soon as they move up and down the organic layer. This high potential for dispersal of fungal propagules into new resources could have effects on organic matter decomposition.

Moore et al. (1988) showed that Folsomia candida preferred some species of fungi as food over others and the actively metabolizing fungi are favoured as to the less active hyphae.

Singh et al. (1989) and Raghubanshi et al. (1990) stated that the reduced microbial biomass and increased microbial turnover in the wet period may result from feeding by expanded microbivore populations.

Ponge (1991) establishes a direct influence of some soil animals upon plant material through digestion processes.

#### **Effect of Isopods and Microbes on the Nutrient Release from Litter**

Tukey (1971) demonstrated that living tissue damaged by herbivory leached more organic and inorganic compounds than undamaged tissues.

Jensen (1974) has indicated that litter mass loss

was generally 25-80% greater per annum in presence of fauna than its absence.

Cornaby et al. (1975) reviewed these elemental studies and reported that of the total annual nutrient input to the forest floors, 2-11% of the calcium and 3-28% of the potassium is ingested by the soil-litter fauna.

Cromack et al. (1975) stated that fungal mycelium in soil contain a major proportion of nitrogen, potassium and other cations in the soil pool and thus the sensitivity of fungal hyphae to microarthropod grazing may well have considerable significance for nutrient mobilization (Hanlon and Anderson 1979, Parkinson et al., 1979).

Gist and Crossley (1975) and Cornaby et al. (1975) developed elemental budgets and models for soil communi-  
cates on white pine and hardwood forest watersheds, although  
the role of invertebrates was not experimentally tested.

Ausmus et al. (1976) suggested that a major proportion of potentially limiting nutrients (N.P.K.) in the soil may be maintained within the fungal population.

Witkam (1976) concluded that the net result of a series of cycles of growth and decline of decomposer biomass was a release of nutrients in proportion to the loss of weight by the material.

Fenchel and Harrison (1976) suggested that increased microbial activity could be that the animals through their movements increase the transfer of nutrients and oxygen to the bacterial cells. The grazing by animals could also result in a more active microbial population as a result of decreased microbial biomass and thus less competition for available energy, nutrients and space.

Crossley (1976) has summarized the role of arthropods in forest soils as being the regulator of decomposition of organic matter and the direct or indirect regulation of nutrient release from decomposing organic matter. He pointed out that although interesting suggestions have been made on the effect of arthropod grazing on rates of organic matter decomposition and nutrient cycling, as yet less data is available to allow clear definition of these affects.

Sneh and Lockwood (1976) suggested that the nutrient shortage in the soil is sufficient to cause fungistasis but the inhibition of microbial activity by microbial exudates has also been demonstrated on several occasions. The importance of isopods, however, in relation to the influence of grazing on microbial activity during the different stages of litter decomposition, have yet to be established.

Coleman et al. (1977) demonstrated that direct consumption by soil animals of bacterial population stimulates total CO<sub>2</sub> evolution and mineralization of nitrogen and phosphorus in microcosm.

Anderson et al. (1981) have suggested that the low C : N ratio in the microbial biomass causes microbial feeding animals to excrete most of the ingested nitrogen. Excreted nitrogen could then stimulate microbial growth and hence substrate utilization.

Edward et al. (1981) stated that despite the small contribution that saprophytic microarthropods make towards total carbon mineralization in forest and woodland soils they have an important role in affecting the quantity, community competition and activity of the soil microflora.

Coleman et al. (1983) showed that the nutrient immobilization and mineralization is mainly caused by the soil microflora, however, the microbial nutrient turnover is considered to be driven by the activity of the soil fauna.

Parker et al. (1984) found that decomposition rates, C mineralization and biomass C were reduced significantly when microarthropods were eliminated and that these processes were reduced even further when fungi were

eliminated together with soil microarthropods.

Ingham et al. (1985) suggested that in semiarid habitats, the effects of microbivores grazing on the microflora and mineralizing nitrogen may be particularly important when nutrient demand by the plant exceeds the rate of mineralization by the microflora alone.

Mitchel and Nakas(1986) and Rusek (1986) stated that in the forests and grasslands, the importance of faunal activities is well recognized, they influence decomposition systems, rates of nutrient regeneration, soil structure, and so forth.

Coleman et al. (1988) concluded that the system and the feeding mode of the various faunal groups affect nutrient transformations and rates of turnover of organic matter.

Schaefer (1990) pointed out that the phosphorus limitation of microbial growth combined with high activity of soil invertebrates may be a key component ensuring the high productivity of the beechwood studied.

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**STUDY SITES AND CLIMATE**

Two forest stands dominated by alder (Alnus nepalensis) were selected for the present study at Upper Shillong 5.5 Km away from Shillong, the capital of Meghalaya. The study area lies between 25°34' N latitude, 91°56' E longitude and at an altitude of 1500 m MSL.

The topography of the two sites was similar and both of them were adjacent to each other. The sites are closely comparable, the functional differences are attributed to the age of the plantation. These two stands are covered with alder plantations on the same face of the hill slope. Each of the two sites chosen was subdivided into closed and open forest stands. The closed forest comprised of 35 years old alder plantation being the original ~~undisturbed~~ stand and the other one the young open forest stand of 20 years old exposed to disturbances such as cutting twigs and collection of wood for fuel by local inhabitants. Tree densities per hectare were 1160 and 380 in closed and open stands, respectively.

Greater Shillong falls under the Gondwana (Mesozoic) period. The plateau is constituted by the rocks of the precambrian age consisting of rocks of hard crystalline granite, gneiss and granulites (Singh, 1989). The



PLATE I - CLOSED FOREST STAND OF ALDER (ALNUS NEPALENSIS)



PLATE II - OPEN FOREST STAND OF ALDER (ALNUS NEPALENSIS)

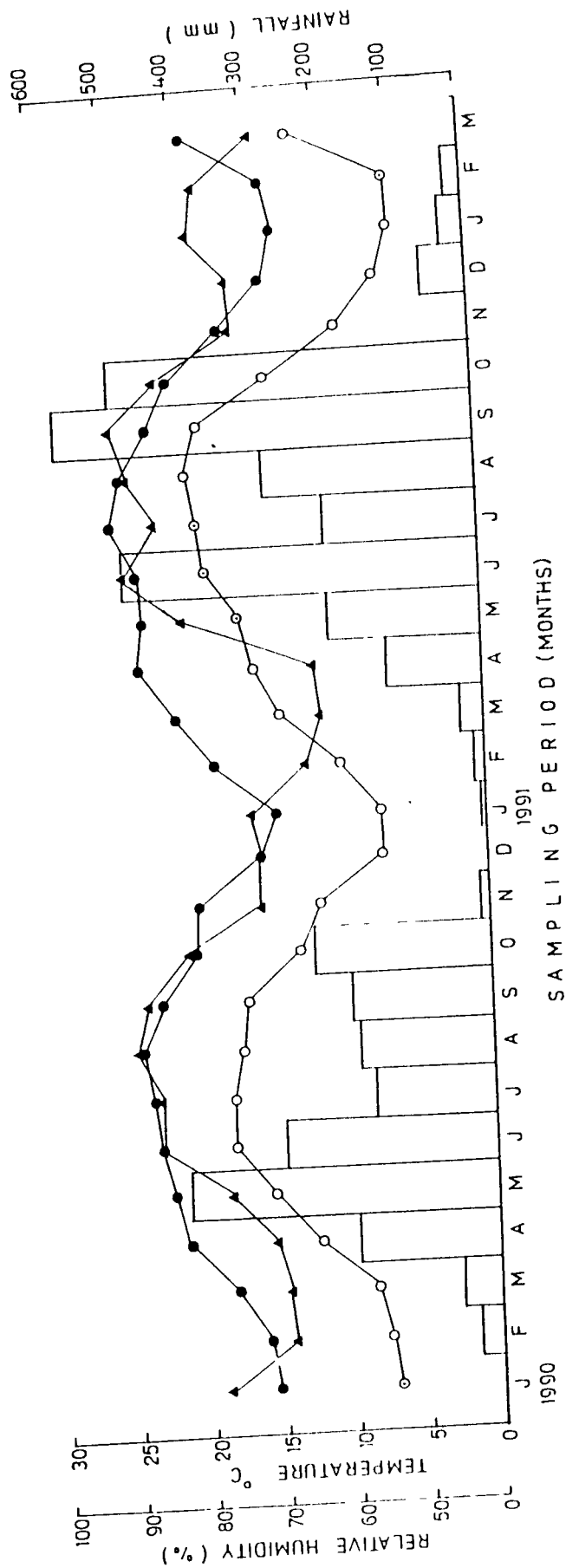


Fig 1. Monthly variation in rainfall (  $\square$  ), Maximum (  $\bullet$  ), minimum (  $\circ$  ) temperature and relative humidity (  $\blacktriangle$  ) of the study area.

general soil type of these sites is red loamy with fine silt and gravel constituting the major fractions (Sand 54.1%, Silt 25.9% and Clay 20%) and acidic in reaction.

The climate of Shillong is of sub-tropical monsoonic type, largely controlled and influenced by the seasonal winds, like the south west monsoonic wind and the north east winter ones. On the basis of general meteorological conditions, the year is divisible into four seasons. The spring season starts from the month of March to April, summer (rainy) from May to September, autumn from October to November and winter from December to February.

During March and April, the atmosphere gradually warms up and relatively it is dry. From the middle of May to the end of July, the temperature reaches the maximum. The maximum temperature recorded during 1990 and 1991 was 24.3°C and 24.6°C respectively. The average minimum temperature was 15.5°C in 1990-1991. The rains start in the middle of April becomes intense in June and August-September and sometimes even upto the middle of October after which it gradually decreases. The annual rainfall during 1990 and 1991 ranged from 2.1 mm to 421.2 mm and 0.1 mm to 574.0 mm respectively. Similarly, the average humidity ranged from 59% to 89% in 1990 and 64.5% to 90%

in 1991. During winter months the climate is very cold and dry and the range of minimum temperatures was from 7.1 to 7.7°C in 1990 and from 4.8 to 8.7°C in 1991 (Fig.1). The low temperature of the winter results into frost during December to January months.

The predominant understorey vegetation of both the selected forest stands was fairly similar. Several species like Rubus ellipticus, Litsca cubeba, Osbeckia crinata, O. nepalensis, Cassia mimosoides, Arundinella khasiana, Houttyunia cordata, Hedychium aurantiacum, Trichoranthus sp. and Eupatorium adenophorum were common to both the sites.

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CHAPTER I

SEASONAL STUDY ON AIR SPORA OF THE ALDER  
(ALNUS NEPALENSIS) FOREST STANDS

## **Introduction**

The pattern of incidence of air spora differs considerably from place to place and season to season. The knowledge of seasonal variation of the atmospheric spores is of prime importance for forecasting and controlling the diseases of that particular locality. The air spora includes both saprophytic and parasitic fungi. The pathogenic forms of microorganisms among the settlers of air spora are responsible for disease incidence on the leaf surface. A wide variety of organisms constitute the air spora of any region (Sreeramulu, 1967) and these biological components follow a definite trend, starting from the source of organisms, to their release, dispersion and finally their impact on the plant or animal systems, including human beings. The concentration of fungal spores in the atmosphere is mostly determined by the local conditions like rainfall, humidity, temperature etc. Some of these fungal spores are seriously pathogenic causing various diseases in crop plants as well as allergic diseases in human beings. Thus an understanding of the nature, periodicity and density of the fungal propagules in the air is much helpful in making a forecast regarding the occurrence of fungal diseases and the quantum of variable pathogenic propagules likely to cause infection.

The fungal content of the air spora has been examined in several parts of the world, particularly in relation to the occurrence of human allergens and the distribution of spores of plant pathogens in disease epidemiological studies. From these studies in Europe and North America, a pattern has gradually emerged of the fluctuating nature of the fungal content of the atmosphere which includes sudden occurrence of certain spores in vast numbers and of the apparent numerical dominance by comparatively few taxa.

There is little information on the type of fungi in the air spora of hilly regions of north eastern states of India. While most of the existing aerobiological studies in India reveal the qualitative features (Kalra and Dumbrey, 1957; Lakhanpal and Nair, 1958; Karnik, 1962) quantitative studies with reference to seasons are scanty (Sreeramulu, 1967). Further these studies have been conducted in plain regions of India, where more or less similar results have been obtained.

Two major methods have been used in these surveys, exposure of sterile media in plates and identification of colonies developing after incubation. The exposed plate method was used to trap air borne fungal spores of selected sites and a culture method to reveal the variation

in abundance but the method also provide information in the relative abundance of numerous genera, particularly unspecialized saprophytes, and their numerical fluctuations (Gregory, 1961). The exposed plate method has been used extensively in air spora investigations around the world, particularly in Europe and North America. There have been surveys of the air spora at some Australian cities (Clarke & Mason, 1964; Derrick and McLennon, 1963; Frey and Durie, 1962; Ress, 1964) and at several tropical locations of the world (e.g., Alvarez and Castro, 1955; Cammack, 1955; Dransfield, 1966; Myers, 1956; Rajan, Nigam & Shukla, 1952; Taylor & McFadden, 1962; Turner, 1966).

The present work was undertaken to survey the aeromycoflora of the two forest sites. Simultaneously the phylloplane studies were also done and the impact of air borne fungi on phylloplane mycoflora was assessed.

## **MATERIALS AND METHODS**

### **Isolation of air mycoflora**

Two sites i.e., open and closed alder (Alnus nepalensis) forest stands at Upper Shillong, East Khasi Hills at an altitude of 1500 m MSL, latitude 25°34'N and longitude 91°56'E were selected for the study of air mycoflora.

The sampling of the air fungi was done fortnightly

simultaneously along with phylloplane mycoflora. The nutrient plates containing Czapek's Dox agar medium were exposed horizontally at 5 places of each forest stand for 5 minutes at a height of 1 metre and brought to the laboratory on the same day in a sterilized polythene container to minimise the contamination. The plates were incubated for 6 days at  $25 \pm 1^\circ$  in BOD incubator. The number of fungal propagules was counted. The slow growing fungal colonies that appeared in the plates were examined for 20 days. Identification of fungi was done with the help of standard literatures (Barnett, 1955; Subramanian, 1971; Gregory, 1973).

The percentage frequency of occurrence was calculated for each fungus based on the following formula.

$$\text{Frequency of fungi} = \frac{\text{Total number of individual species of fungus}}{\text{Total number of all the species of fungi}} \times 100$$

The results have been expressed as an average of 5 replicates for the period of 2 years (1990-1992).

### Statistical Analysis

The population of air mycoflora was correlated with climatic conditions and fungal population of phylloplane and litter of Alnus nepalensis in both the forest stands by using Pearson's product moment correlation coefficient (r) (Zar, 1974).

## RESULTS

The air fungi isolated from the atmosphere of the two alder forest stands are listed with their frequency of occurrence (Tables 1-4). A total number of 30 fungal species belonging to 21 genera was recorded during the present investigation. The air spora of both the sites belonged to 3 main groups of fungi viz., Mucorales, ascomycetes and hyphomycetes out of which maximum number of species belonged to Hyphomycetes. ✓

Monthly variation of air fungal population of both the forest sites is given (Fig. 2). It is clear from the Tables (1-4) that some genera were relatively more abundant at open forest site than the other ones. Phycomycetes, ascomycetes and sterile mycelial forms were poorly represented in the air of both the sites. In general, deuteromycetes contributed to about 80% of the total fungal population in the air of open forest stand. Cladosporium herbarum, Aspergillus spp., Alternaria spp. and Trichoderma spp., were dominant species at open forest site. C. herbarum was present throughout the year but showed its higher population during the winter months when temperature was low (Fig. 3a). Low population of air mycoflora was observed during the rainy season. Species of Penicillium and Aspergillus were common throughout the sampling period. Fusarium

was most common during a period of heavy rain in September and October (Fig. 3a and b). Curvularia showed a rapid increase in the beginning of rainy season. Helminthosporium oryzae showed a pronounced peak between August and September. The incidence of Nigrospora was observed in relative dry hot months with a peak before the onset of heavy rain. The population of T. viride also varied remarkably at the different sampling period, however, a peak was observed in the months of July and August (Figs. 3a and b). In addition, Alternaria and Penicillium were present almost throughout the year and their frequency pattern varied remarkably at different sampling periods (Figs. 3a. and b).

Population of Cladosporium sp. and Penicillium sp showed a distinct variation in their abundance corresponding to those at the open forest site. Fusarium, Geotrichum, Penicillium and Trichoderma were favoured by the closed forest site. Penicillium has been recognised as the major component of the air spora in this site (Figs. 3c and d). Fusarium appeared irregularly throughout the year of investigation and was more abundant in the closed site at latter part of wet season (Figs. 3c and d). Geotrichum was most abundant in the middle of the wet season while Cladosporium was dominant all through the dry season. Species of Trichoderma were most abundant during the summer

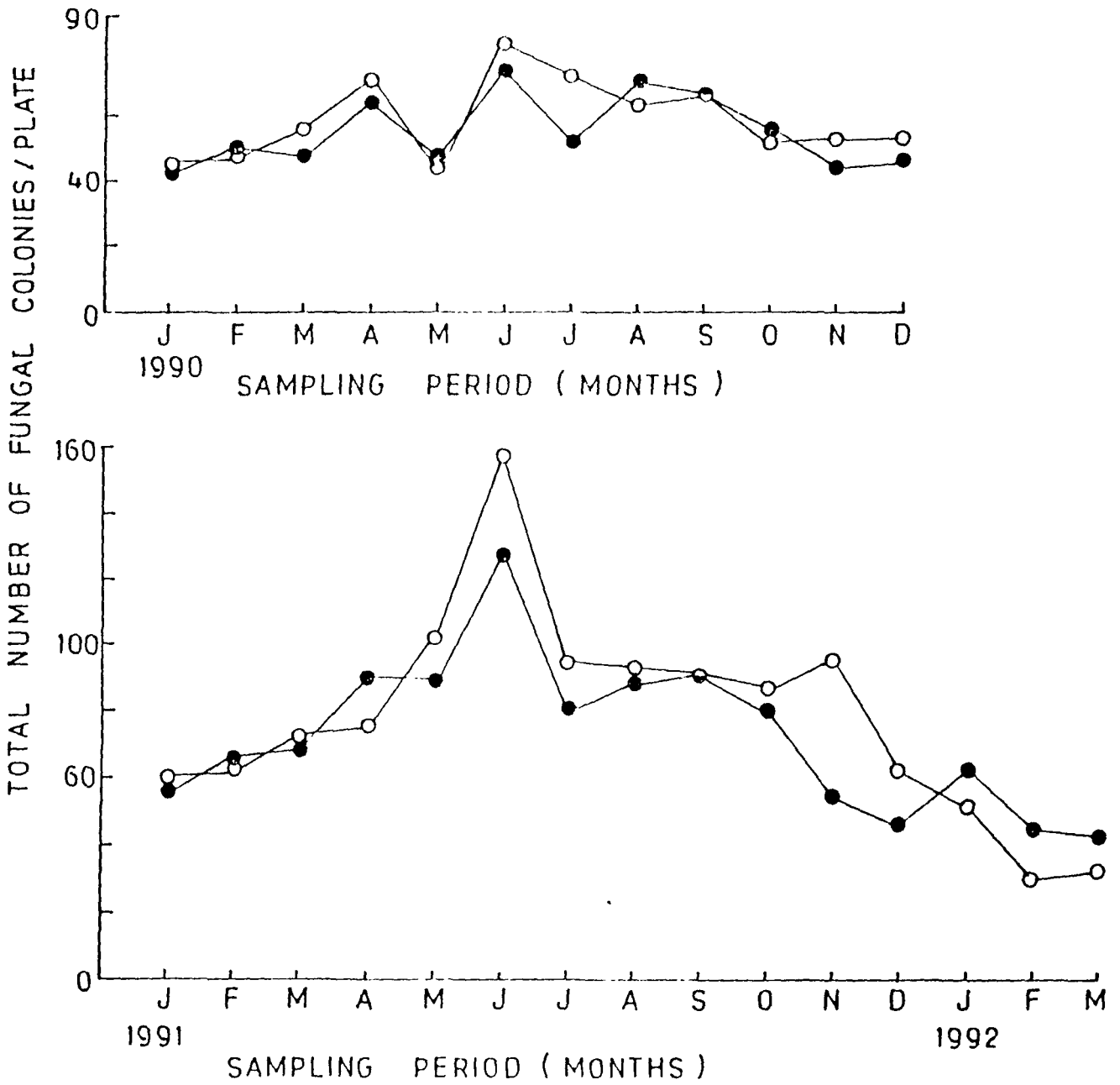


Fig 2. Average monthly variation of fungal spores at two forest stands of *Alnus nepalensis* (●—● closed, ○—○ -open).

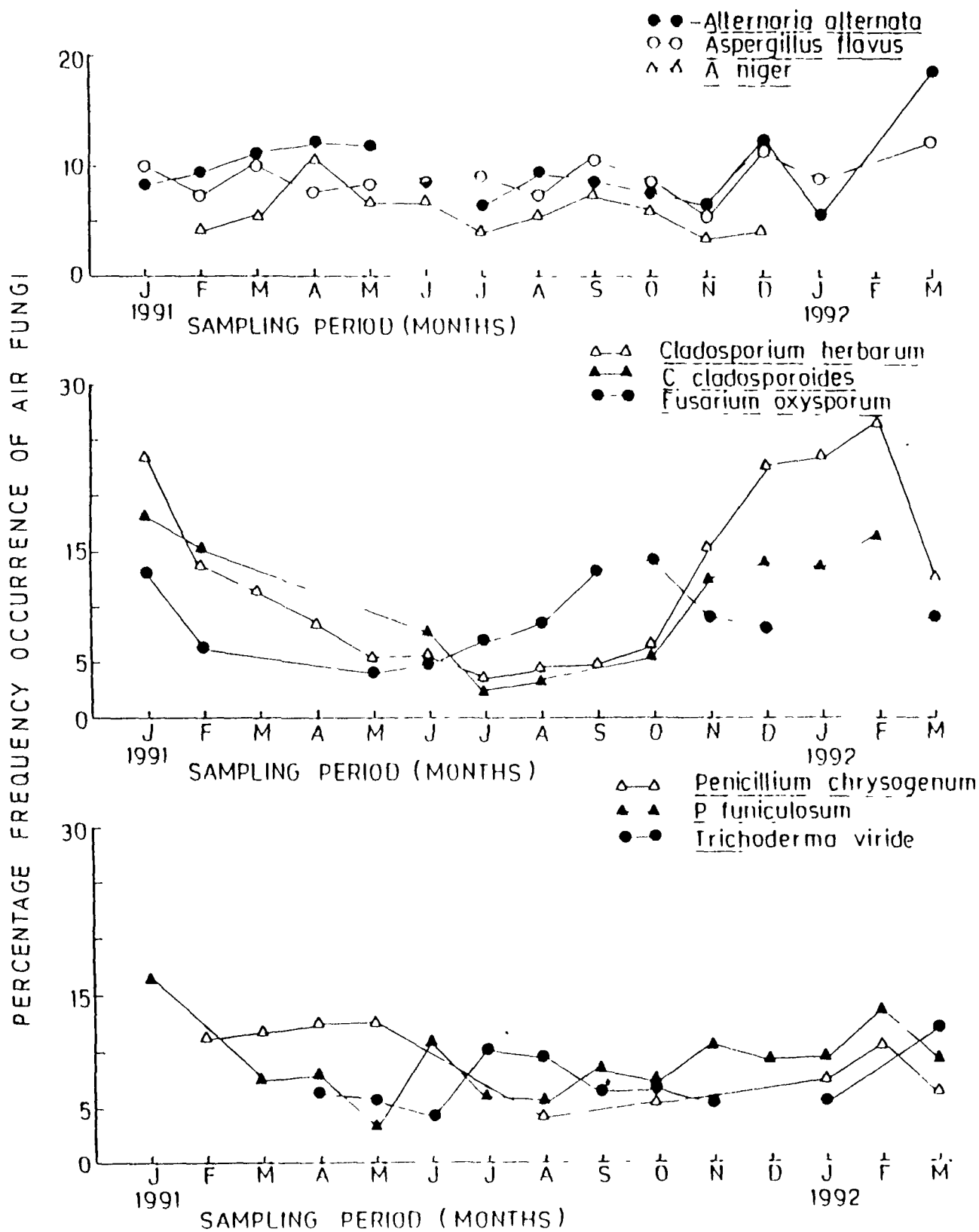


Fig 3.(a) Average monthly variation in frequency of fungal spores in the air of open forest stand of Alnus nepalesis.

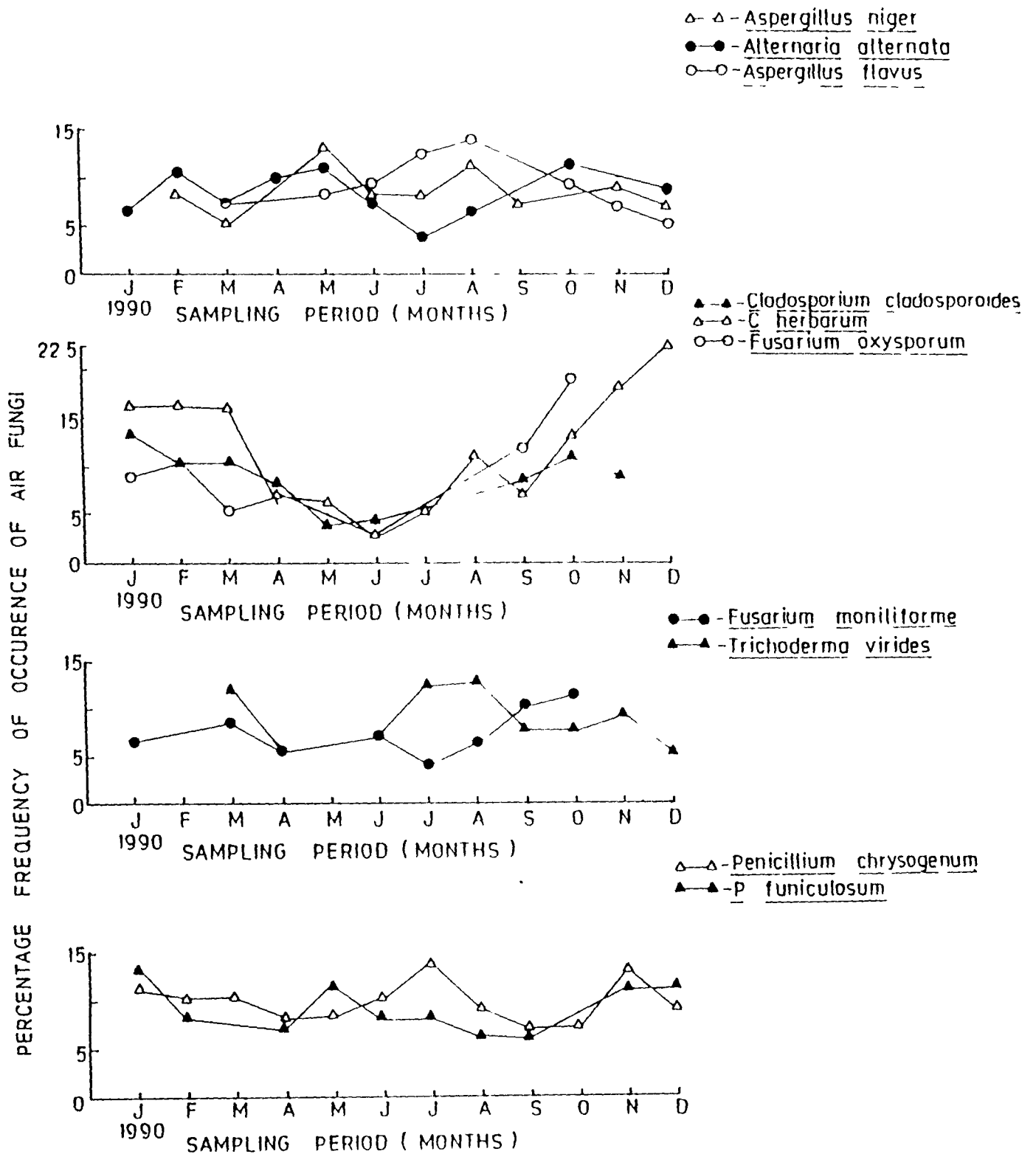


Fig 3.(b) Average monthly variation in frequency of fungal spores in the air of open forest stand of Alnus nepalensis.

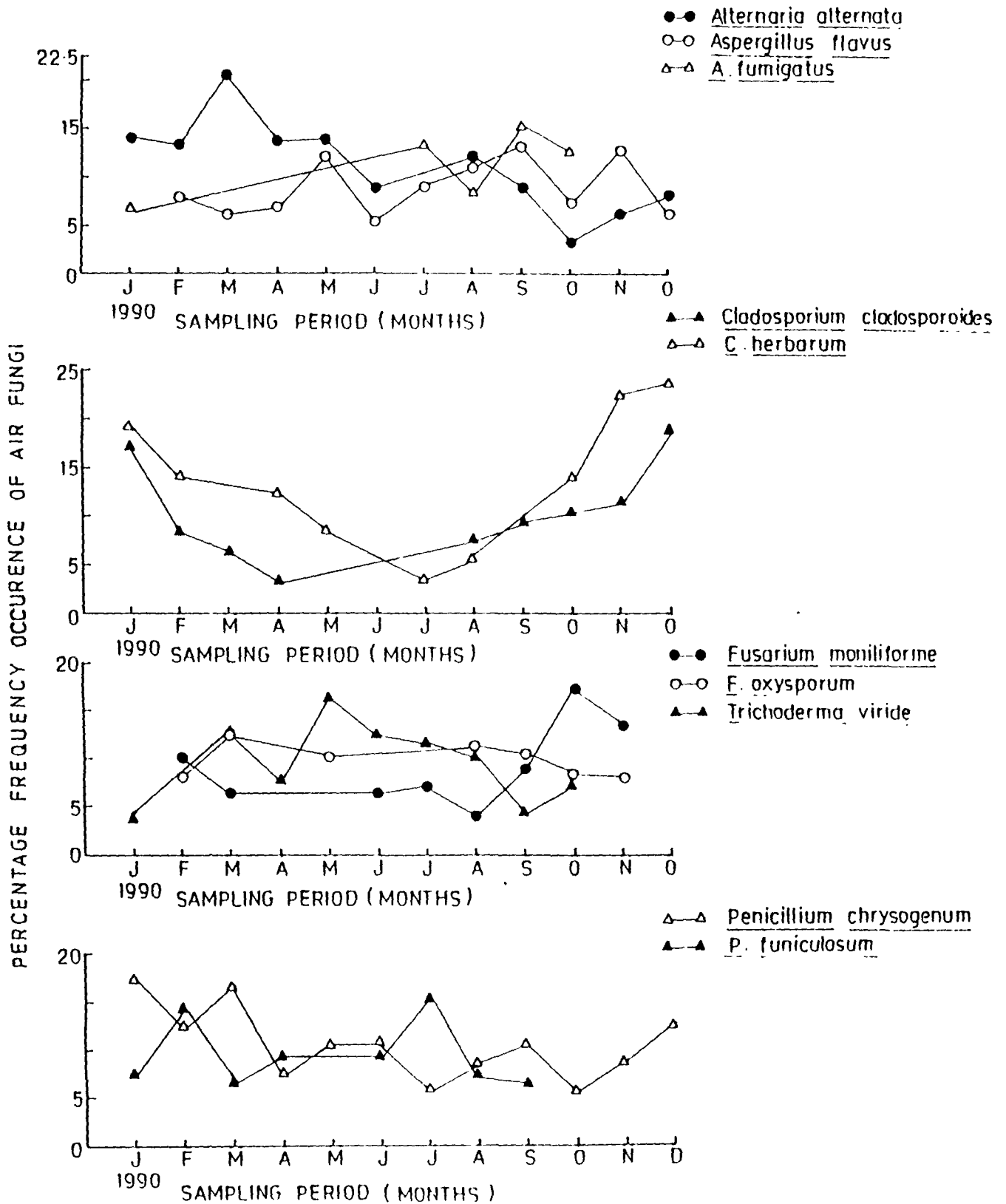


Fig 3. (c) Average monthly variation in frequency of fungal spores in the air of closed forest stand of *Alnus nepalensis*.

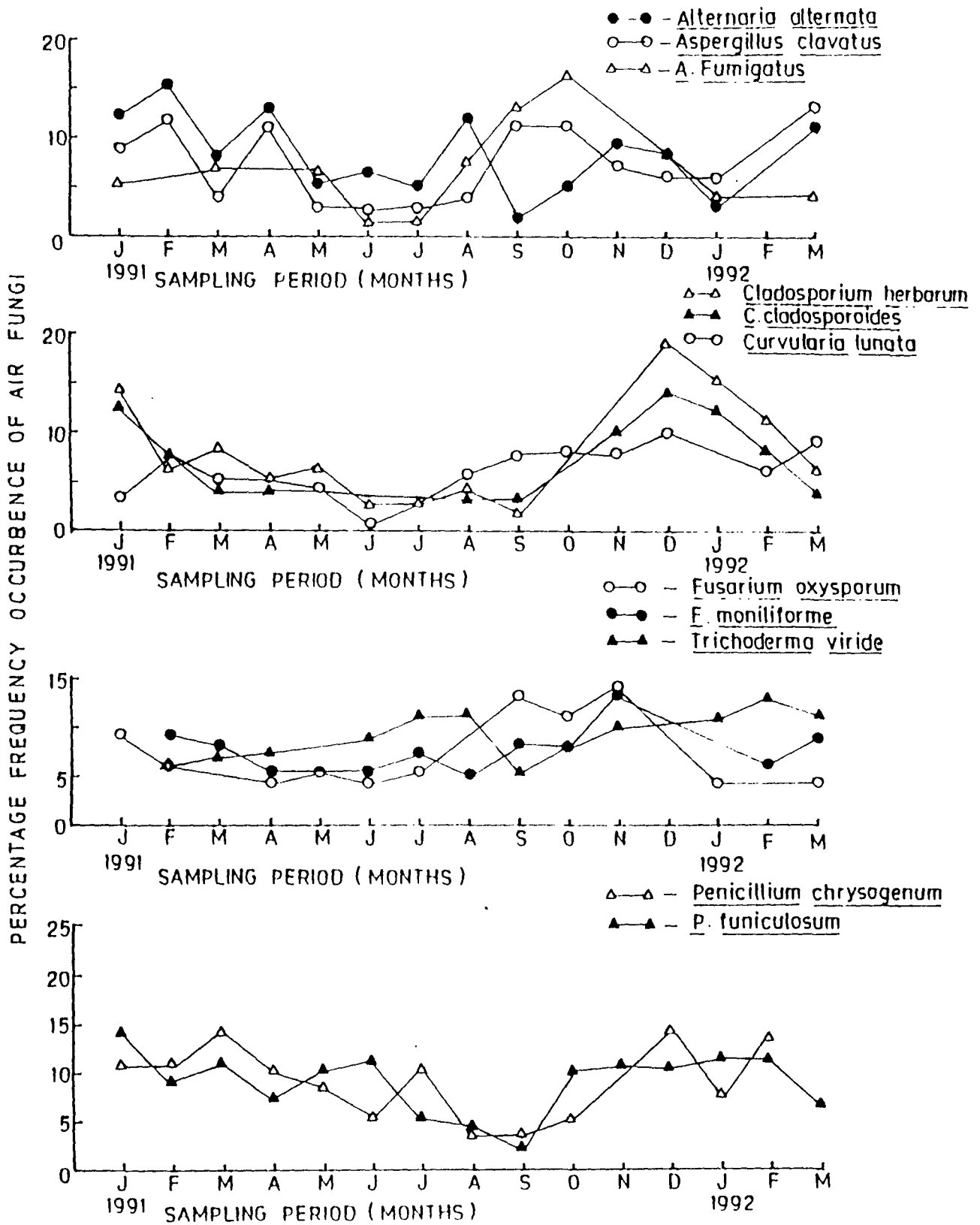


Fig 3.(d) Average monthly variation in frequency of fungal spores in in the air of closed forest stand of Alnus nepalensis.

Table 1 - Average Frequency of Fungal Spores in the Air of Open Forest Stand (1990).

FUNGAL SPECIES	SAMPLING PERIOD											
	J	F	M	A	M	J	J	A	S	O	N	D
<u>Mucor hiemalis</u>	4 65	-	-	-	-	3.65	4.22	3.17	-	-	5.66	7.54
<u>Alternaria alternata</u>	6.97	10.20	7.14	10 00	11.11	7.31	4.22	6.34	-	11.76	-	9.43
<u>A. solani</u>	-	-	-	5.71	-	2.43	2.81	-	-	-	-	-
<u>Aspergillus flavus</u>	-	-	7.14	-	8.88	9.75	12.67	14.28	-	9.80	7.54	5.66
<u>A. niger</u>	-	8.16	5.35	-	13.33	8.53	8.45	11.11	7.69	-	9.43	7.54
<u>Cladosporium cladosporoides</u>	13.95	10.20	10.71	8.57	4.44	4.87	-	-	9.23	11.76	9.43	-
<u>C. herbarum</u>	16.27	16.32	16.07	7.14	6.66	3.65	5.63	11.11	7.69	13.72	18.86	22.64
<u>Curvularia lunata</u>	6.97	8.16	7.14	12.85	13.33	6.09	-	-	4.61	-	-	-
<u>C. pullescens</u>	-	-	-	8.57	-	-	-	-	3.07	-	-	-
<u>Fusarium oxysporum</u>	9.30	10.20	5.35	7.14	-	3.65	-	-	12.30	19.60	-	-
<u>F. moniliforme</u>	6.97	-	8.92	5.71	-	7.31	4.22	6.34	10.76	11.76	-	-
<u>Helminthosporium oryzae</u>	-	-	-	-	-	4.87	7.04	9.52	12.30	-	-	-
<u>Monilia sp.</u>	-	-	-	-	-	2.43	8.45	6.34	7.69	-	5.66	7.54
<u>Nigrospora oryzae</u>	-	14.28	8.92	12.85	15.55	-	-	-	-	5.88	3.77	1.88
<u>Penicillium chrysogenum</u>	11.62	10.20	10.71	8.57	8.88	10.97	14.08	9.52	7.69	7.84	13.20	9.43
<u>P. funiculosum</u>	13.95	8.16	-	7.14	11.11	8.53	8.45	6.34	6.15	-	11.32	11.32
<u>Trichoderma viride</u>	-	-	12.50	5.71	-	7.31	12.67	12.69	7.69	7.84	9.43	5.66
<u>Verticillium albostrum</u>	6.97	-	-	-	-	-	-	-	-	-	5.66	7.54
<u>Geotrichum sp.</u>	-	-	-	-	6.66	4.87	4.22	3.17	-	-	-	-
<u>White sterile mycelia</u>	2.32	4.08	-	-	-	3.65	2.81	-	3.07	-	-	3.77

Table 2 - Average Frequency of Fungal Spores in the Air of Open Forest Stand (1991-1992).

FUNGAL SPECIES	SAMPLING PERIOD														
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M
<u>Mucor hiemalis</u>	-	-	-	-	4.42	3.16	-	3.19	-	-	-	-	3.92	13.33	-
<u>Pythium</u>	-	6.34	4.28	-	-	-	1.05	-	-	-	-	-	-	-	-
<u>Chaetomium bostrychodes</u>	-	-	-	-	3.53	1.89	2.10	-	-	-	4.16	-	-	-	-
<u>Melanospora zamae</u>	-	-	-	-	-	2.53	5.26	2.12	-	-	-	-	-	-	-
<u>Phoma glomerata</u>	-	-	4.28	5.12	5.30	-	-	-	-	-	-	-	-	-	-
<u>Alternaria alternata</u>	8.33	9.52	11.42	12.92	12.38	8.22	6.31	9.57	8.70	7.86	6.25	12.90	5.88	-	18.38
<u>A. solani</u>	-	-	-	-	7.07	3.70	4.21	6.38	-	-	-	-	-	6.66	-
<u>Aspergillus clavatus</u>	-	6.34	4.28	5.12	1.76	-	-	-	-	-	-	11.76	-	-	-
<u>A. flavus</u>	10.00	7.73	10.00	7.99	8.34	8.86	9.47	7.44	10.98	8.38	5.20	11.20	9.80	-	12.12
<u>A. niger</u>	-	4.76	5.71	10.25	6.19	2.22	4.21	5.31	7.99	6.74	3.12	-	-	-	-
<u>Cladosporium cladosporioides</u>	18.33	15.87	-	-	-	-	7.59	2.10	3.10	-	5.91	12.50	14.51	13.72	16.66
<u>C. herbarum</u>	23.33	14.28	11.42	8.97	5.30	5.99	3.15	4.25	4.30	6.74	15.92	22.58	23.52	26.90	12.12
<u>Curvularia lunata</u>	-	-	8.57	6.11	5.30	5.99	10.52	5.31	-	-	-	-	-	13.33	15.15
<u>C. pullescens</u>	-	-	-	-	-	3.79	7.36	6.38	-	-	-	-	-	-	-
<u>Fusarium oxysporum</u>	-	-	-	-	-	6.96	-	-	9.80	11.23	9.37	-	-	-	-
<u>F. moniliforme</u>	13.33	6.34	-	-	4.42	5.06	7.36	8.51	13.18	14.50	9.37	8.06	-	-	9.09
<u>Helminthosporium oryzae</u>	-	-	-	-	-	-	6.31	4.25	10.98	-	7.29	-	-	-	-
<u>Paecilomyces variata</u>	-	-	-	-	-	3.79	7.36	5.31	7.69	8.08	-	-	-	-	-
<u>Nigrospora oryzae</u>	-	12.69	8.57	10.25	8.84	-	-	2.12	-	-	5.20	-	-	-	-
<u>Penicillium chrysogenum</u>	-	11.11	11.42	12.82	12.38	-	-	4.25	-	5.61	-	-	7.84	10.00	6.06
<u>P. funiculosum</u>	16.66	-	7.14	7.69	3.53	10.12	6.31	5.31	8.79	7.86	10.41	9.67	9.80	13.33	9.04
<u>P. citrinum</u>	-	-	4.28	3.84	5.30	3.16	-	-	-	-	-	-	-	-	-
<u>Trichoderma viride</u>	-	-	-	6.41	5.30	4.43	10.52	9.57	6.59	6.74	5.20	-	5.88	-	12.12
<u>Verticillium alboastrum</u>	-	-	8.57	-	-	3.16	-	-	-	-	6.25	4.83	3.92	-	-
<u>Geotrichum</u>	-	-	-	-	-	3.79	4.21	4.25	3.29	3.37	-	-	-	-	-
Black sterile mycelia	10.00	4.76	-	-	-	-	-	-	6.59	5.61	-	11.29	-	-	-
White sterile mycelia	-	-	-	2.56	-	1.89	2.10	3.19	1.09	-	-	-	3.92	-	5.06

Table 3 - Average Frequency of Fungal Spores in the Air of Closed Forest Stand (1990).

FUNGAL SPECIES	SAMPLING PERIOD												
	J	F	M	A	M	J	J	A	S	O	N	D	
<u>Absidia glauca</u>	-	-	-	-	-	-	-	-	6.06	5.35	4.44	4.25	
<u>Mucor hiemalis</u>	12.19	-	-	4.68	4.16	6.66	5.88	-	-	-	-	6.38	
<u>Chaetomium bostrychodes</u>	-	-	-	-	-	4.00	9.80	1.42	-	-	-	-	
<u>Phoma glomerata</u>	-	-	-	6.25	-	4.00	-	-	-	-	-	-	
<u>Alternaria alternata</u>	14.63	14.00	20.49	14.06	14.58	9.33	-	12.85	9.09	3.57	6.66	8.51	
<u>Aspergillus flavus</u>	-	8.00	6.12	7.81	12.50	5.33	9.80	11.42	13.63	7.14	13.33	6.38	
<u>A. fumigatus</u>	7.31	-	-	-	-	-	13.72	8.57	15.15	12.5	-	-	
<u>Cladosporium cladosporioides</u>	17.07	8.00	6.12	3.12	-	-	-	7.14	9.09	10.71	11.11	19.14	
<u>C. herbarum</u>	19.51	14.00	-	12.50	8.33	-	3.92	5.71	-	14.28	22.22	23.40	
<u>Fusarium oxysporum</u>	-	8.00	12.24	-	10.41	-	-	11.42	10.60	8.92	8.88	-	
<u>F. moniliforme</u>	-	10.00	6.12	-	-	6.66	7.84	4.28	9.09	17.85	13.33	-	
<u>Humicola sp.</u>	-	-	-	-	-	8.00	5.88	2.85	-	-	-	-	
<u>Nigrospora oryzae</u>	-	12.00	8.16	10.93	12.50	-	-	-	3.03	-	4.44	6.38	
<u>Paecilomyces variata</u>	-	-	-	-	4.16	6.66	-	-	-	-	-	-	
<u>Penicillium chrysogenum</u>	17.07	12.00	16.32	7.81	10.41	10.66	5.88	8.57	10.60	5.35	8.88	12.76	
<u>P. funiculosum</u>	7.31	14.00	6.12	9.37	-	9.33	15.68	7.14	6.06	-	-	-	
<u>Trichoderma viride</u>	4.87	-	12.24	7.81	16.66	12.00	11.76	10.00	4.54	7.14	-	-	
<u>Verticillium albostrum</u>	-	-	6.12	10.93	6.25	9.33	-	-	-	-	6.66	8.51	
<u>Geotrichum sp.</u>	-	-	-	-	-	8.00	9.80	4.28	-	7.14	-	-	
White sterile mycelia	-	-	-	4.68	-	-	-	4.28	3.03	-	-	4.25	

Table 4 - Average Frequency of Fungal Spores in the Air of Closed Forest Stand (1991-1992).

FUNGAL SPECIES	SAMPLING PERIOD														
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M
<u>Absidia glauca</u>	-	-	-	-	2.24	2.32	-	-	3.33	5.00	-	-	4.76	4.44	-
<u>Mucor hiemalis</u>	-	-	-	-	4.49	4.65	5.00	-	2.22	-	-	-	6.34	6.66	6.97
<u>Chaetomium bostrychnodes</u>	-	-	-	-	4.49	4.95	3.75	2.24	1.11	-	-	-	-	-	-
<u>Phoma glomerata</u>	-	-	-	3.33	3.37	2.32	-	2.24	-	-	-	-	-	-	-
<u>Alternaria alternata</u>	12.72	15.38	3.32	13.33	5.01	9.20	5.00	12.35	2.22	5.00	9.99	3.91	3.17	-	11.92
<u>A. solani</u>	7.27	4.01	-	5.55	-	5.42	9.25	9.74	-	-	-	-	2.17	9.99	-
<u>Aspergillus clavatus</u>	9.09	12.30	4.41	11.11	3.37	3.10	3.75	4.49	11.11	11.25	7.27	9.38	9.34	-	13.45
<u>A. fumigatus</u>	5.45	-	7.35	-	9.74	2.22	2.90	7.39	3.33	16.25	-	-	4.76	-	1.99
<u>Microsporum saigosporoides</u>	12.72	7.69	4.41	4.44	-	-	-	3.37	3.33	-	10.99	14.39	12.99	8.38	4.95
<u>C. carpaeum</u>	14.54	6.15	3.92	5.55	9.74	3.10	3.75	4.49	2.22	-	-	19.14	15.37	11.11	6.97
<u>Curvularia lunata</u>	3.03	7.69	5.38	5.55	4.49	1.55	3.75	9.74	7.77	7.5	7.27	10.63	-	9.99	9.30
<u>Fusarium oxysporum</u>	9.09	6.15	-	4.44	6.74	4.65	6.25	-	13.33	11.25	14.54	-	4.76	-	4.05
<u>F. moniliforme</u>	-	9.23	8.32	5.55	-	5.42	7.50	5.61	8.38	8.75	14.54	-	-	9.99	9.30
<u>Helminthosporium oryzae</u>	-	-	-	7.77	3.37	3.10	-	-	-	-	-	-	-	-	-
<u>Humicola</u>	-	-	-	-	-	5.42	5.00	3.37	3.33	2.5	-	-	-	-	-
<u>Monilia</u>	-	-	-	-	6.74	3.87	7.50	4.49	8.88	-	3.63	6.38	3.17	-	-
<u>Nigrospora oryzae</u>	-	4.61	-	3.33	4.49	2.32	-	-	-	-	-	-	-	-	4.65
<u>Paecilomyces varieta</u>	-	-	-	-	-	3.37	3.75	2.24	-	-	-	-	-	-	-
<u>Penicillium chrysogenum</u>	10.90	10.76	14.70	10.00	8.98	5.42	10.00	3.37	3.33	5.00	-	14.89	7.93	13.33	-
<u>P. funiculosum</u>	14.54	9.23	11.76	7.77	10.11	11.62	5.00	4.49	2.22	10.00	10.90	10.63	11.11	11.11	6.97
<u>Trichoderma viride</u>	-	6.15	8.82	8.38	-	9.30	11.25	11.23	5.55	8.75	10.90	-	11.11	13.33	11.92
<u>Verticillium albostrum</u>	-	-	7.35	3.33	-	1.55	-	4.49	-	-	7.27	8.51	4.76	8.38	-
<u>Geotrichum</u>	-	-	5.88	-	4.49	4.65	10.00	6.74	7.77	6.25	-	-	-	-	-
<u>White sterile mycelia</u>	-	-	2.94	-	2.24	3.10	-	3.37	-	2.50	3.63	-	-	2.22	4.95

Table 5(a) - Correlation coefficient (r) for air spora and phylloplane fungi of different isolating techniques at two forest stands of Alnus nepalensis.

Sources of variation	D.F.	Air spora			
		Species		Colonies	
		Open	Closed	Open	Closed
Direct observation	9	0.287	0.332	0.101	0.293
Impression plate	9	0.170	0.284	0.163	0.312
Moist chamber	9	0.467	0.194	0.533*	0.142
Washed leaves	9	0.315	0.362	0.216	0.193
Dilution plate	9	0.350	0.515	0.150	0.419

\*, \*\*, \*\*\*,  $p < 0.1, 0.05$  and  $0.01$  respectively.

Without asterisk statistically insignificant at these levels.

Table 5(b) - Correlation coefficient (r) for air spora, litter fungal population and climatic conditions at two forest stands of Alnus nepalensis.

Source of variation	D.F.	Litter fungal population	D.F.	Temperature	Relative Humidity (RH%)	Rainfall (mm)
Air spora (Open)	12	0.643***	25	0.567***	0.433**	0.582***
Air spora (Closed)	12	0.795***	25	0.629***	0.446**	0.673***

\*, \*\*, \*\*\*,  $p < 0.1$ , 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

months. A. clavatus and A. fumigatus occurred in high concentrations during September and October (Fig. 3c and d). Monilia was dominant during the summer months and Paecilomyces in the hot wet months.

At both the forest stands, the population of air fungi exhibited a positive correlation with climatic conditions and phylloplane fungi isolated by moist chamber technique (Tables 5a and b) whereas the correlations were not significant statistically with the rest of isolation techniques of both the stands. However, the fungal population of the litter of Alnus nepalensis showed a significant positive correlation with air spora of both the sites (Table 5b).

## DISCUSSION

The composition of air spora differed in both the forest stands. The majority of the forms isolated from the air belonged to deuteromycetes which accounted for approximately 80 per cent of the total mycoflora. Similar observations were made by Mishra and Tewari (1976a) and Dixit and Gupta (1980). Species of Cladosporium, Alternaria, Penicillium and Aspergillus were the dominant forms in the air of both the forest sites which was attributed to their ability to produce and discharge large number of spores (Pady, 1971; Mishra and Tewari, 1976b). A fall

in number of Cladosporium propagules during hot months was correlated to high temperature and low humidity as has been reported by Frey and Durie (1960) and Taylor and MacFadyen (1962) which was in contrast to the findings of Hyde and William (1953), Kramer et al. (1959), Morrow (1947) and Targow and Plunkett (1951). Further, from the present studies it is also clear (Figs. 3a, b, c and d) that population of C. herbarum in air was maximum during winter season indicating the resistance of the fungus to low temperature.

Dominance of species of Alternaria, Aspergillus and Penicillium in closed forest than open ones was attributed to the inhibition of spore dissipation due to physical barrier of high density of trees in the earlier site than the later ones. Sreeramulu and Seshavataram (1962) have also noticed high number of spores of Penicillium in paddy fields than open fields. In temperate climates, Penicillium generally shows a peak in winter and spring (Di Menna, 1955; Pawsey and Heath, 1964). Aspergilli and Penicillia are known to be quite frequent occurrence in the air of different localities (Dickinson, 1967). Some species such as A. niger and A. flavus occurred frequently from the air of both the forest sites. Alternaria alternata was dominant during warm months as has also

been reported by earlier workers (Sinha, 1971; Dickinson, 1967, 1981).

Curvularia showed a rapid rise at the beginning of warm and moist season. The fungus has been reported as a facultative or secondary parasite on grasses, sorghum, maize, millet and sugarcane (Dickinson, 1956; Lam Quang-Bach, 1964). Sreemulu and Seshavataram (1962) mentioned that the peak of Curvularia was a little earlier than Cladosporium in paddy fields and suggested that it is favoured by warmer conditions, perhaps because of its host range growing in that period.

Fusarium was most common during later part of rainy season (September and October) (Figs. 3c and d) at closed forest site. Pady (1957) also reported an increase in Fusarium sp. after rain. Sreeramulu and Seshavataram (1962) found that 14.4% of the total air spores belonged to the genus Helminthosporium was rare and showed its peak in August and September in the open forest site and in April, May and June in closed site when temperature and relative humidity were high. The observations were supported by Sreeramalu and Seshavataram (1962) and were in contrast to Gregory and Hirst (1957) who related to the two peaks of H. avena conidia in the atmosphere with the primary and secondary phases of oat leaf spot.

Nigrospora was favoured by dry period in the open site and it exhibited a peak before the onset of heavy rain. The increased number of spores of the fungus in Western Nigeria was correlated to the availability of suitable hosts and climatic factors (Cammack, 1955). Trichoderma was dominant during hot and wet season in both the sites corresponding to the increase in the population on the leaves which might have been released into the air. Monilia was also reported<sup>as</sup> a major component of the air spora at Honolulu (Myer, 1956) and Johannesburg (Ordman and Etter, 1956).

Fungi belonging to Phycomycetes, Ascomycetes and sterile mycelial forms were poorly represented in the air which may probably be due to their poor survival in the air.

In general, the present investigation revealed that the total air fungal population increased with increase of phylloplane fungi of the plant leaves of both the forest sites. The total number of fungal species fluctuated at different sampling periods similar to the reports of earlier workers (Mishra and Srivastava, 1971a; Mishra and Tewari, 1976a; Kumar and Gupta, 1980). The fungal spores produced on the leaf surface might be getting released into the air which<sup>is</sup> attributed to the richness

of air spora of the open forest than closed ones. The distribution of air fungi was also affected by the climatic condition especially during rainy season (Dickinson, 1967). Seasonal variation in frequency of occurrence has been established for a number of fungi, but it appears that macroclimatic conditions alone are not responsible for the variations. The fall in number of fungi during the hot and wet months was supported by observation of Taylor and McFadden (1962). Most of the air fungal spores were washed down and only a few of them like *Aspergilli* and *Penicillia* occurred throughout the sampling period. The increase in fungal population in the air after rains may be due to their release from the leaf surface. Temperature changes in atmosphere had considerable influence on seasonal variation possibly due to its influence on spore viability in general and larger spores in particular (Gregory, 1961; McDonald, 1962) which could account for reduced numbers excluding Penicillium and Aspergillus during hot and wet months. Factors such as humidity and wind velocity were also contributed to the diurnal periodicities of fungal spores in the air (Mishra and Tewari, 1976a and Kumar and Gupta, 1980).

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CHAPTER II

ASSESSMENT OF THE FUNGAL POPULATION FROM THE  
PHYLLOPLANE REGION OF ALNUS NEPALENSIS LEAVES

## Introduction

The term phylloplane and phyllosphere have been used imprecisely, with different meanings or as synonyms, to designate the leaf as a particular habitat (Last, 1955; Ruinen, 1961; Diem, 1973). Here phylloplane is used according to the definition of Kerling (1958) to indicate the area in direct contact with the leaf surface, and phyllosphere in a wide sense, as used by Carrol et al. (1977) to indicate the leaf as a whole, including the interior.

The aerial surfaces of higher plants growing under natural conditions are usually colonized by large and varied populations of microorganisms forming an ecological niche term the phylloplane. The leaf surface traps different spores present in the air through various devices, viz., hairs, sticky surfaces etc. The host species themselves play an important role in microbial colonization by providing different ecological niches to the microorganisms (Dickinson, 1981). The ecological habitat of the plant surface is of different nature. Cuticle, epidermis etc. of different plants vary and the leaves of different species of plants support a distinctive microbial population (Lamb and Brown, 1970). The leaf surface forms

a unique habitat since it supports a definite microbial community by providing a complex of niches to various organisms viz., bacteria, actinomycetes, yeasts and fungi. The different microbial populations in such a community interact with one another by competing for space and nutrients, by production of secondary metabolites and also the antibiotics (Fokkema, 1973; Hudson, 1978). Host specificity is known to be controlled both by the physical nature of the leaf surfaces, the chemical nature of exudates. Weather may also play an important role in the distribution of fungi of leaf surface both qualitatively and quantitatively.

The colonization of leaf surfaces by fungi presents an interesting study with regard to substrate relationships (Pugh and Buckley, 1971). The fungal colonizers on leaf surface come either from the air or from soil. The occurrence of many fungi on aerial surfaces may be directly related to inoculations from the atmosphere, which in turn is related to the production of the deciduous propagules elsewhere (Dickinson, 1976).

Considerable interest has developed recently (Narula and Mehrotra, 1981; Khara and Singh, 1981; Rao and Manoharachary, 1981; Pennycook and Newhook, 1981; Ahuja and Payak, 1981; Kumar and Singh, 1981; Miller and

Roy, 1982; Gupta and Mukerji, 1982) in the investigation of the various types of microorganisms which occur on leaf surface at various stages of plant growth and also during the changing environments.

Thus, the studies on phylloplane mycoflora are important because of their role in biological control of certain epidemic diseases, active decomposition and enhancement of activity of phylloplane nitrogen fixers (Singh and Sinha, 1962). The fungi on the leaf surface have been investigated by a variety of techniques. The merits and demerits of cultural techniques commonly used in phyllosphere studies have been reviewed by Dickinson (1971) who emphasized the use of suitable combination of various cultural methods along with the direct or indirect microscopic examination of leaf surface.

Despite numerous investigations reviewed by Hudson (1968) and more recently by several authors in Dickinson and Pugh (1974), there are still some aspects of the fungal colonization of leaf surfaces which require examination. Seasonal surveys such as those of Dickinson (1967) and Pugh and Buckely (1971) adequately describe the species involved, but in common with other reports deal mainly with general trends and make little attempt to provide a detailed analysis of the structure of the fungal community.

The phylloplane mycoflora of green and decaying leaves have been studied by many workers (Hogg and Hudson 1966; Sinha, 1971; Rai, 1973; Sherina and Dwivedi, 1975; Kumar and Gupta, 1976; Sinha and Dayal, 1983; Garg and Sharma, 1985; Mathur et al., 1985 and Adhikari, 1990). Present studies were undertaken to gather information of the saprophytic fungal community of the phyllosphere, seasonal pattern and variation in populations in relation to age and/or physiological condition of the leaf surface attached to the tree and after leaf fall. The overall aim of the investigation was to determine the nature of initial colonizers on leaf surface and their role in Alnus nepalensis litter decomposition under natural condition after leaf fall. ✓

#### **MATERIALS AND METHODS**

The leaves of Alnus nepalensis of both the selected forest sites were used as the plant material throughout the course of the present investigation.

##### **Collection of samples**

The survey extended from March 1990 when the leaves bud unfolded to January 1991 when they were senescent and falling. Samples were taken at intervals of 15 days from both the selected stands. Five Alnus nepalensis trees of almost equal height were selected to collect the leaf

samples throughout the study period. From each tree, samples were collected at random from approximately equal height of the trees. Five replicates sample were collected when the leaves were at different degrees of maturity after flushing as folding (bud). Unfolding (buds opened) and young expanding green leaves upto the leaves which entered into the litter stage.

The samples collected from the month of November 1990 upto January 1991 were found to be senescent. Collection of the leaves as Alnus nepalensis litter was followed from the month of January 1991 to March 1992.

During each collection the samples were collected in previously sterilized polythene bags using sterilized scissors and forceps. The bags were properly sealed and transported to the laboratory and the leaves were used on the same day for the isolation of mycoflora. On a few occasions when isolations could not be done on the same day, the sample were stored overnight at 4°C (Ruscoe, 1971). Additional samples were collected for the determination of moisture content and pH.

The leaf surface was examined for mycoflora by different techniques.

## 1. Direct techniques

(i) Cellotape impression technique (Edward and Hartman, 1952). Strips of cellotape was pressed gently against the leaf surfaces and the cellotape strips with the impression of the mycoflora were stained in cotton blue-lactophenol and the total number of spores and mycelia were recorded in five different fields of microscope and average values were calculated.

(ii) Nail polish impression technique (Masurovsky and Jordan, 1960). Nail polish of light colour was coated smoothly on the leaf surface and when the layer dried it was peeled off and mounted in lactophenol with 0.10% acid fuschin. Such peels containing the impression of leaf surface mycoflora were observed under a microscope and the total number of spores and mycelia were recorded under five different fields of microscope and their average values were calculated.

## 2. Moist chamber (Keyworth, 1951)

Ten leaves were kept in moist chambers prepared by moisting 3-4 filter papers kept in petri-plates with sterile distilled water. These were incubated at  $25 \pm 1^{\circ}\text{C}$  and observations taken at intervals of 5 days till one month.

### 3. Impression plate (Potter, 1910)

Leaves were gently pressed against nutrient agar in petriplates with the help of a sterilized forceps. The leaf was subsequently removed leaving its impression on the nutrient agar (Czapek's Dox agar and cellulose agar medium) contained in a petriplate. The surface of the leaves were pressed separately in different petriplates containing the nutrient agar. Replicates for each were maintained and incubated at  $25 \pm 1^\circ\text{C}$  for 6 days and observations were taken by counting the number of colonies of different fungi occurring on the plates.

### 4. Dilution plate (Dickinson, 1971)

This method was employed for quantitative estimation of fungi (Dickinson, 1971; Sharma et al., 1974). The colonies were counted irrespective of their origin from a spore or mycelium. 1 g of leaves were cut into small segments with the help of a sterilized scissor and the leaf segments were put in 250 ml conical flask containing 99 ml of sterilized distilled water and then kept on a mechanical shaker for 30 mins to get a uniform shaking. 1 ml of this washing was taken and diluted 10 times by adding sterilized distilled water. The plating of  $10^{-3}$  dilution was found to be appropriate for colony counting. 1 ml of this aliquot was pipetted on nutrient agar (Czapek's

Dox agar and cellulose agar mediums). Replicates were maintained for each. Plates were incubated at  $25 \pm 1^\circ\text{C}$  for 6 days and the fungal colonies were counted.

#### **5. Washed leaves plating**

The leaf segments which were washed as given in the dilution plate technique were taken out and transferred into another 250 ml flask containing sterilized distilled water and again were given thorough 20 washings of sterilized distilled water. These leaf pieces were teased into smaller fragments with the help of sterilized forceps and needle and dried on sterilized blotting paper. Five such pieces were inoculated in each petriplate containing nutrient agar (Czapek's Dox agar and cellulose agar mediums). Five replicates were maintained for each site. The petriplates were incubated at  $25 \pm 1^\circ\text{C}$  for 6 days and the leaf segments were examined for fungal colonies occurring on them and the observations were taken till no new forms appeared.

#### **Nutrient media used for isolation of fungi**

Several media were employed during the present investigation such as potato dextrose agar, Czapek's Dox agar, cellulose agar to achieve isolation of maximum number of fungi.

### 1. Potato dextrose agar

Potato peeled and sliced - 200 g, D-Glucose - 20 g, Agar - 15 g, water - 1 litre.

Peeled sliced potatoes were boiled for 1 hr in one litre of water. This was filtered through cheese cloth and glucose was added to it and the medium was sterilized at 15 lb for 15 mins.

### 2. Czapek's Dox agar (Raper and Thom, 1949)

Agar - 15 g, Sucrose - 30 g,  $\text{NaNO}_3$  - 3 g,  $\text{K}_2\text{HPO}_4$  - 1 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.5 g, KCl - 0.5 g,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  - 10 mg, Distilled water - 1 litre, Streptomycin - 30 mg.

### 3. Cellulose Agar (Eggins and Pugh, 1962)

Agar - 20 g, Cellulose (powdered) - 10 g,  $(\text{NH}_4)_2\text{SO}_4$  - 0.5 g, L - Asparagine - 0.5 g,  $\text{KH}_2\text{PO}_4$  - 1 g, KCl - 0.5 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.2 g,  $\text{CaCl}_2$  - 0.1 g, Distilled water - 1 litre, Streptomycin - 30 mg.

### Isolation of fungi

The plates were examined regularly and slow growing forms were transferred to fresh nutrient plates to avoid over running of fast growing ones. Efforts were made to get pure cultures of various fungi occurring in moist chambers, impressive plates, dilution plates and washed leaves plating techniques and these were raised on tube

slants containing Czapek's Dox agar, cellulose agar and potato dextrose agar media. The fungi were identified and pure cultures maintained. ✓

### Physical analysis of the leaves

#### 1. pH

pH of the leaves was determined by electric pH meter. 10 g of fresh leaves were crushed in 25 ml of double distilled water and filtered. The filtrate was then used for determining pH. ✓

#### 2. Moisture content

The moisture content of the leaves was determined by drying 10 g of fresh leaves in hot air oven at 105°C for 24 hours until constant weight was obtained.

### Qualitative estimation

The qualitative estimations were expressed as percentage frequency of occurrence. The latter was calculated for moist chamber, impression plate and washed leaves plating techniques.

(i) The percentage frequency of occurrence was calculated for each fungus based on the formula given by Tresner et al. (1954).

$$\text{Percentage frequency of occurrence} = \frac{\text{Number of samples of occurrence}}{\text{Total number of samples of occurrence}} \times 100$$

### Quantitative estimation

For quantitative estimation the number of fungal propagules was expressed as fungal population/g dry weight of leaves. It was calculated as:

$$\frac{\text{Total number of colonies} \times \text{dilution of the aliquot } (10^3) \times \text{inoculum}}{\text{Dry weight of leaves (g)}}$$

The fungal population was calculated in case of dilution plate technique where colony numbers could be counted easily. Determination of percentage relative abundance and percentage frequency of fungi.

The percentage relative abundance of a fungal species was calculated from the formula as detailed below.

$$\frac{\text{Total number of individual species of fungus}}{\text{Total number of individual of all species of fungi}} \times 100$$

The term 'frequency' is used to refer the frequency of a fungus during the entire sampling period and is represented by the number of samplings in which the fungus appeared, expressed in percentage. Based on percentage frequency the fungi are grouped as dominant, 80-100%, common 61-80%, frequent 41-60%, occasional 21-40% and rare 1-20% (Vittal, 1976).

### Statistical analysis of the data

The data collected during the present investigation

by dilution plate technique were statistically analysed by using analysis of variance.

Further, pH and moisture content of the leaves were correlated with the fungal population at different growth stages of the plant leaves by using Karl Pearson's Coefficient of Correlation (Zar, 1974). ✓

## RESULTS

Based on the observation from the five methods of isolation of fungi (Table 6) a total of 36 species belonging to 25 genera of fungi were isolated from the phylloplane of Alnus nepalensis at different growth stages of the leaves in the selected sites. It has been observed that maximum fungal species were isolated in moist chamber and dilution plates followed by impression plate, washed leaves plating and least by using direct observation methods (Fig. 5).

The fungal population of the phylloplane increased gradually from folded to senescent stage and the peak of fungal population was attained at the maturity of the leaves. The least fungal population was obtained at the bud or folded stage soon after flushing.

The moisture content of leaves at different stages of maturity varied. The moisture content of leaves at

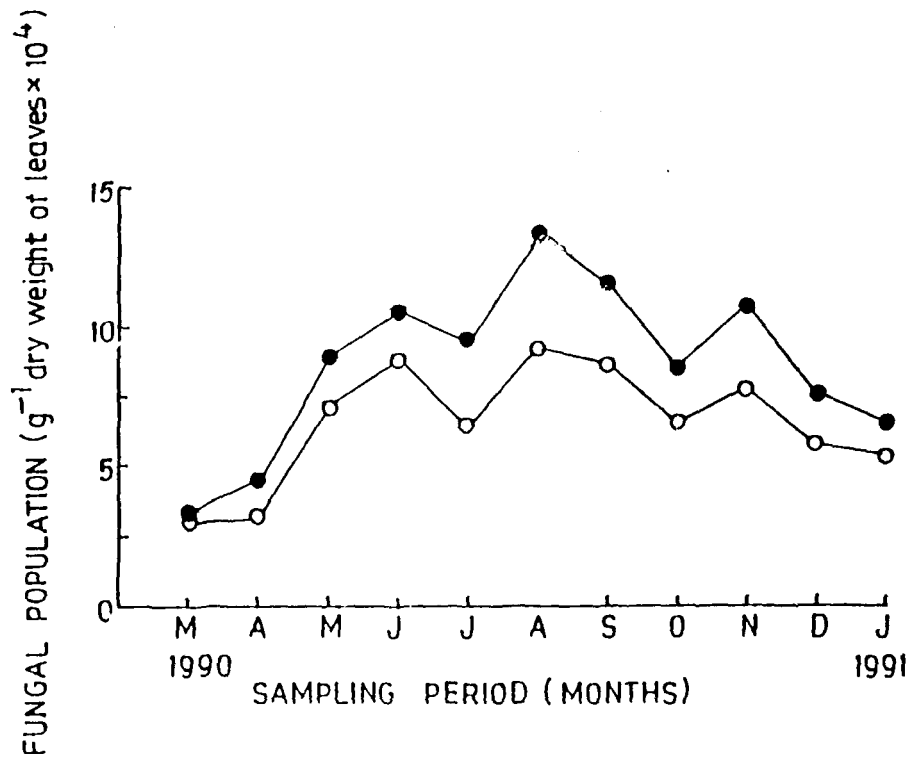


Fig 4. Variation in fungal population from phylloplane of *Alnus nepalensis* during different ages of the leaves at two forest stands (○-○ - open, ●-● -closed)

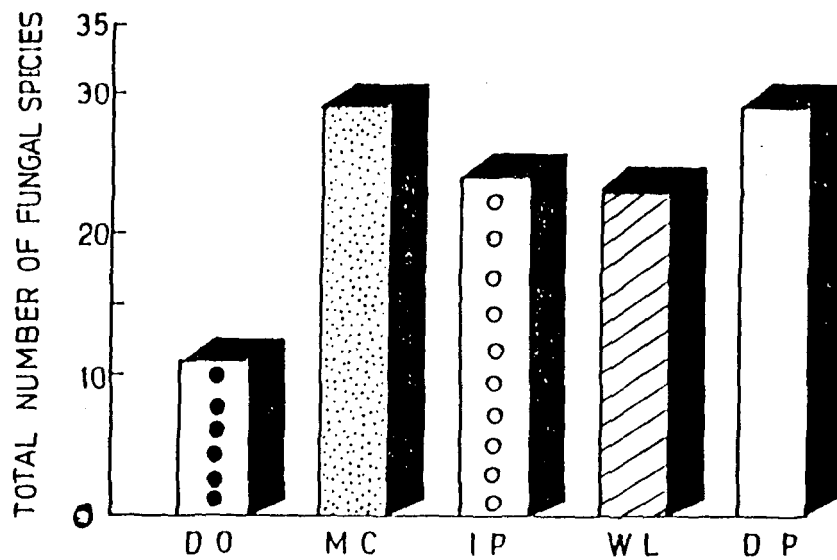


Fig 5. Different techniques used for isolation of phylloplane fungi in two forest stands (D.O - Direct Observation, M.C.-Moist Chamber, I.P.- Impression plate, W.L.-Washed leaves and D.P.-Dilution Plate).

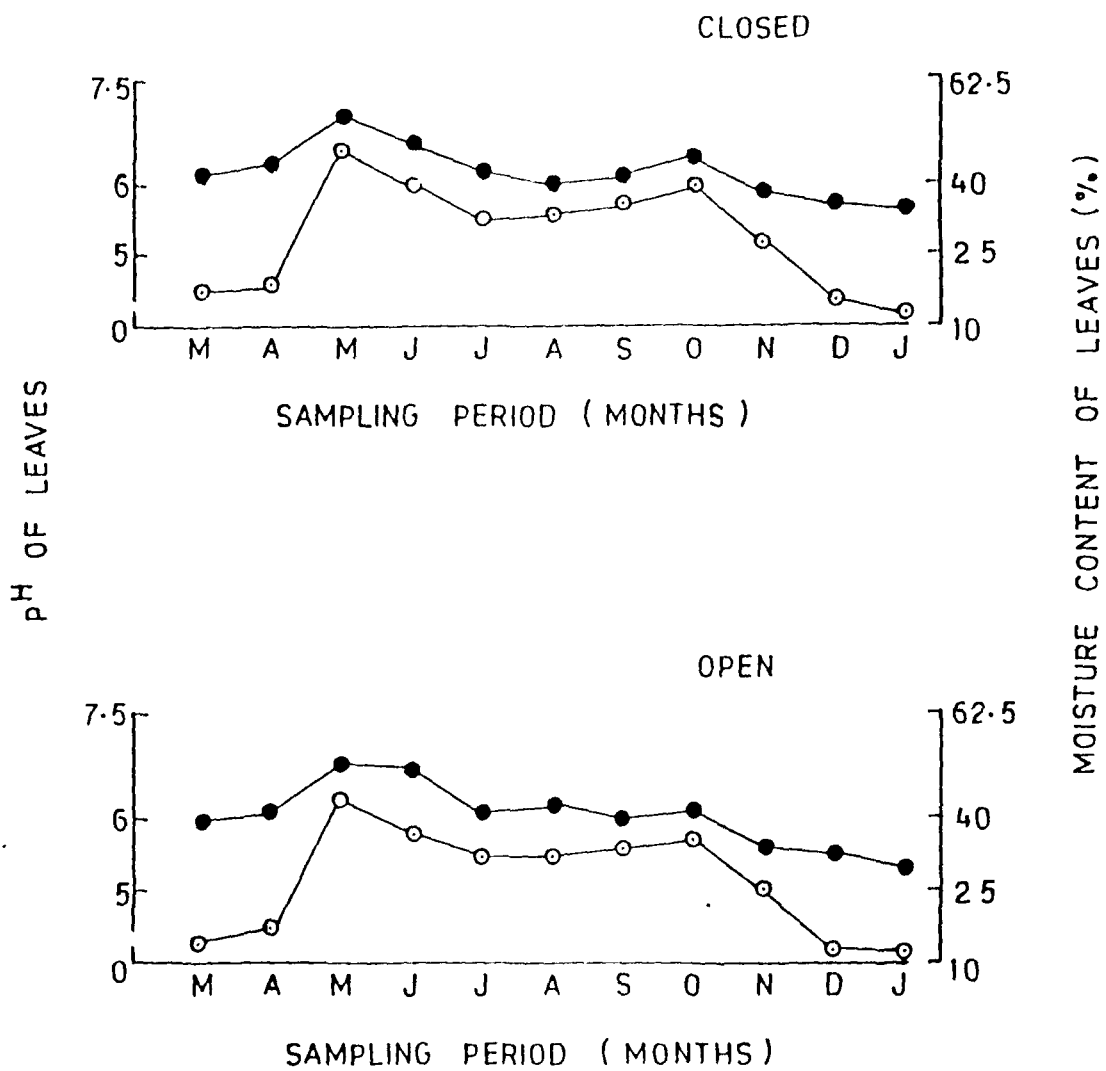


Fig 6.(a) Average monthly variation in moisture content (○—○) and P<sup>H</sup> (●—●) of Alnus nepalensis leaves at two forest stands.

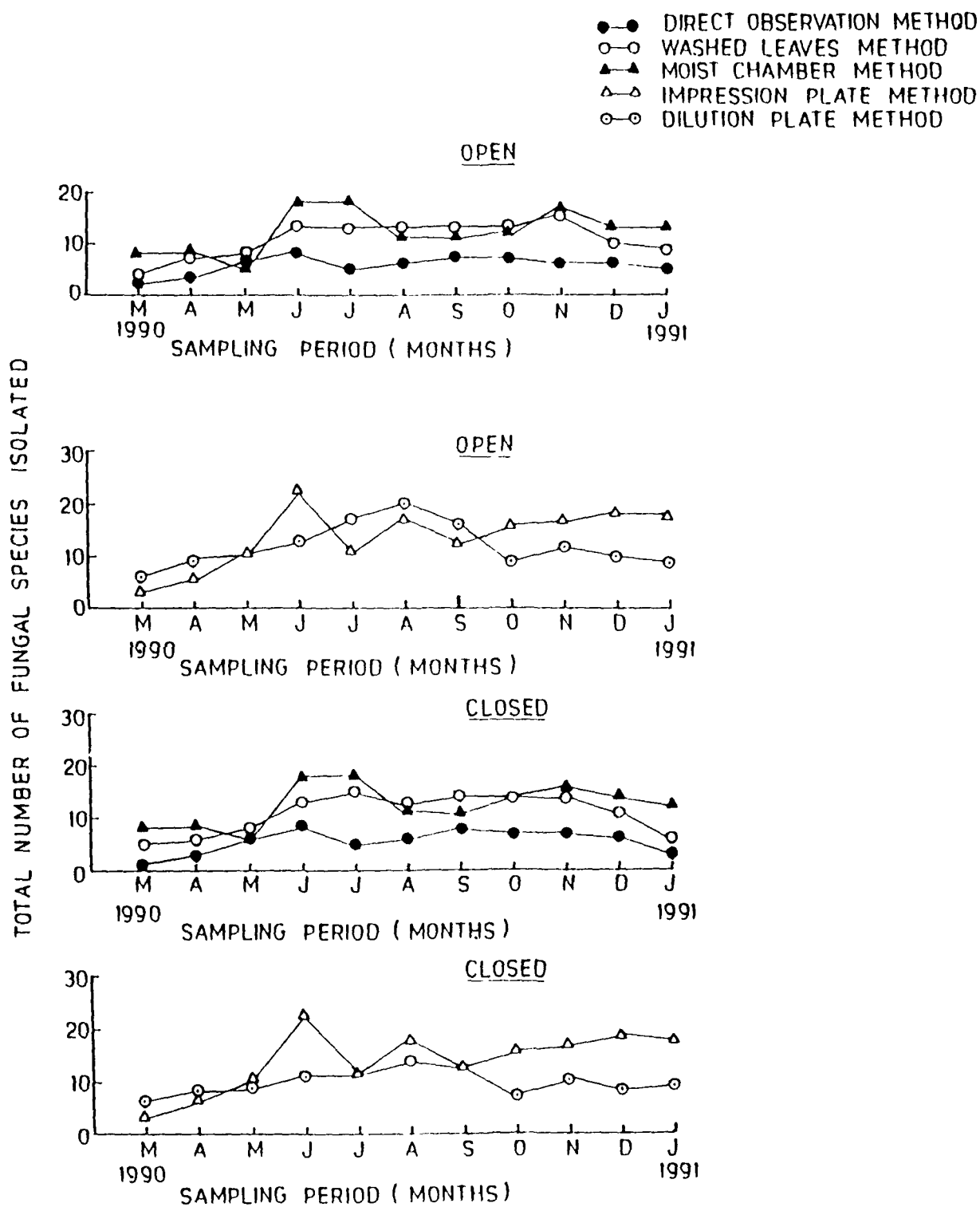


Fig 6.(b) Total number of fungal species isolated from the phylloplane of *Alnus nepalensis* at different ages of the leaves in two forest stands.

Table 6 - The comparison of the five techniques used for isolation of fungi at two forest stands of Alnus nepalensis.

Fungal Species	Isolating Techniques				
	D.O.	I.P.	W.L.	M.C.	D.P.
<u>Mucor hiemalis</u>	-	-	+	+	-
<u>Rhizopus nigricans</u>	-	+	+	+	+
<u>Pythium sp.</u>	-	+	+	-	+
<u>Chaetomium bostrychodes</u>	-	+	+	+	+
<u>C. globosum</u>	-	-	-	+	+
<u>Collectrichum capsicum</u>	-	-	-	+	-
<u>Melanospora zamae</u>	-	+	+	+	+
<u>Phoma glomerata</u>	-	+	+	+	+
<u>Rhizoctonia solani</u>	-	+	-	+	-
<u>Alternaria alternata</u>	+	+		+	+
<u>A. solani</u>	-	+	-	-	+
<u>Arthrimum sp.</u>	-	+	-	+	+
<u>Aspergillus clavatus</u>	-	-	-	-	+
<u>A. flavus</u>		-	+	+	+
<u>A. fumigatus</u>	-	-	-	-	+
<u>A. nidulans</u>	+	-	+	+	+
<u>A. niger</u>	+	+	+	+	+
<u>Cladosporium cladosporoides</u>	-	+	+	+	+
<u>C. herbarum</u>	+	+	+	+	+
<u>Curvularia lunata</u>	+	+	+	+	+
<u>C. pullescens</u>		-	-	+	-
<u>Fusarium oxysporum</u>	+	-	-	-	-
<u>F. moniliforme</u>	-	+	+	+	+
<u>Gliocladium pencilloides</u>		+	+	+	+
<u>Humicola prisea</u>	-	+	+	+	+
<u>Monilia sitophylla</u>	-	-	+	-	-
<u>Nigrospora oryzae</u>	+	+	+	+	+

Table 6 (Contd.)

Fungal Species	Isolating Techniques				
	D O	I.P.	W.L.	M.C.	D.P.
<u>Paecilomyces veruta</u>	-	-	-	-	+
<u>Penicillium funiculosum</u>	+	+	+	+	+
<u>P. chrysogenum</u>	-	+	+	+	+
<u>Torula herbarum</u>	-	+	-	+	+
<u>Trichoderma viride</u>	+	+	+	+	+
<u>T. roseum</u>	-	+	+	+	+
<u>Verticillium albo-atrum</u>	-	+	+	+	+
White sterile mycelia	+	+	+	+	+
Orange sterile mycelia	-	-	-	+	-

D.O. = Direct observation technique

I.P. = Impression plate technique

W.L. = Washed leaves plating technique

M.C. = Moist chamber technique

D.P. = Dilution plate technique

both the forest sites was higher in rainy months and lower in winter and during senescent stage the moisture content abruptly dropped to lowest (Fig. 6).

#### Qualitative assessment

Direct observation methods (Table 7) yielded only those forms which were dominant species on the phylloplane viz., Cladosporium herbarum, Penicillium spp. and Alternaria spp. which were exclusive to this method in both the forest stands. By impression plate method (Table 8) only those fungi which were occurring superficially on the leaf surface such as Penicillium spp., Alternaria alternata, Cladosporium spp. and Fusarium moniliforme were isolated dominantly in both the forest stands. While Aspergillus spp. and Trichoderma roseum were also of common occurrence. Other fungi like Rhizoctonia solani, Gliocladium pencilloides, Humicola grisea and Nigrospora oryzae occurred rarely in both the forest stands of Alnus nepalensis.

By plating washed leaves some idea about the actual activity of fungi which had penetrated the leaves could be obtained. It was observed (Table 9) in both the sites that fungi like Alternaria solani and Cladosporium herbarum were the dominant colonizers in addition to fungi like Aspergilli and Penicillia which occurred mostly as dominants.

Moreover, fungi like Arthrinium sp. and Trichoderma viride were of common occurrence and fungi like Mucor hiemalis, Rhizopus nigricans and Pythium sp. were isolated occasionally. Whereas fungi like Melanospora zamae, Chaetomium bostrychodes, Gliocladium penicilloides, Humicola grisea and Nigrospora oryzae were of rare occurrence.

The mycoflora recorded on leaf surface of both the forest stands by moist chamber technique (Table 10) exhibited that Aspergilli, Mucorales and a few Hyphomycetes colonized the young leaves and dematiaceous, Hyphomycetes and Ascomycetes were frequent on matured leaves and later on as the leaves started senescing few species of Ascomycetes like Chaetomium bostrychodes and more Hyphomycetes like Alternaria alternata, Aspergilli, Cladosporium cladosporoides, C. herbarum, Curvularia lunata, Fusarium moniliforme, Penicillium spp., Trichoderma viride and Verticillium alboatrum appeared. These species seem to be foremost and frequent colonizers of senescent leaves with high percentage frequency of occurrence.

Some fungi such as Mucorales, Ascomycetes like Melanospora zamae, Colletotrichum capsicum and Hyphomycetes like Aspergilli, Humicola grisea showed very low percentage frequency of occurrence with age of green leaves. White and orange sterile mycelia were also isolated by the different techniques.

Table 7 - Phylloplane fungi of Alnus nepalensis at two forest stands of different growth stages of leaves isolated by direct observation technique.

FUNGAL SPECIES	Fold- ing bud		Unfold- ing bud		Young Green		Matured Green Leaves		Senescent Leaves		Fre- quency				
	MAR	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC		JAN			
	O	C	O	C	O	C	O	C	O	C		O	C		
<u>Alternaria alternata</u>	+	-	+	+	+	+	+	+	+	+	+	-	D	C	
<u>Aspergillus niger</u>	-	-	+	+	+	+	+	+	+	-	+	-	-	F	C
<u>Cladosporium herbarum</u>	-	-	+	+	+	+	+	+	+	+	+	+	+	D	D
<u>Curvularia lunata</u>	-	-	+	+	+	+	+	+	+	+	+	+	-	F	C
<u>Fusarium oxysporum</u>	-	-	-	+	+	+	+	+	+	+	+	+	+	C	C
<u>Nigrospora oryzae</u>	-	-	+	+	-	-	-	-	-	-	-	-	-	O	O
<u>Penicillium funiculosum</u>	-	-	-	+	+	+	+	+	+	+	+	+	+	D	C
<u>Trichoderma viride</u>	-	-	-	-	-	-	-	-	-	+	+	+	+	O	F
White sterile mycelia	+	+	-	+	-	-	+	+	+	-	-	-	+	F	O

O = open, C = closed, + = presence of a fungus.

D = Dominant (81 - 100% Frequency), C = Common (61 - 80%), F = Frequent (41 - 60% Frequency), Occasional (21 - 40%), R = (1 - 20% Frequency).



Table 8 (Contd.)

FUNGAL SPECIES	Fold- ing bud		Unfold- ing bud		Young Green Leaves			Matured Green Leaves			Senescent Leaves			Fre- quency				
	MAR		APR		MAY	JUNE		JULY	AUG		SEPT	OCT			NOV	DEC	JAN	
	O	C	O	C	O	O	C	O	C	O	C	O	C		O	C	O	C
<u>Curvularia lunata</u>	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	F
<u>Fusarium moniliforme</u>	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	D
<u>Gliocladium pencilloides</u>	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	+	R
<u>Humicola grisea</u>	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	R
<u>Nigrospora oryzae</u>	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	R
<u>Penicillium funiculosum</u>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D
<u>P. chrysogenum</u>	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	F
<u>Torula herbarum</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Trichoderma viride</u>	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	F
<u>T. roseum</u>	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	C
<u>Verticillium albo-atrum</u>	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	F
White sterile mycelia	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	F
Orange sterile mycelia	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	O

D = Dominant (81 - 100% Frequency), C = Common (61 - 80% Frequency), F = Frequent (41 - 60% Frequency),  
O = Occasional (21 - 40%), R = Rare (1 - 20% Frequency).



Table 9 (Contd.)

FUNGAL SPECIES	Fold- ing bud		Unfold- ing bud		Young Green Leaves			Matured Green Leaves			Senescent Leaves			Fre- quency			
	MAR		APR		MAY	JUNE		JULY	AUG		SEPT	OCT	NOV		DEC	JAN	
	O	C	O	C	O	C	O	C	O	C	O	C	O		C	O	C
<u>C. pullescens</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Fusarium moniliforme</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F
<u>Gliocladium pencilloides</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Humicola grisea</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Monilia sitophylla</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F
<u>Nigrospora oryzae</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Penicillium funiculosum</u>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	D
<u>P. chrysogenum</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Trichoderma viride</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F
<u>T. roseum</u>	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	O
<u>Verticillium albo-atrum</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	O
White sterile mycelia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F

D = Dominant (81 - 100% Frequency), C = Common (61 - 80% Frequency), F = Frequent (41 - 60% Frequency),  
O = Occasional (21 - 40%), R = Rare (1 - 20% Frequency).

Table 10 - Percentage frequency of occurrence of phylloplane fungi of Alnus nepalensis at the forest stands of different growth stages of leaves isolated by moist chamber technique.

FUNGAL SPECIES	Folding bud		Unfolding bud		Young Green Leaves			Matured Green Leaves			Senescent Leaves			Frequency																			
	MAR		APP		MAY			JUNE			JULY				AUG			SEPT			OCT			NOV			DEC			JAN			
	O	C	O	C	O	C	O	C	O	C	O	C	O		C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C
<u>Mucor hiemalis</u>	-	-	10	10	20	20	-	-	20	20	-	-	-	-	-	-	-	-	10	10	-	-	10	10	-	-	10	10	-	-	10	10	F
<u>Rhizopus nigrigans</u>	10	10	10	10	-	-	10	20	20	20	-	-	-	-	-	-	-	-	10	10	-	-	10	10	-	-	-	-	-	-	-	-	F
<u>Chaetomium bostrychodes</u>	-	-	-	-	-	-	10	10	10	10	10	10	-	-	-	-	-	-	20	20	-	-	20	20	-	-	20	20	-	-	20	20	F
<u>C. globosum</u>	-	-	-	-	-	-	-	-	20	10	-	-	-	-	-	-	-	-	-	-	-	-	10	10	-	-	-	-	-	-	-	-	R
<u>Colletotrichum capsicum</u>	-	-	-	-	-	-	-	-	20	20	10	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Melanospora zamae</u>	-	-	-	-	-	-	-	-	10	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Phoma glomerata</u>	-	-	-	-	-	-	20	10	-	-	10	10	-	-	10	10	-	-	-	-	20	20	30	20	20	20	20	20	20	20	20	20	F
<u>Rhizoctonia solani</u>	-	-	10	20	-	-	20	20	-	-	-	-	-	-	10	10	20	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	O
<u>Alternaria alternata</u>	-	-	10	10	20	10	10	10	20	30	20	10	-	-	10	-	-	-	20	20	40	20	20	20	20	20	50	30	30	30	30	30	D
<u>Arthrinium sp</u>	-	-	-	-	-	-	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
<u>Aspergillus flavus</u>	10	10	-	-	10	20	20	30	20	10	20	20	60	50	-	-	-	-	40	50	20	10	10	10	10	10	10	10	10	10	10	10	D
<u>A. nidulens</u>	20	10	30	20	-	-	20	20	30	10	-	-	10	10	20	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	F
<u>A. niger</u>	-	-	-	-	10	10	20	10	30	40	-	-	20	10	40	30	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	C
<u>Cladoporium cladosporoides</u>	20	10	10	10	-	-	10	10	-	-	-	-	20	20	30	40	40	10	50	40	10	50	40	40	40	40	40	40	40	40	40	40	C

Table 10 (Contd.)

FUNGAL SPECIES	Folding bud		Unfold- ing bud		Young Green Leaves		Matured Green Leaves						Senescent Leaves			Fre- quency								
	MAR		APR		MAY		JUNE		JULY		AUG		SEPT		OCT		NOV		DEC		JAN			
	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O		C	O	C	O	C	O	C	O
<u>C. herbarum</u>	20	20	10	20	-	-	20	10	-	10	20	30	30	60	50	80	100	90	70	100	80	-	-	D
<u>Curvularia lunata</u>	10	20	-	-	-	-	20	10	30	20	-	20	20	10	10	-	-	-	-	-	-	-	-	F
<u>C. pullescens</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	10	-	-	-	-	R
<u>Fusarium moniliforme</u>	-	-	-	-	-	-	10	20	20	20	-	10	10	-	20	10	10	20	10	40	40	20	-	F
<u>Humicola grisea</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	10	-	-	-	-	-	-	-	R
<u>Penicillium funiculosum</u>	10	20	20	20	10	20	30	30	50	60	40	40	60	80	30	40	20	30	40	30	50	70	-	D
<u>P. chrysogenum</u>	-	-	-	-	-	-	-	-	-	10	20	20	20	10	10	-	-	-	-	-	-	-	-	O
<u>Torula herbarum</u>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	10	10	-	-	-	-	R
<u>Trichoderma viride</u>	10	10	-	-	-	-	10	10	20	10	10	30	-	-	10	10	60	80	50	40	90	100	-	C
<u>T. roseum</u>	-	-	-	-	-	-	10	20	20	10	10	-	-	-	-	-	-	-	-	-	-	-	-	O
<u>Verticillium albo-atrum</u>	-	-	-	-	-	-	-	-	20	20	40	30	60	50	30	10	40	40	60	40	50	40	-	C
White sterile mycelia	-	-	-	-	-	-	10	10	20	-	-	-	-	-	-	-	10	10	20	10	10	-	-	O
Orange sterile mycelia	-	-	-	-	-	-	-	-	10	20	-	-	-	-	10	-	-	-	-	-	-	-	-	R

D = Dominant (81 - 100% Frequency), C = Common (61 - 80% Frequency), F = Frequent (41 - 60% Frequency), O = Occasional (21 - 40%), R = Rare (1 - 20% Frequency).

Table 11 - Percentage relative abundance and frequency of phylloplane fungi of *Alnus nepalensis* at two forest stands of different growth stages of leaves isolated by dilution plate technique

FUNGAL SPECIES	Folding bud		Unfolding bud		Young Green Leaves		Mature Green Leaves		Senescent Leaves		Frequency													
	MARCH		APRIL		MAY		JUNE		JULY			AUGUST		SEPTEMBER		OCTOBER		NOVEMBER		DECEMBER		JANUARY		
	O	C	O	C	O	C	O	C	O	C		O	C	O	C	O	C	O	C	O	C	O	C	O
<i>Ascochyta blightens</i>	-	-	8 82	17 85	8 51	5 00	-	2 03	8 88	-	-	-	-	-	-	-	-	-	5 06	-	-	-	-	-
<i>Penicillium</i> sp.	-	-	-	-	-	-	6 34	10 71	-	6 00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Craetophilum nosterhodoc</i>	-	-	-	-	-	-	-	-	7 46	6 00	5 31	3 22	6 06	5 26	-	-	-	-	-	-	-	-	-	-
<i>C. globosum</i>	-	-	-	-	-	-	-	-	5 00	4 44	3 48	-	4 00	-	-	-	-	-	-	-	-	-	-	-
<i>Metanospora zamae</i>	-	-	-	-	-	-	-	-	-	-	2 32	3 44	-	-	-	-	-	-	-	-	-	-	-	-
<i>Phoma glomerata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Leghämoglobin</i>	14 28	11 14	7 14	10 63	-	-	8 02	-	7 44	-	-	-	-	-	8 88	2 38	-	3 38	4 2	6 2	3 6	3 6	3 6	
<i>Ascochyta blightens</i>	-	-	-	-	-	-	-	2 03	8 88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Penicillium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Craetophilum nosterhodoc</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. globosum</i>	-	-	5 8	10 71	-	-	-	-	-	-	-	-	-	14 03	-	-	-	-	-	-	-	-	-	
<i>Metanospora zamae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	5 6	10 52	-	-	-	-	-	-	-	-	
<i>Phoma glomerata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Leghämoglobin</i>	21 42	25 92	17 85	14 9	51 10	0	-	5 7	-	-	3 50	3 50	-	-	-	-	-	-	-	-	-	-	-	
<i>Ascochyta blightens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Penicillium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Craetophilum nosterhodoc</i>	14 28	7 40	8 82	-	6 38	-	-	2 45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>C. globosum</i>	17 85	11 14	11 76	10 71	10 63	10 00	0	2	-	-	5 33	2 6	11 76	16 66	1 75	2 25	3 7	14 00	20 58	17 39	-	-	-	
<i>Metanospora zamae</i>	-	-	8 82	-	17 02	-	12 50	-	-	-	6 30	-	7 84	-	-	-	-	-	-	-	-	-	-	
<i>Phoma glomerata</i>	-	-	-	-	8 51	15 00	7 9	-	-	-	3 00	17 54	3 30	10 04	2 5	16 94	1	24 00	17 24	15 21	-	-	-	
<i>Leghämoglobin</i>	-	-	-	-	-	-	-	-	-	-	8 00	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Ascochyta blightens</i>	-	-	-	-	-	-	-	-	-	-	4 00	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Penicillium</i> sp.	-	-	-	-	-	5 4	7 14	-	-	3 48	3 83	-	3 40	5 88	-	-	-	-	-	-	-	-	-	
<i>Craetophilum nosterhodoc</i>	-	-	-	-	-	-	-	-	5 00	3 48	3 50	4 00	8 77	-	-	-	-	-	-	-	-	-	-	
<i>Phoma glomerata</i>	21 42	20 62	14 7	21 42	12 76	17 50	12 50	19 04	8 35	22 22	3 30	10 35	10 66	15 78	19 60	21 42	14 10	20 33	11 11	16 00	-	-		
<i>Leghämoglobin</i>	10 71	-	11 76	7 14	-	-	6 34	5 35	7 46	-	-	-	-	-	-	21 56	11 90	12 82	6 77	-	-	-		
<i>Ascochyta blightens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Penicillium</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Craetophilum nosterhodoc</i>	-	-	17 85	-	17 5	9 52	12 5	7 40	17 7	6 97	14 51	0 33	8 77	-	-	10 25	15 25	15 87	24 00	15 51	17 39	-		
<i>Phoma glomerata</i>	-	-	-	-	-	-	-	-	-	3 48	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Leghämoglobin</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Ascochyta blightens</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Penicillium alboatrum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>White sterile mycelia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

D = Dominant (81 - 100% Frequency), C = Common (61 - 80%), F = Frequent (41 - 60% Frequency), Occasional (21 - 40%), R = (1 - 20% Frequency)

### Quantitative assessment

Quantitative estimations as done through dilution plate technique accounts for the fungal propagules present on the leaf surface only and ignores the actual activity of fungi which had penetrated the leaves.

The composition of the phylloplane colonizers of both the forest stands at different ages of the leaves as observed by the dilution plate method (Table 11) revealed that fungi like Alternaria alternata, Cladosporium herbarum, Penicillium funiculosum and Trichoderma viride dominated the entire phylloplane region at different age of the leaves. Higher counts of fungal population was observed during summer (rainy) period (August and September) on green matured leaves of Alnus nepalensis of both the forest stands (Fig. 4). Penicillium funiculosum, Alternaria alternata and Aspergillus nidulans were the dominant forms immediately after flushing (Table 11). When the leaves were under going maturation and entered into the senescent stage Cladosporium herbarum, Trichoderma viride and Fusarium moniliforme become the dominant form (Table 11).

In addition, Rhizopus nigricans, Phoma glomerata, Aspergillus flavus, Paecilomyces veruta, Verticillium alboatrum and white sterile mycelia were also frequently

Table 11(b) - Analysis of variance for fungal population of young, mature and senescent stage under two plantations and in different sampling periods.

Sources of variation	F value		
	Young stage	Mature stage	Senescent stage
Plantation	9.73 ns	57.52*	20.24**
Sampling period	84.10*	16.69**	20.65**

\*  $p < 0.01$ ; \*\*  $p < 0.05$ ; ns not significant.

Table 12 - Correlation coefficient (r) for pH, moisture content and fungal population of various stages of Alnus nepalensis leaves at two forest stands.

Sources of variation	D.F.	Fungal Population	
		Open	Closed
pH	9	0.230	0.162
Moisture content	9	0.656**	0.699**

\*, \*\*, \*\*\*,  $p < 0.1, 0.05$  and  $0.01$  respectively.

Without asterisk statistically insignificant at these levels.

occurring species with less percentage frequency of occurrence, other forms though considerably high in number were less frequent and were cultured occasionally (Table 11).

The variation in the fungal population of the young stage of Alnus nepalensis leaves between the plantation was insignificant but statistically significant between different sampling period. The variation in the fungal population between plantation and between different sampling period of mature and senescent stage was statistically significant (Table 11b).

At both the forest stands, the fungal population of both the leaves of Alnus nepalensis showed a positive correlation with moisture contents. However, the correlation of pH with fungal population of both the leaves was not significant (Table 12).

## DISCUSSION

The result obtained from different methods of isolation although gives some picture about the colonization of different fungi on the phylloplane region but they may entice misleading speculations about successional patterns. As there is no single method which can selectively remove the mycelium directly from the leaf surface

for culture purpose. The information gained from all the different techniques used simultaneously was taken as a measure of fungal activity in vivo as suggested by earlier workers (Dickinson, 1971; Sharma et al., 1974).

The mycoflora recorded on leaf surface of both the forest sites in different stages of the leaves, exhibit that these were colonized by almost similar species of fungi. In present study the representatives of Phycomycetes and Ascomycetes fungi were found to be weak colonizers, whereas Deuteromycetes were strong colonizers, showing better adaptability and higher competitive saprophytic ability (Rai, 1973; Garg and Sharma, 1985). An increase in number of fungi was recorded with age of green leaves of both the forest stands which may possibly be due to availability of more nutrients on older leaves (Kumar and Gupta, 1976; Sinha and Dayal, 1983 and Adhikari, 1990). The number of phylloplane mycoflora of matured green leaves was higher than their corresponding senescent leaves possibly due to lower relative humidity and moisture content during winter (November - February).

#### **Qualitative assessment**

Low population on the folded (buds) leaves of Alnus nepalensis to young green leaves was perhaps due to the presence of thick waxy cuticle on the leaves. Turkey

and Morgan (1963) pointed that although young leaves appear delicate and fragile, they are less susceptible to leaching than are the older leaves. An increase in moisture content of the leaves at matured stages encouraged the leaching of nutrient on the surface of the leaves which favoured the growth of fungal population vigorously. Sinha (1971) observed a similar increase in the microflora on older leaves than young leaves of some agricultural crop plants. However, Schoch (1955) stated that mature tissue approaching senescence is very susceptible to leaching. Such leaves with increased nutrient status are more favourable habitat for the growth of the microbes.

The senescent leaves were found to be colonized by Aspergilli, species of Curvularia, Alternaria alternata, Cladosporium cladosporoides, C. herbarum, Chaetomium botrychodes, Fusarium moniliforme, species of Penicillium, Trichoderma viride, Verticillium alboatrum and white sterile mycelia. Mishra and Srivastava (1970a) reported that Alternaria alternata, Aspergillus spp., Cladosporium cladosporoides, Fusarium sp., Phoma sp. and Trichoderma viride were found to be dominant on senescent leaves. The results of the present investigation stand in conformity with the findings of the above workers. ✓

The dominant<sup>2c</sup> of Fusarium moniliforme, Trichoderma

viride, Penicillium spp., Verticillium alboatrum and other fungi (Table 11) in later stages of maturity of leaves indicates their preparatory role for the ensuing senescent stage. All these fungal species have been reported to colonize senescing plant parts (Lamb and Brown, 1970; Bainbridge and Dickinson, 1972 and Ericker, 1976). Dickinson (1976) also advocated that increased activity of saprophytes on senescent leaves may be primarily a result of alteration in leaf physiology.

The presence of Cladosporium herbarum with fairly high frequency on Alnus nepalensis leaves immediately after flushing especially on senescent leaves reveals that lower atmospheric temperature, scanty rainfall and presence of higher amount of dew on leaf surface probably favoured the growth of this species during winter (Tables 10 and 11). The fungus is of wide occurrence in colder regions as observed in the present studies and it is a major constituent of the mycoflora of plant surface (Mishra and Srivastava, 1970a). The dominant of Cladosporium sp. on the leaf surface has been reported by many workers (Kerling, 1958; Dickinson, 1965; Hislop and Cox, 1969; Sinha, 1971; Mishra and Srivastava, 1970a, 1971b, Di Menna, 1971). An occasional higher counts in the fungal population on senescent leaves was recorded in winter due to the increase in the population of Cladosporium herbarum.

In addition, Alternaria alternata, Cladosporium herbarum, Penicillium sp. and other forms mentioned earlier are fast growing, heavily sporulating and some of them produce deeply pigmented conidia or pigmented mycelia which account for their successful survival in adverse circumstances (Tewari, 1973). Hudson (1971) also observed that most of the forms mentioned above as residents were more adapted to adverse conditions and reported them to be the primary colonizer of the senescent leaves.

The results of the present investigation indicate that with minor exceptions the dominant members of different successional series are almost the same in both the forest stands of Alnus nepalensis. Alternaria alternata, Cladosporium spp., Mucorales and Phoma glomerata were found to be the first colonizers of leaves and most of them remained throughout the various growth stages of the leaves similar to the findings of Hogg and Hudson (1966), Lindsey and Pugh (1976) and Tsuneda and Skoropod (1978).

#### **Quantitative assessment**

Quantitatively the fungal population in the beginning when the leaves were in bud and young stages was low increased gradually with the age of the leaves whereas the fungal population was very low during the month of

December and January. It was due to extreme desiccation and low relative humidity in these months. The highest number of leaf surface fungi was recorded in the month of August and September in matured green stages of both the forest stands of Alnus nepalensis probably due to favourable temperature, relative humidity and moisture content of leaves (Kangsen and Kapooria, 1979; Mathur et al., 1985). However, at senescent stage in the month of November onward there is an increase in percentage frequency of saprophytic fungi like Fusarium moniliforme, Trichoderma viride, Penicillium spp., Verticillium albo-atrum and white sterile mycelia which indicates their preparatory role for the ensuing senescent stage. The marked increase at maturity might be due to the availability of the maximum surface area, maximum atmospheric moisture and an increase at senescence can be explained on the basis of increased dead tissues which provides niches to saprophytes which multiply there. ✓

pH of the leaves which varied from folded to senescent stage (Fig. 6) did not affect much the pattern of colonization of Alnus nepalensis leaves by different fungi. But many workers have suggested that pH of the leaves may play an important role in the distribution of fungi both quantitatively and qualitatively (Last and Deighton, 1965; Dickinson, 1967; Rao and Manoharachary, 1981).

Meteorological factors such as atmospheric temperature, humidity and rain are important factors (Fig. 1) influencing the microorganisms on the leaf surface and is in conformity with the findings of earlier workers (Gregory, 1950, 1961; Last, 1955a; Hirst and Stedman, 1963; Mishra, 1968; Heuvel, 1970; sinha, 1971; Ahuja and Payak, 1981; Kumar and Singh, 1981; Pennycook and Newhook, 1981 and Hayes, 1982). The total number of microorganisms and also the specific composition of the microflora were affected by the weather conditions some being present throughout the growing period whereas others were exclusively associated with a particular set of climatic factors (Mishra and Srivastava, 1970a; Sinha, 1971; Sharma and Mukherji, 1976; Garg *et al.*, 1978; Khara and Singh, 1981; Hayes, 1982 and Adhikari, 1990).

Thus the distribution of fungi on leaf surface was dependent mainly on host species, leaf maturity and weather changes. The number of fungi increased with increasing leaf age (Fig. 4) similar to the finding of earlier workers (Hudson, 1962; Last and Deighton, 1965; Dickinson, 1967; Lamb and Brown, 1970). The increase in fungi could be related to change in the nutritional status of leaves with age because there is often an increase in nutrient containing leachates from aerial plant parts (Webster

and Dix, 1960; Tukey, 1971 and Sinha, 1971). When leave senesce there are numerous changes in their morphology, physiology and biochemistry which may account for the upsurge of fungal populations (Narulla and Mehrotra, 1981). The increasing trend of total fungal population may be due to weathering of surface waxes (Forester, 1977), decreased production of phyto-alexins (Bailey, 1971) and the expanding leaves may provide more surface areas to be occupied by the microorganisms. In addition, prevalence of significance superficial colonist of fungi on different age of the leaves reflected the abundance of the species in the air during different sampling periods (Gregory and Hirst, 1957).

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CHAPTER III

SEASONAL CHANGES IN THE POPULATION OF MICROBES  
AND ISOPODS DURING DECOMPOSITION OF  
LEAF LITTER OF ALNUS NEPALENSIS

## INTRODUCTION

Decomposition is a consequence of the growth and succession of microbial communities. Decomposition of different plant debris, notwithstanding their peculiarities, follows a general pattern i.e. change in chemical composition, a gradual decrease in the weight loss and change in the succession of soil organism. Microbial decomposition of litter and rate of nutrient release are dependent on the temperature, moisture and substrate quality (Kshattriya et al., 1992).

The succession of microorganisms in litter decomposition is primarily dependent on their ability to utilize available organic compounds and adopt themselves to the changed environmental conditions. It plays an important role in the functioning of the ecosystems and cycling of the mineral nutrients particularly when decomposers are an important biotic component. The sequence of fungal succession upon a natural substratum reflects a complex interaction of nutritional relationship between each individual and of substratum along with competition between different fungi (Macauley and Thrower, 1966). Fungi are the most important agents of decomposition amongst the various organisms involved (Webster, 1970).

The role of fungi in decomposing process has been reviewed by Hudson (1968), Pugh (1980), Wicklow and Carroll (1982) and Kjoller and Struwe (1982) but only few studies have considered the role of bacteria (Sundman, 1970; Hisset and Gray, 1973; Kauri, 1983; Shelley et al., 1983 and Kjoller et al., 1985). This is mainly due to their secondary role in the decomposition of litter. However, bacterial population plays an important role in the turn over of nitrogen (Struve and Kjoller, 1985).

The biotic relationship of organisms living in the soil have a great influence on the twin environmental processes of nutrient cycling and energy flow and have been subjected to considerable study. Despite this fact many aspects of soil biology, especially the trophic inter-relationship of the several hundred species of arthropods which may be found in a square metre of soil are little understood. A considerable amount of information has been accumulated concerning the behavioral responses of woodlice to micro climate conditions in the laboratory (Cloudsley-Thompson, 1951; Edney, 1954 and Peitsalmi, 1974).

Studies have shown that soil arthropods are non-randomly distributed in the soil. A review of the factors causing this non-randomness indicated that the patchy distribution of either food or soil water was the most

likely cause of these aggregation (Usher, 1976). An important question that remains unanswered is the relation between organic matter input and soil fauna populations.

The nature and number of soil organisms play a vital role in soil productivity because of their contribution to organic matter accumulation, nutrient cycling and structural stability. The studies of distribution of soil fauna (Santos et al., 1978 and Franco et al., 1979) and litter disappearance soil fauna relationship (Santos and Whitford, 1981; Whitford et al., 1982) suggest that for soil organisms, an adequate energy and nutrient supply in the form of plant litter is more important than water as a factor affecting numerical responses of soil fauna.

Studies on the interaction between microbes and isopods during litter decomposition in subtropical forests are lacking. Therefore, the present investigation was carried out to study the seasonal changes in the succession of fungi, bacteria and isopods. Their relationship with environmental conditions, litter quality and interdependence on each other was also studied during the progressive decomposition of alder litter under field condition.

## **MATERIALS AND METHODS**

### **Selection of site**

Two sites of alder forest stands i.e., open and

closed stands were selected for the present study at Upper Shillong at an altitude of 1500 m MSL, latitude 25°34'N and longitude 91°56'E. The age of the plant was about 20 and 35 years in the open and closed forest stand respectively. The tree density was 180 per hectare and 1160 per hectare in earlier and latter stand respectively. The dominant tree species in both the forest stands was Alnus nepalensis which was selected for the present investigation.

### Succession of microbes and isopods on alder litter

#### (i) Collection of the litter samples

Nylon bag technique of Bocoek et al. (1960) was followed to assess the saprophytic activity of microflora (fungi and bacteria) and isopod population during the decomposition of leaf litter. Nylon litter bags (20 x 20 cm size) of normal mesh bag (1 mm pore) which exclude the soil macrofauna were spread randomly on the forest floor and coarse mesh bags (3 mm pore) <sup>which</sup> allowed soil isopods access to litter but minimised fragmentation loss, were buried randomly at 2-5 cm depth on the sites as described above. The two types of litter bag were collected aseptically at monthly intervals and brought to the laboratory and kept at 4°C till further processing (Ruscoe, 1971). Three litter bags collected on each sampling were used

for the assessment of the microflora and isopods. The remaining ones were used for the determination of pH, moisture content, dry weight and nutrient contents of litter.

#### **(ii) Extraction of soil isopods**

The buried litter (2-5 cm depth) of coarse mesh bags and as many 0.2 m<sup>2</sup> soil samples of depth 0-2 cm and 5-10 cm with duplicate samples were taken during each time of collection at each site and was emptied into separate Tullgren funnel to extract the associated isopods in litter bags and soil cores respectively. Extracted isopods were collected in 70% ethanol, counted and identified. After extraction was completed each of the litter bags was stored at 4°C until analyzed for nutrients.

#### **(iii) Isolation of microflora from the leaf litter**

Dilution plate method (Waksman, 1922) was followed for the isolation of fungi and bacteria from the decomposing leaf litter. 1 g of leaf litter was cut into small pieces (1.0 cm) with the help of sterilized scissor and transferred into a 250 ml sterilized conical flask containing 100 ml of sterilized distilled water. Litter suspension of 1:100 dilution was then obtained. 10 ml of this litter suspension was transferred aseptically into another sterilized 250 ml conical flask containing 90 ml of sterilized

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distilled water to get a dilution of 1:1000. The process was repeated once more to get a suspension of 1:10,000 dilution. For the estimation of fungi and bacteria 1:1000 and 1:10,000 dilutions respectively were found suitable for counting their population. Enumeration of fungal and bacterial population was done on Rose Bengal Agar (Martin, 1950) and Nutrient Agar medium (Difco-manual, 1953) respectively.

0.5 ml of litter suspension was transferred aseptically from 1:1000 and 1:10,000 dilution into each of the sterilized petri plate containing 15 ml of sterilized solidified Rose Bengal Agar and Nutrient Agar media for fungi and bacteria respectively. The plates were shaken gently in order to disperse the suspension uniformly over the surface of the media. Three replicates were maintained in each case. The entire isolation procedure was carried out inside a Laminar flow chamber. For the isolation of fungi and bacteria, the plates were incubated at  $25 \pm 1^{\circ}\text{C}$  and  $30 \pm 1^{\circ}\text{C}$  in B.O.D. incubator for five days and 24 hours respectively. ✓

The total number of fungi and bacteria was calculated taking into consideration the dilution factor and moisture content of the litter. The pure cultures of fungi were maintained on slant of Malt Extract and Czapek's

Dox Agar media. The fungi were identified by consulting the manuals of Gilman (1957), Subramanian (1971) and Barnett and Hunter (1972).

#### (iv) Relative abundance of fungi

The percentage relative abundance of fungi was calculated by using the following formula:

$$\text{Relative abundance (\%)} = \frac{\text{Total number of colonies of the individual species}}{\text{Total number of colonies of all the species}} \times 100$$

#### (v) Species diversity of Isopods

Species diversity (Also called species heterogeneity) a characteristics unique to the community level of biological organization was analysed using the following measures.

#### Margalef's Index

The simplest measure of species diversity is the number of species or the species richness, which was calculated after Margalef (1968) as modified by Brower and Zar (1977).

$$D_a = \frac{(S - 1)}{\text{Log}N}$$

Where  $D_a$  = Margalef's index

S = Number of species, and

N = Total number of individuals.

**(vi) Determination of pH and moisture content of the litter and soil samples**

pH of the decomposing leaf litter samples during different sampling periods was determined with the help of a digital pH meter. 10 g of leaf litter was mixed in distilled water in a ratio 1:5. The litter was ground and left for 1 hour and then the pH of the resulting extract was read with a digital pH meter.

The moisture content of the decomposing litter was determined by drying 1 g of litter at 80°C for 24 hours.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where,  $W_1$  = Initial weight of the litter.

$W_2$  = Weight of the litter after drying at 80°C in oven.

**(vii) Soil and air temperature**

Temperature were measured by an ordinary centigrade thermometer (0° - 100°C). Air temperature was taken at 1 m height above the ground. Soil temperature was measured from soil core (0-2 cm) litter depths (2-5 cm) and 5-10 cm soil depths during each time of collection.

**(viii) Collection of data on climate of the study sites**

Data on the climate of study sites were collected

from Meteorological Office, Shillong and are represented in figure (1).

#### (ix) Statistical Analysis of the data

The coefficient of correlation ( $r$ ) was calculated using Karl Pearson's Coefficient of correlation (Zar, 1974).

### RESULTS

#### Microbial Population

Seasonal changes in the population of bacteria and fungi during leaf litter decomposition of Alnus nepalensis were observed (Figs. 7 and 8). Bacterial population was higher compared to fungal population in both the forest stands. It showed a steady increase with the progress of litter decay. Initially, lower bacterial count was recorded in fine mesh bag (1 mm) from both the stands which increased consistently from May onwards and attained a peak in June with a slight decrease in July and August which increased significantly to a maxima in September exhibiting a second peak. Thereafter, the population decreased towards the end of the decomposition. Similarly, in coarse mesh bag (3 mm) the bacterial peak was observed in September after which it decreased to a low level towards the end of the process. The bacterial count in both types of mesh bags was remarkably low in winter.

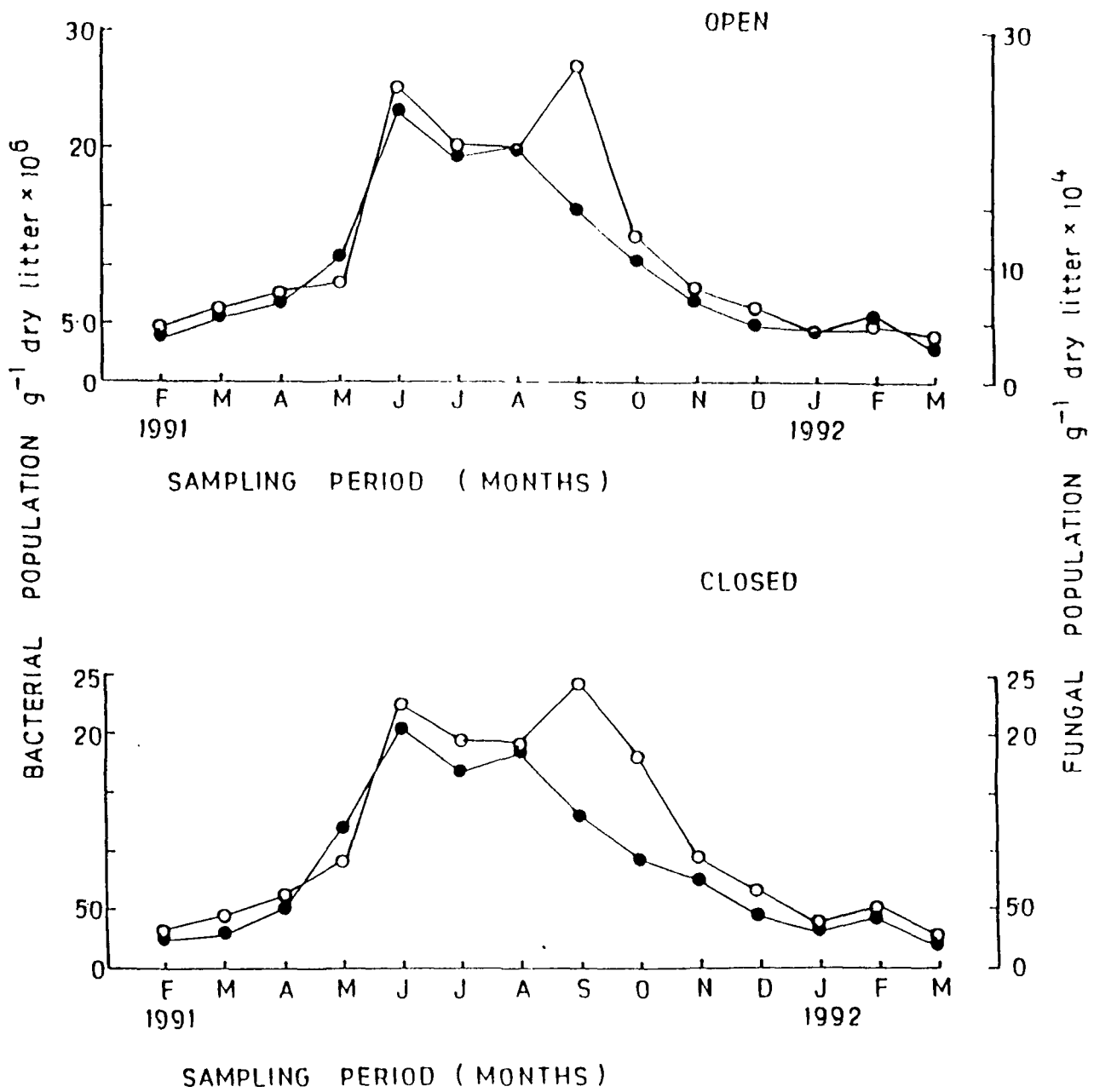


Fig 7. Monthly variation in fungal (●—●) and bacterial (○—○) population of leaf litters at two forest stands of Alnus nepalensis

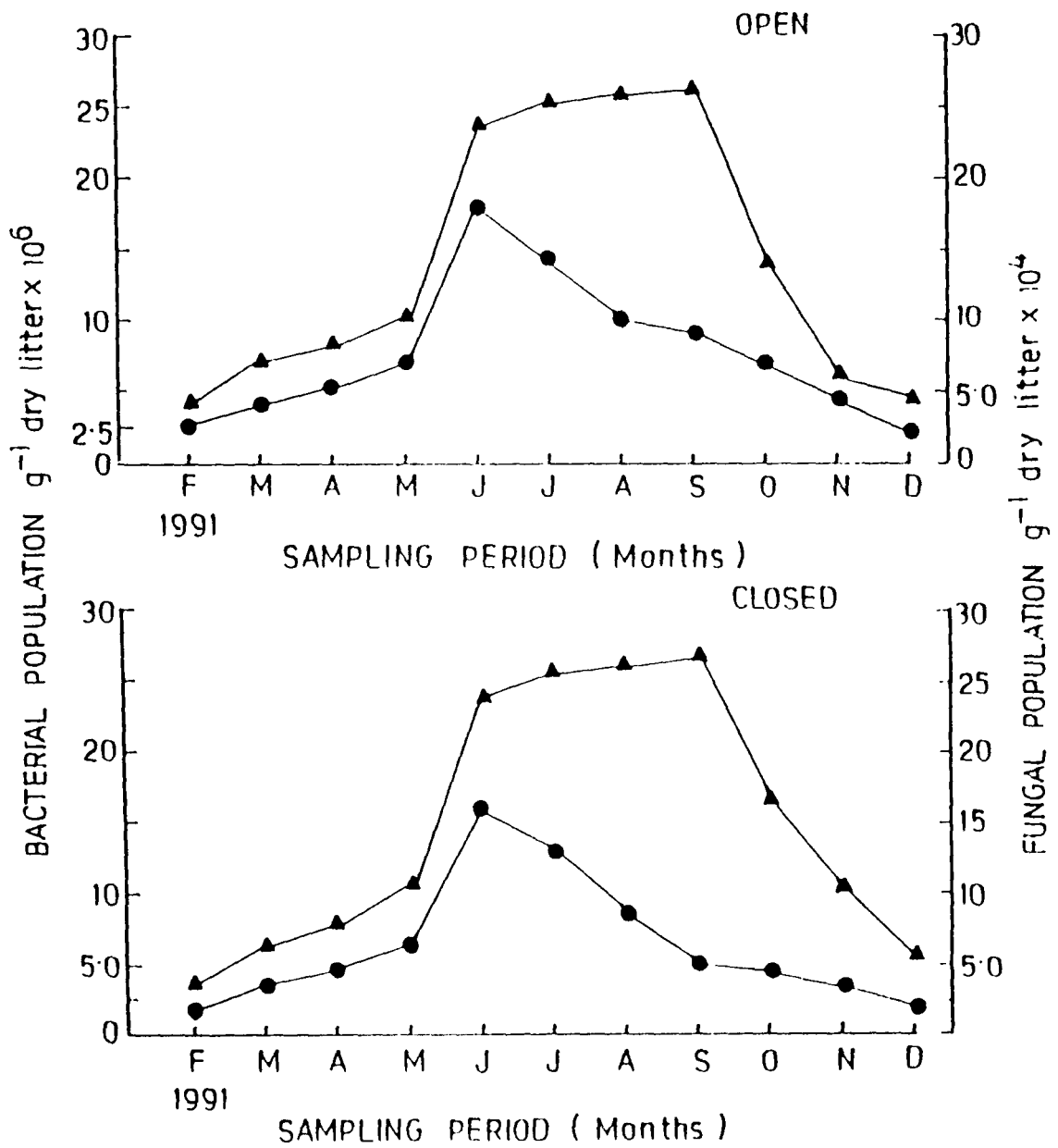


Fig 8. Monthly variation in fungal (●—●) and bacterial (▲—▲) population of buried leaf litters at two forest stands of Alnus nepalensis.

Bacterial population showed a significant positive correlation with moisture content and pH of the leaf litter (Tables 17 and 18).

The fungal population exhibited more or less similar trend of monthly variation in both the forest stands. However, higher number of fungi was associated with leaf litter in open forest stand as compared to closed forest stand. The fungal population was minimum in the fresh litter which increased with the progress of litter decomposition and subsequently decreased towards the end of the decomposition in both the forest stands.

Two peaks in fungal population were recorded in fine mesh bag (1 mm) of both the stands. The first peak of fungi was observed in June after which it decreased in July and again exhibited a second peak in August. Similarly, in coarse mesh bag (3 mm) the fungal peak was observed in June in both the stands. Like bacteria, the fungal population too dropped sharply towards the end of the decomposition in both the cases.

Altogether 19 fungal species were isolated from leaf litter of both the stands, Absidia cylindrospora, Mucor hiemalis, Rhizopus stolonifer, Pythium sp., Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium

Table 13 - Distribution of percentage relative abundance of fungal species of Alnus nepalensis leaf litter of open forest stand during 1991-1992.

FUNGAL SPECIES	F	M	A	M	J	J	A	S	O	N	D	J	F	M
<u>Absidia cylindrospora</u>	6.9	7.2	-	-	-	6.6	-	5.9	-	6.0	-	7.2	-	-
<u>Mucor hiemalis</u>	5.5	9.2	9.8	-	-	-	8.3	6.6	-	-	3.5	-	8.8	-
<u>Rhizopus stolonifer</u>	11.1	10.3	-	8.1	5.1	-	10.7	-	7.8	-	8.2	-	7.7	7.6
<u>Pythium</u> sp	-	-	-	7.5	6.8	11.1	9.5	-	6.2	-	-	5.4	10.0	10.2
<u>Alternaria alternata</u>	-	-	-	5.6	4.5	-	-	5.1	-	-	7.0	-	-	-
<u>Aspergillus flavus</u>	8.3	11.3	6.2	6.2	8.5	-	13.0	15.5	13.3	12.9	-	10.9	6.6	12.8
<u>A. niger</u>	4.1	6.1	10.7	-	7.4	7.7	-	7.4	8.6	8.6	-	-	-	10.2
<u>Cladosporium cladosporoides</u>	9.7	-	7.1	6.2	-	8.8	7.1	-	11.0	6.8	-	9.0	5.5	-
<u>Fusarium oxysporum</u>	-	-	8.0	9.3	9.7	10.0	5.9	-	-	7.7	9.4	7.2	4.4	-
<u>Curvularia lunata</u>	-	7.2	-	4.3	-	4.4	4.7	-	-	-	5.8	-	-	-
<u>Paecilomyces</u> sp	-	-	3.5	-	3.4	6.6	-	4.4	-	-	4.7	-	3.3	-
<u>Penicillium chrysogenum</u>	13.8	14.4	18.7	19.3	21.1	15.5	17.8	14.0	16.5	14.6	17.6	14.5	16.6	15.3
<u>P. citrinum</u>	-	-	4.4	5.0	8.0	5.5	-	8.8	-	4.3	10.5	10.9	-	10.2
<u>Trichoderma viride</u>	20.8	25.7	22.3	21.8	18.2	17.7	16.6	17.7	19.6	18.1	20.0	21.8	20.0	17.9
<u>T. harzianum</u>	9.7	5.1	-	-	4.0	-	-	8.1	11.8	11.2	11.7	12.7	10.0	5.1
<u>Verticillium</u> sp	6.9	-	5.3	3.7	2.8	-	-	3.7	-	5.1	-	-	6.6	7.6
<u>Geotrichum candidum</u>	-	-	-	-	-	3.3	-	-	4.7	2.5	1.1	-	-	-
<u>White sterile mycelia</u>	2.7	3.0	-	2.5	-	2.2	2.3	2.2	-	1.7	-	-	-	2.5
<u>Black sterile mycelia</u>	-	-	3.5	-	-	-	3.5	-	-	-	-	-	-	-

Table 14 - Distribution of percentage relative abundance of fungal species of Alnus nepalensis leaf litter of closed forest stand during 1991-1992.

FUNGAL SPECIES	F	M	A	M	J	J	A	S	O	N	D	J	F	F
<u>Absidia cylindrospora</u>	-	6.1	6.1	5.3	4.8	-	-	-	5.4	-	-	-	2.3	3.3
<u>Mucor hiemalis</u>	6.6	8.1	-	6.0	-	6.2	11.2	5.6	-	7.6	-	5.7	-	10.0
<u>Rhizopus stolonifer</u>	-	10.2	-	6.8	-	9.2	-	-	-	3.9	5.3	-	9.4	6.6
<u>Pythium sp</u>	-	-	8.0	4.5	8.2	7.6	5.0	8.8	-	-	-	-	10.5	-
<u>Alternaria alternata</u>	-	-	-	-	8.9	6.1	8.7	-	-	-	-	-	-	-
<u>Aspergillus flavus</u>	11.1	4.0	12.0	-	6.2	-	10.0	10.4	12.7	12.0	-	-	7.0	13.3
<u>A. niger</u>	8.8	12.1	4.0	8.3	2.0	3.0	-	8.0	7.2	8.7	4.0	-	5.8	-
<u>Cladosporium cladosporoides</u>	-	-	9.3	7.5	-	4.6	-	4.0	9.0	9.8	10.6	11.5	-	-
<u>Fusarium oxysporum</u>	13.3	-	-	9.0	6.8	10.7	-	10.4	10.0	-	9.3	7.6	11.7	10.0
<u>Paecilomyces sp</u>	-	2.0	-	-	2.0	-	3.7	4.8	3.6	-	2.6	-	-	-
<u>Penicillium chrysogenum</u>	17.7	18.3	17.3	18.9	22.0	16.9	18.7	16.8	14.5	10.9	16.0	15.3	12.9	16.6
<u>P. citrinum</u>	-	6.1	10.6	3.7	4.1	-	7.5	-	6.3	5.4	8.0	13.4	4.7	-
<u>Trichoderma harzianum</u>	15.5	-	5.3	3.7	7.5	-	2.5	8.0	8.1	13.1	12.0	9.6	8.2	-
<u>T. viride</u>	20.0	22.4	21.3	20.4	22.7	20.0	17.5	16.0	18.1	19.7	24.0	23.0	21.1	23.3
<u>Verticillium sp</u>	-	-	-	3.0	2.7	7.6	7.5	3.2	-	6.5	4.0	9.6	-	6.6
<u>Geotrichum candidum</u>	-	4.0	1.3	-	-	-	1.2	1.6	-	2.1	2.6	-	3.5	6.6
White sterile mycelia	6.6	6.1	4.0	2.2	-	4.6	6.2	-	4.5	-	-	3.8	-	-
Black sterile mycelia	-	-	-	-	1.3	3.0	-	2.4	-	-	1.3	-	2.3	-

Table 15 - Distribution of percentage relative abundance of fungal species of buried leaf litter of Alnus nepalensis in closed forest stand during 1991-1992.

FUNGAL SPECIES	F	M	A	M	J	J	A	S	O	N	D
<u>Mucor hiemalis</u>	-	11.86	8.82	-	-	-	11.29	15.38	7.69	-	-
<u>Rhizopus stolonifer</u>	7.5	6.77	-	-	-	7.69	9.67	7.69	-	-	5.88
<u>Absidia cylindrospora</u>	-	-	-	-	5.78	-	-	-	-	-	-
<u>Pythium sp</u>	-	-	5.88	8.43	8.26	8.97	-	11.53	-	-	-
<u>Aspergillus flavus</u>	15.0	-	-	8.43	8.26	8.97	-	11.53	-	-	-
<u>A. niger</u>	10.0	8.47	14.70	14.45	8.26	-	-	19.23	17.94	11.32	11.76
<u>Cladosporium cladosporoides</u>	12.5	-	10.29	-	4.95	-	-	-	10.25	9.43	14.70
<u>Fusarium oxysporum</u>	-	10.16	11.76	10.84	13.22	12.82	-	-	-	13.20	-
<u>Penicillium chrysogenum</u>	25.00	18.64	17.64	19.27	14.87	20.51	19.35	26.92	15.38	16.98	26.47
<u>P. citrinum</u>	-	6.77	4.41	7.22	6.61	11.53	8.06	-	7.69	-	-
<u>Faecilomyces sp</u>	-	-	7.35	-	4.13	6.41	9.67	-	-	3.77	-
<u>Trichoderma viride</u>	17.5	22.03	16.17	18.07	22.31	16.66	20.96	19.23	25.64	26.41	23.52
<u>T. harzianum</u>	7.5	11.86	2.94	9.63	7.43	-	12.90	-	7.69	13.20	11.76
White sterile mycelia	5.0	3.38	-	3.61	4.13	5.12	-	-	2.56	-	5.88

Table 16 - Distribution of percentage relative abundance of fungal species of buried leaf litter of Alnus nepalensis in open forest stand during 1991-1992.

FUNGAL SPECIES	F	M	A	M	J	J	A	S	O	N	D
<u>Mucor hiemalis</u>	6.81	10.14	3.75	9.37	-	-	8.69	5.55	-	-	-
<u>Rhizopus stolonifer</u>	9.09	7.24	6.25	10.41	-	-	6.52	-	-	-	-
<u>Absidia cylindrospora</u>	4.54	5.79	-	7.29	4.76	5.45	-	-	5.17	8.82	-
<u>Pythium</u> sp	-	-	-	6.25	6.80	10.00	-	7.40	-	-	-
<u>Aspergillus flavus</u>	9.09	-	8.75	12.50	8.84	-	8.69	11.11	8.62	7.35	-
<u>A. niger</u>	-	10.14	10.00	-	5.44	3.63	-	3.70	6.89	5.88	4.76
<u>Cladosporium cladosporioides</u>	-	-	7.50	6.25	-	7.27	7.60	7.40	-	11.76	14.28
<u>Fusarium oxysporum</u>	13.63	-	6.25	5.20	10.88	12.72	13.04	14.81	10.34	-	11.90
<u>Penicillium chrysogenum</u>	15.90	17.39	18.75	14.58	14.96	15.45	17.39	22.22	22.41	17.64	16.66
<u>P. citrinum</u>	6.81	8.69	6.25	-	6.12	5.45	5.43	-	-	-	-
<u>Paecilomyces</u> sp	-	7.24	7.50	-	4.76	8.18	6.52	5.55	12.06	8.82	11.90
<u>Trichoderma viride</u>	20.45	14.49	11.25	16.66	19.04	14.54	18.47	18.51	18.96	19.11	26.19
<u>T. harzianum</u>	6.81	8.69	7.50	-	2.04	4.54	-	-	5.17	5.88	-
<u>Geotrichum candidum</u>	-	5.79	-	-	6.12	6.36	5.43	-	-	10.29	7.14
<u>Verticillium</u> sp	4.54	-	-	10.41	4.76	3.63	2.17	3.70	6.89	4.41	4.76
White sterile mycelia	2.27	4.34	3.75	4.16	3.40	2.72	-	-	3.44	-	2.38
Black sterile mycelia	-	-	2.50	-	2.04	-	-	-	-	-	-

Table 17 - Correlation coefficient (r) for pH, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial population of Alnus nepalensis litters at two forest stands.

Sources of variation	D.F.	Fungal Population		Bacterial Population	
		Open	Closed	Open	Closed
pH	12	0.749***	0.753***	0.802***	0.855***
Moisture content	12	0.866***	0.868***	0.896***	0.955***
Total nitrogen	12	0.697***	0.754***	0.664**	0.546**
Phosphorus	12	0.699***	0.212	0.679**	0.101
Cellulose	12	0.306	0.303	0.287	0.186
Hemicellulose	12	0.237	0.173	0.201	0.041
Lignin	12	-0.081	-0.032	-0.058	0.143
Total sugars	12	0.081	0.096	0.041	-0.052
Total amino acids	12	0.098	0.069	0.055	-0.067

\*, \*\*, \*\*\*,  $p < 0.1$ , 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 18 - Correlation coefficient (r) for pH, moisture content, total nitrogen, phosphorus, cellulose, hemicellulose, lignin, total sugars, total amino acids, fungal and bacterial populations in buried litters of Alnus nepalensis.

Sources of variation	D.F.	Fungal Population		Bacterial Population	
		Open	Closed	Open	Closed
pH	9	0.558*	0.510	0.636**	0.730**
Moisture content	9	0.797***	0.685**	0.927***	0.971***
Total nitrogen	9	0.570*	0.663**	0.358	0.330
Phosphorus	9	0.577*	0.694**	0.280	0.284
Cellulose	9	-0.082	0.035	-0.294	-0.370
Hemicellulose	9	-0.181	-0.052	-0.368	-0.435
Lignin	9	0.451	0.269	0.751***	0.828***
Total sugars	9	-0.146	-0.030	-0.326	-0.399
Total amino acids	9	-0.211	-0.003	-0.293	-0.346

\*, \*\*, \*\*\*,  $p < 0.1$ , 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

cladosporoides, Fusarium oxysporum, Curvalaria lunata, Paecilomyces sp., Penicillium chrysogenum, P. citrinum, Trichoderma viride, T. harzianum, Verticillium sp., Geotrichum candidum, white and black sterile mycelia were common to both the forest stand (Tables 13, 14, 15 and 16).

P. chrysogenum, F. oxysporum, A. flavus, A. niger and T. viride were dominant species in both types of mesh bag of the two forest stands (Tables 13, 14, 15 and 16). Pythium sp. and F. oxysporum were frequently isolated towards the rainy season. The genera like Geotrichum, Paecilomyces, Verticillium sp., white and black sterile mycelia were isolated occasionally with low percentage of relative abundance in both the stands. Phycomycetes forms in general were poorly represented as compared to the deuteromycetes ones.

In both the cases, positive significant correlation between fungal population, litter moisture content and pH was established (Tables 17 and 18).

### Isopod population

The seasonal variation in the total population of isopods in the two forest stands of A. nepalensis was observed (Fig. 9). A definite seasonal fluctuation in the distribution of different species of Burmoniscus at

different soil depths was clear. Their number was more in the upper litter surface in spring (March-April) and deeper in winter (November-February) and summer (May-October). Maximum population count in buried litter was recorded in the month of July and minimum in December in both the forest stands. While in the lower layer (5-10 cm), maximum population was counted in October in both the forest stands.

The species of Burmoniscus started to migrate from the litter layer to downward into the lower layer of litter-soil (5-10 cm) in October and was deepest in December and January. It moved up in February so that from March to October, it again inhabited the litter layer in both the forest stands. In the summer the species underwent a more marked downward migration than they did in drier months (Fig. 9a).

Among the three species of isopods represented irrespective of the depths, Burmoniscus new species A was most abundant followed by B.n.sp. B. Both the species were recorded throughout the study period in both the forest stands of alder. While B.n.sp. C was confined to the depth of litter layers (2-5 cm). It was represented by low number of individuals in few months of the year (July-Nov) (Fig. 9b).

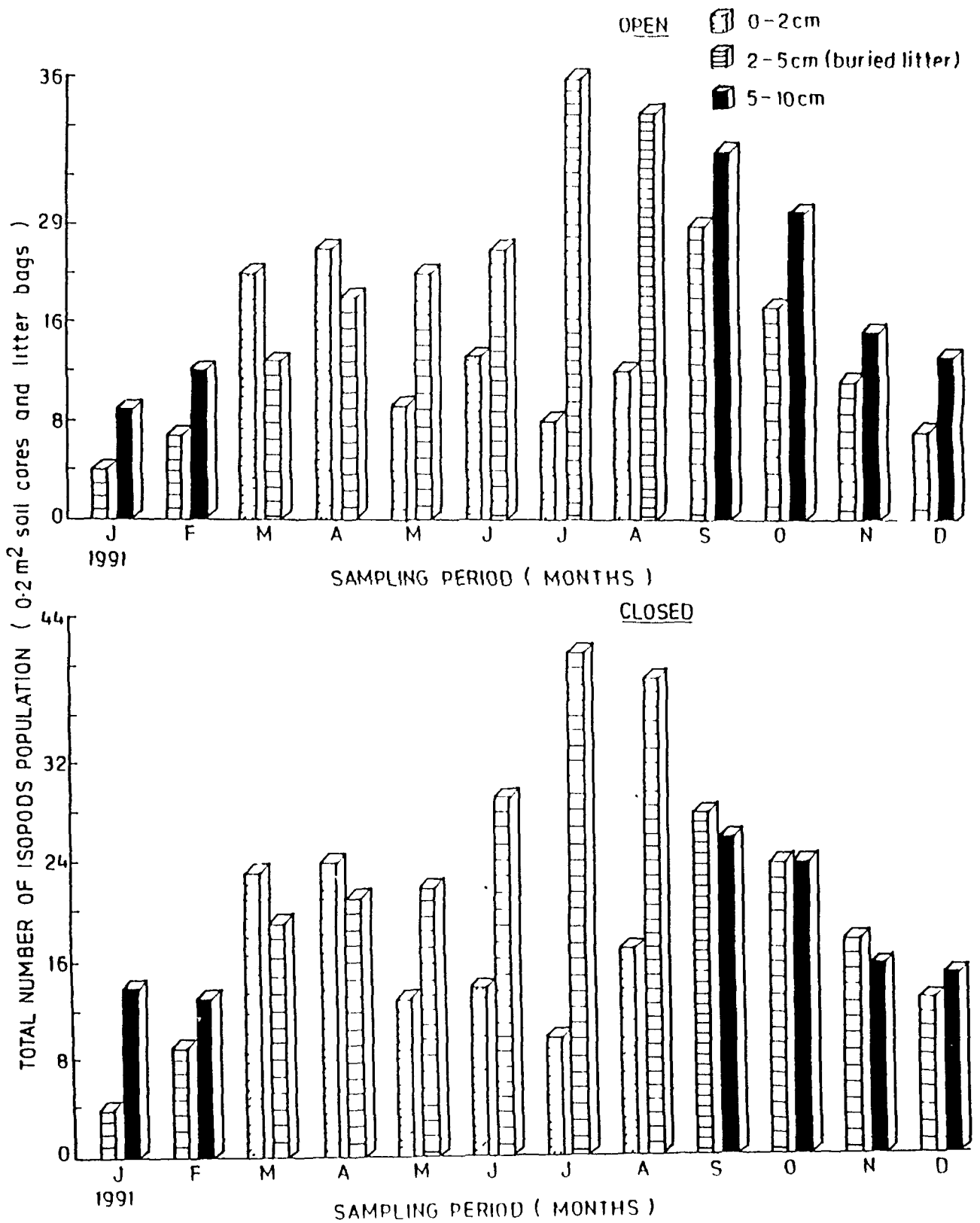


Fig 9. (a) Monthly variation in isopods population at two forest floors of *Alnus nepalensis*.

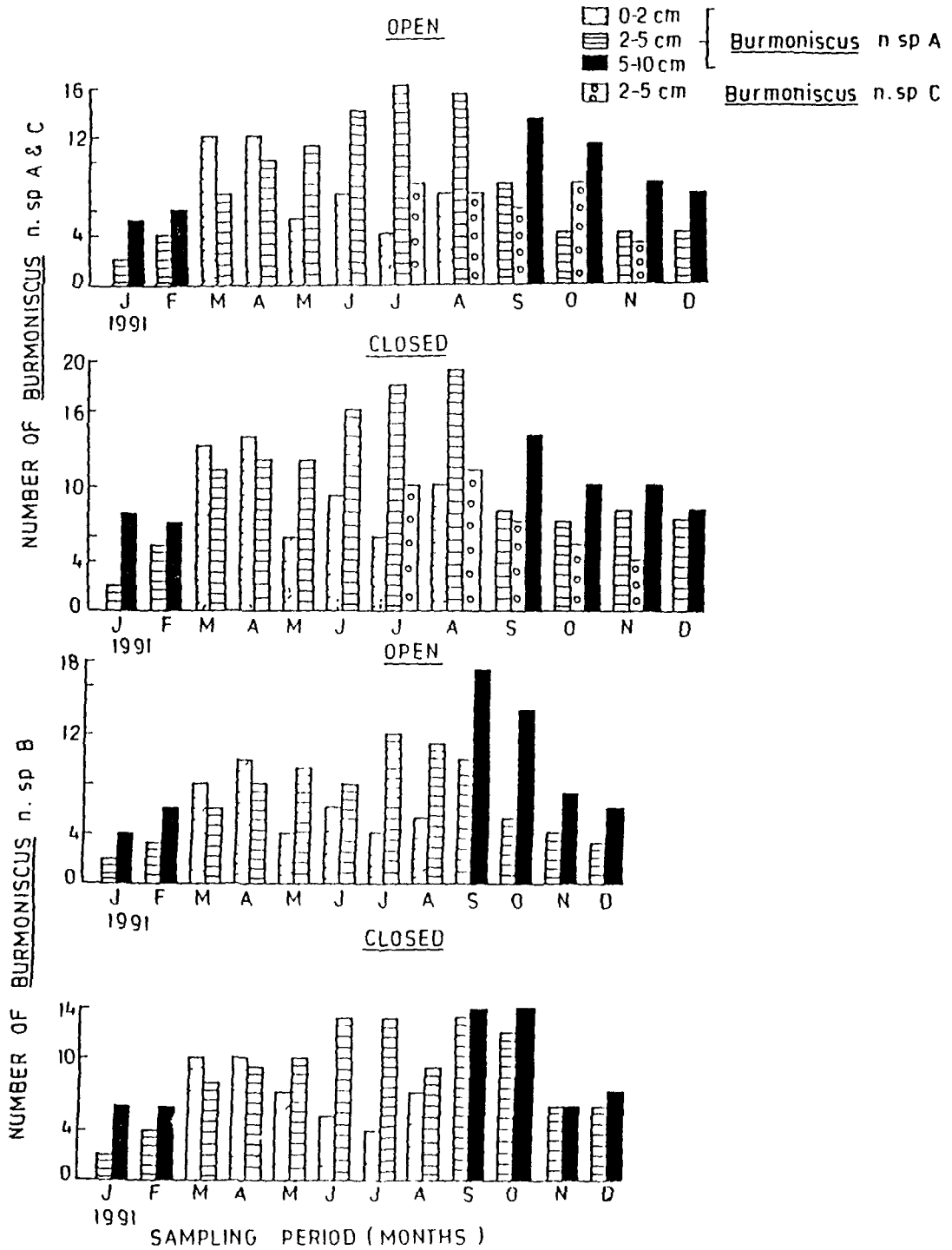


Fig. 9(b) - Monthly variation in Burmoniscus species at two forest floors of *Alnus nepalensis*

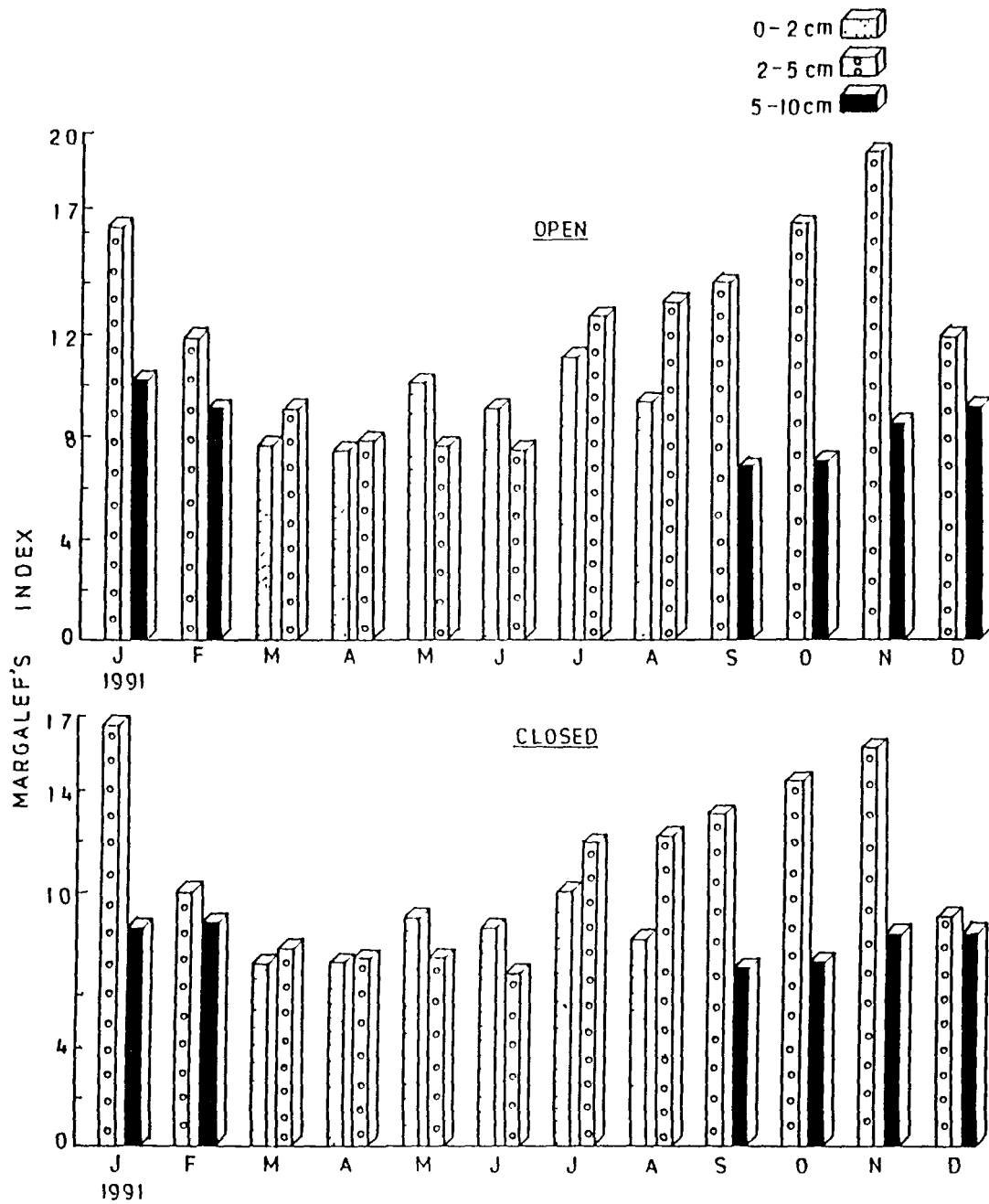


Fig. 9(C) - Monthly variation in the number of species, Margalef's Index of isopods in the 0-2 cm, 2-5 (buried litter) and 5-10 cm depths of the two forest stands

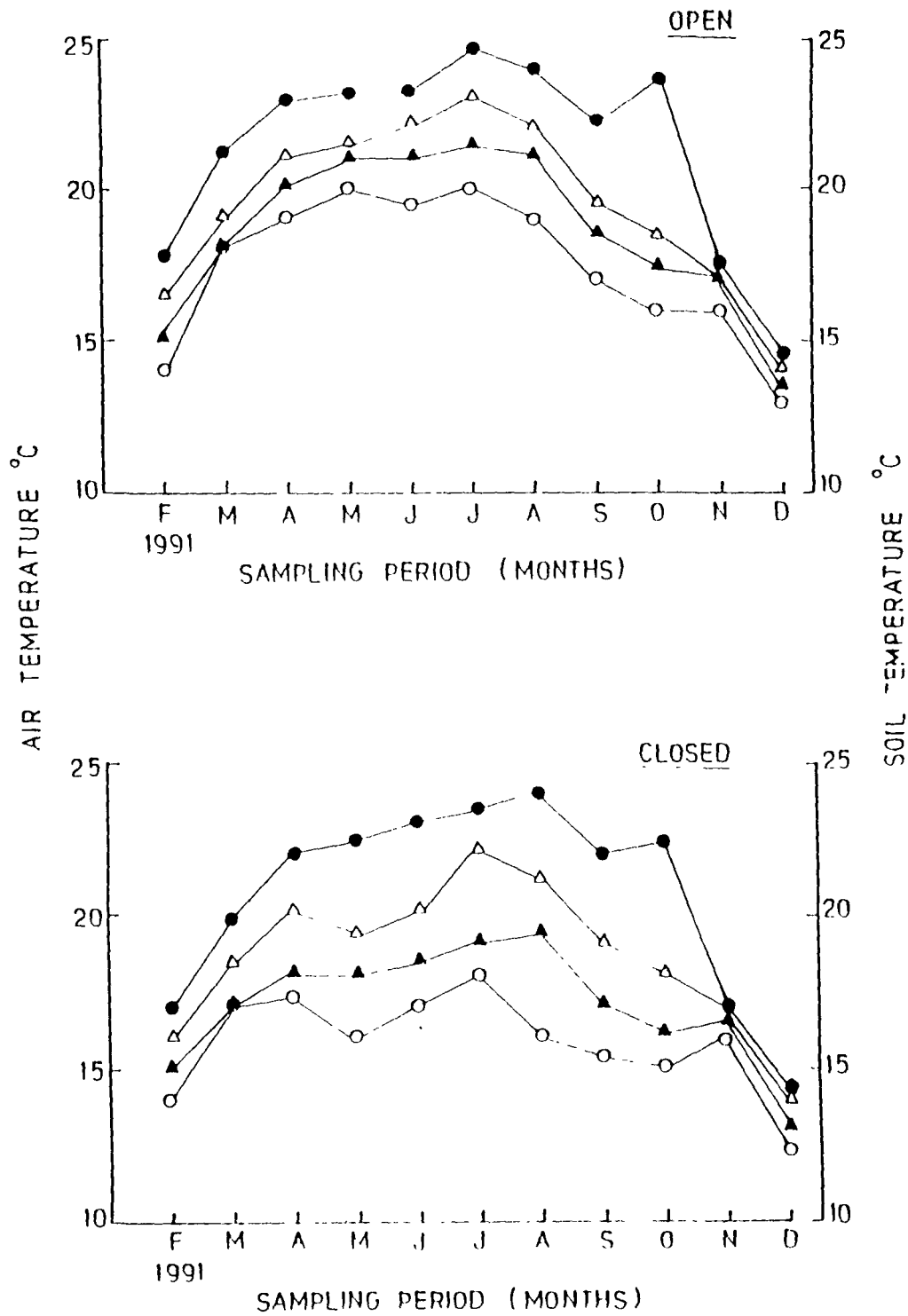


Fig 10. Monthly variation in air temperature (●—●) and soil temperature (△—△ = 0 - 2 cm, buried litter (2-5cm) =▲—▲, O—O = 5-10cm) at two forest stands of Alnus nepalensis.

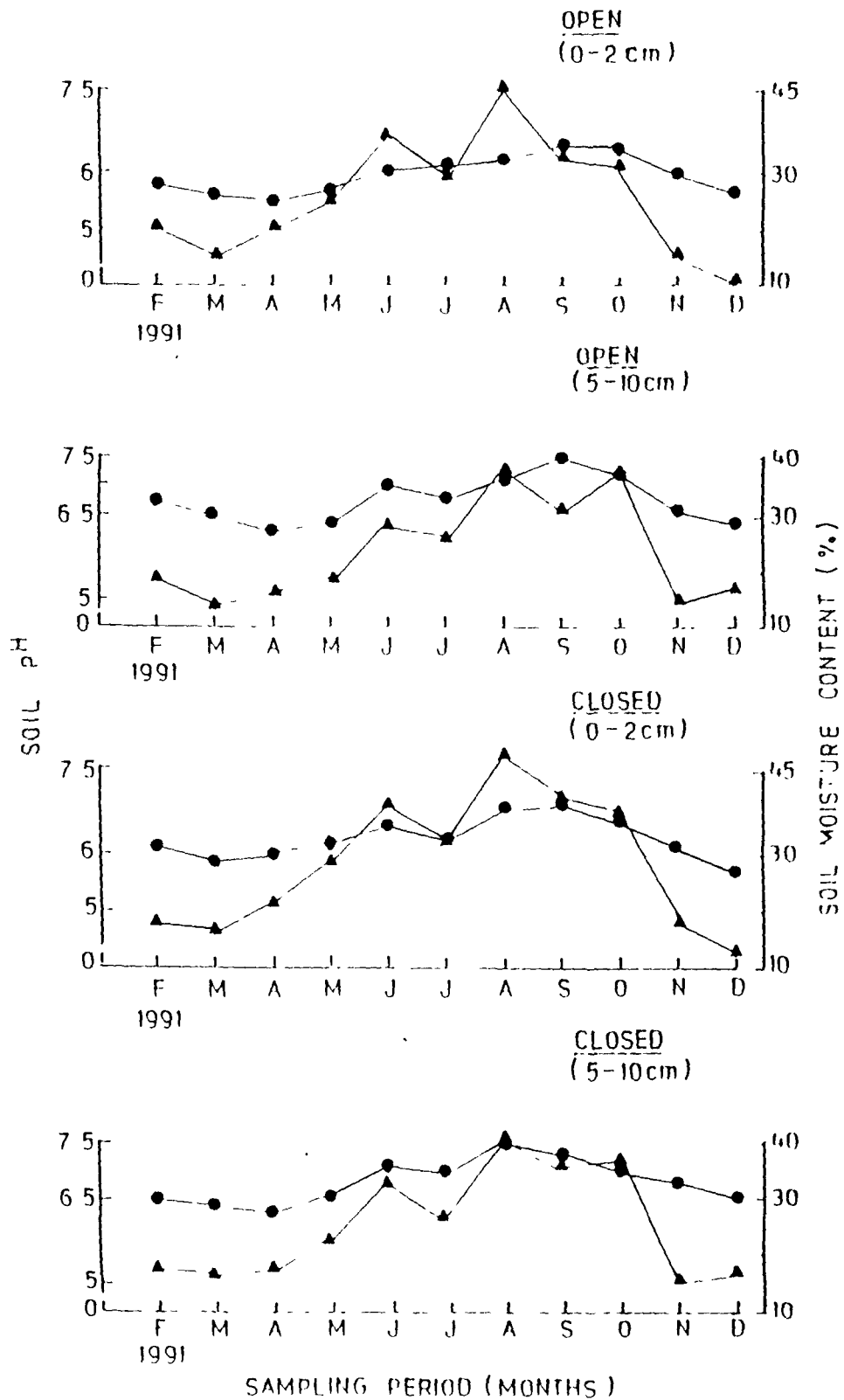


Fig 11. Monthly variation in soil  $P^H$  (●—●) and moisture content (▲—▲) of 0 - 2 cm and 5 - 10cm depths at two forest stands of Alnus nepalensis.

Population of isopod <sup>(was)</sup> decreased during drier and cold season in both the forest stands (Fig. 9a). They remained deeper down during winter, moving up again in warmer season. It did however migrate up into the lower layer (5-10 cm) for a short period during summer season, moving up again with the decline of rainfall. Margalef's index in both the forest stands ranged from 0.72-1.11 in 0-2 cm depth, 0.62-1.92 in buried litter of 2-5 cm depth and 0.68-1.05 in 5-10 cm depth (Fig. 9c).

The results of air and soil temperature, pH and moisture of content (%) of litter are represented in Fig. 10 and 11. In general, the air and soil temperatures of open forest stand were slightly higher than the closed forest stand. Maximum temperature was recorded in July and minimum in December. The closed forest soils always showed higher moisture content than the open forest stand. The availability of moisture tended to decrease with the approach of winter months recorded during Nov-Feb. and also during March and April due to hot and dry weather and tended to increase at both the sites from May onwards with the arrival of precipitation. ✓

At both the sites, the upper layer (0-2 cm) was found to be more acidic and pH increased with greater depth (Fig. 11).

In both the forest stands the isopod population showed a significant positive correlation with fungal and bacterial population of the buried litter of A. nepalensis (Table 19). However, isopod population was not correlated with the absolute weight loss of both the stands.

## DISCUSSION

Low counts of fungi and bacteria in the initial stages of leaf litter decomposition may be due to the unavailability of soluble nutrients which were still in a complex form (Kshattriya, 1990). The peaks of fungi and bacteria in rainy season indicated more availability of the specific substrate (Garrett, 1951 and Nikhra, 1981) and conducive moisture and temperature. However, low microbial count have been attributed to moisture stress and low temperature (Witkamp, 1963; Edmonds, 1979 and Rai and Srivastava, 1982).

The improvement in litter moisture level, moderation of temperature and higher relative humidity (Fig. 1) favoured the build up of the microbial population in June-September (Fig. 7 and 8) particularly bacterial population which was also correlated to rate of decomposition indicating the mobilization of the nutrients from the litter required for their growth (Das, 1980; Kshattriya, 1990). This indicates that the prevailing temperature and moisture

Table 19 - Correlation coefficient (r) for air temperature, soil temperature, pH, moisture content, absolute weight loss, isopod population, fungal and bacterial population in three different depths of the two forest stands of Alnus nepalensis.

Sources of Variation	D.F.	OPEN				CLOSED		
		Isopod Population				Isopod Population		
		0-2 cm	Buried litter (2-5 cm)	5-10 cm		0-2 cm	Buried litter (2-5 cm)	5-10 cm
Air temperature	9	-	0.824***	-	-	0.818***	-	
Soil temperature	9	0.576*	0.840***	-0.609**	0.601*	0.802***	-0.612**	
pH	9	-0.563*	0.530*	0.552*	-0.122	0.563*	0.188	
Moisture content	9	0.091	0.786***	0.263	0.063	0.864***	0.167	
Absolute weight loss	9	-	0.151	-	-	0.290	-	
Fungal population	9	-	0.760***	-	-	0.765***	-	
Bacterial population	9	-	0.890***	-	-	0.900***	-	

\*, \*\*, \*\*\*,  $p < 0.1$ , 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

conditions were favourable to allow bacteria and fungi to grow and multiply in decomposing litter. The decline in populations of fungi and bacteria after September signified the adverse effect of low temperature and moisture during winter as has also been observed by others (Witkamp, 1963; Holm and Jensen, 1972; Das, 1980; Deka and Mishra, 1982 and Kshattriya, 1990).

The high fungal and bacterial population at open forest stand compared to the closed ones may be due to the dominance of temperature influence over moisture effects (Shanks, 1954 and Witkamp, 1963). The reduced fungal population during excess moisture content of litter may be due to the lack of aeration (Mikola, 1954; Witkamp, 1966 and Das, 1980). Under the existing environmental conditions of forest soils, fungi viz., deuteromycetes and ascomycetes constituted the primary decomposer population of leaf litters suggesting that they could utilize locked up nutrients in litters while the appearance of phycomycetes as late successional species could only utilize the simpler nutrients either as fungal products of soluble forms. The difference in fungal species composition during different stages of decomposition of litters may be due to the fact that a variety of organic substances were released which favoured certain <sup>g</sup> groups of fungi while adversely affected the others (Garrett, 1951 and Alexander, 1961).

Genera like Paecilomyces, Geotrichum and sterile mycelia were rarely isolated while Penicillium, Trichoderma and Fusarium appeared with high frequency throughout the sampling period. The discontinuity of occurrence may be governed by the change in the microbial balance, nutrient status and their release from the decomposing litters and antagonistic factors (Lockwood, 1964 and Brandsberg, 1969). ✓

Qualitatively, the fungal species composition of both the forest stands was similar with a minor variation though the overlying vegetation was quite dissimilar. This was attributed to the similarity in soil characters and litter resource quality. ✓

There was seasonal changes in isopod densities between the three different depths. The isopod population showed a general trend of increase in both the forest stands with the advent of the warm and rainy season and tended to decrease with the approach of dry and cold season (Mukherjee and Singh, 1970 and Price, 1973). The changes in the seasonal variations of soil isopods may be due to the vertical movements of the population within the soil. The influence of vertical migration as an influence of estimate of seasonal changes in abundance was noted (Price, 1973). During the present study, the vertical

distribution of the isopods between the three different depths showed a higher concentration in the buried litter layer (Fig. 9).

Forces outside the soil community which influence population densities are the climatic factors (Drift, 1963). The effect of temperature and precipitation has been reported by Usher (1971). Kevan (1962) reported that moisture content of the soil is of vital importance to the soil fauna. The high population of soil isopods in rainy season corresponded to excessively high moisture content of the soil and litter and during the winter minima due to desiccation of the soil combined with low temperature (Choudhuri and Roy, 1967). The present study showed that the population of isopods in both the stands was correlated significantly with the moisture content of the buried litter (Table 19).

Kevan (1962) reported that temperature is probably less important to soil animals than it is to surface dwelling species. The present study showed a significant positive correlation between isopod population to the air and soil temperature. However, isopod population was considerably reduced in the winter months (Nov.-Feb.). This may be due to decrease in temperature, coupled with decreased moisture content of the soil, having adverse

effects on the population density of the soil isopods. The results were supported by Niijima (1971) who found that most soil fauna, low temperature results in the reduction of activity. Further recurrence of warm temperature and rainfall during April-May brought about suitable conditions for multiplication of isopod and other major group of soil fauna which also corroborated by the observation of Niijima (1971).

The relation of pH to the distribution of certain groups of soil arthropods have been reported by Kevan (1962). In contrast, some investigators believe that pH conditions have little effect upon many of the soil fauna (Christiansen, 1964). The present study revealed that in open stand a positive correlation existed between pH of the soil and densities of isopods. However, in closed forest stand it correlates only with the buried litter (Table 19).

Populations of all three species of isopods exhibited a highly aggregated distribution patterns forming a dynamic mosaics of grazing intensities on the soil and litter microflora. The grazing by soil animals could also result in a more active microbial population as a result of decreased microbial number and biomass and thus less competition for available energy, nutrients and space

(Bååth et al. 1981). The results suggest that the microbes combined or interrelated with faunal attacks could be important link in the decomposition pathway of alder litters. Comminution of uningested organic matter by isopods increased the surface area for microbial colonization and may expose previously unexploited areas of the resource (Visser, 1985).

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CHAPTER IV

MASS LOSS AND NUTRIENT MINERALISATION FROM  
ALDER LITTER IN RELATION TO  
THE MICROBES AND ISOPODS

## INTRODUCTION

Decomposition of litter under natural conditions is regulated by a number of variables including the litter's physical properties, climate, microbes, macro and micro-faunal responses. In spite of the complexity of the process two most important attributes are the prevailing environmental conditions and the decomposers community. Rate of litter decomposition differs in sites with different soil types (Witkamp, 1960).

Decomposition processes are intimately coupled to mineralization and immobilization of nutrients. Mineralization is the conversion of organic molecules to inorganic ionic states readily available for plant use. It is carried out by a diverse range of soil organisms including bacteria, fungi and invertebrate animals. These organisms release inorganic N, P, S and many other elements from soil organic matter. In natural ecosystems, these processes are the major ways excluding biological N fixation in which the essential elements are made available to the plant.

The soil on which the litter decomposes may also influence the process by acting as a reservoir of micro-

organisms which colonize the litter. In forest soils decomposition of organic plant debris to yield inorganic plant nutrients is performed largely by fungi, bacteria and actinomycetes (Jensen, 1974) and can become a rate determining step in the nutrient cycling process with the potential to alter ecosystem primary productivity (Miller et al. 1979).

The release of nutrients from forest litter through natural decomposition process is recognized as being a very important part of the nutrient cycle whereby essential mineral elements tied up in the plant biomass are made available for further plant growth. The decrease in dry weight of plant litter is a composite measurement attribute to leaching decomposition by microorganisms and animals; fragmentation by biotic and abiotic breakdown processes and export.

Fungi are considered to be the most important primary microbial decomposers of lignified litter because of their ability to degrade lignin (Ander and Erikson, 1978, Kirk, 1971).

The soil fauna may affect rate of mass loss from decomposing substrate, directly by ingestion and indirectly by grazing on microbes. The <sup>e</sup>ffects of substrate communi-

tion are major phenomena in forest systems, where a large fraction of mass loss can be attributed directly or indirectly to the present<sup>ce</sup> of soil fauna.

Faunal<sup>l</sup> affects on litter decomposition have been investigated using litter bags with varying mesh size (Edwards and Heath, 1963; Heath et al. 1964; Madge, 1969) by treatment of substrate with insecticides (Kurcheva, 1960) or a combination of both (Witkamp and Crossley, 1966, Cromack, 1973; Ward and Wilson, 1973).

Many workers have studied the role of soil animals in litter decomposition in natural (Edward et al. 1970;

Edward, 1974; Lofty, 1974; Mason, 1974; Wood, 1976; Anderson et al. 1983; Seastedt, 1984) and agriculture ecosystems (Coleman et al., 1984; House and Parmelee, 1985; Coleman, 1985).

Much attention has recently been paid to devise the techniques for studying decomposition of litter (particularly leaf litter) and its rate of decomposition under different climatic zones (Edwards and Heath, 1963; Weighert and Evans, 1964; Witkamp, 1966; Howard, 1967; Van Cleve, 1971; Anderson, 1973; Gosz et al., 1973; Heal and French, 1974; Howard and Howard, 1974; Jensen, 1974; Suffling and Smith, 1974; Wood, 1974).

However, there is need to investigate the role of various groups of organisms in the decomposition of litter and nutrient release. Particularly of isopods and of soil microbes during litter degradation which has received little attention to North East India (Kshattriya, 1990) as most of the work having done in Europe (Edwards, 1974).

The present study aims to evaluate the rate of decomposition of alder leaf litter as influenced by the microbes and isopods in open and closed forest stands of Alnus nepalensis.

## MATERIALS AND METHODS

### (i) Rate of leaf litter decomposition

Nylon bag technique of Bock et al. (1960) with two varying mesh size (20 cm x 20 cm; mesh sizes, 1 mm and 3 mm) was followed to estimate the rate of litter decomposition (Details are given in Chapter III).

On each sampling, six leaf litter bags were collected for each of the two mesh size from both the sites, three bags were recovered to assess the loss in litter on dry weight basis. The litter bags were open<sup>ed</sup>, carefully separated and washed on 200  $\mu$ m mesh sieve to clean the <sup>of</sup> the adhered soil particles. The litter was dried in a

hot air oven at 60°C until a constant dry weight was obtained. Final dry weight of the litter samples was taken and percentage weight loss was calculated on the oven dry weight basis. The decay constant (K) was calculated using Olson's (1963) decay model with the following formula:

$$\frac{X_t}{X_0} = e^{-kt}$$

Where  $X_0$  = Initial weight

$X_t$  = Weight after time 't'

K = The annual experimental (base e) decay coefficient.

#### (ii) pH and moisture content of the litter

The remaining 3 bags were used for the determination of pH and moisture content of the litter (details given in Chapter III).

#### (iii) Estimation of organic constituent of leaf litter

Cellulose, hemicellulose, lignin, total soluble sugars and total amino acids of litter were estimated by the method of Peach and Tracey (1955).

#### (a) Cellulose and Hemicellulose

0.5 g of ground litter was treated with 25% KOH

aqueous solution (W/V). The mixture was centrifuged at 3000 rpm for 15 minutes. The decant obtained was used for detection of hemicellulose. The residue left was washed several times with distilled water. Thereafter, it was dried at 105°C in oven for 24 hours and cooled at room temperature in a desicator and weighed. The amount thus obtained was total cellulose.

The decant obtained was neutralized with equal amount of glacial acetic acid and ethanol. The precipitate was filtered, washed, dried and weighed as above for determination of total hemicellulose. Three replicates were taken in each case. ✓

#### **(b) Lignin**

0.5 g of dried litter powder was taken in a test tube and treated with 20 ml of 72%  $H_2SO_4$  and kept in deep freeze for 24 hours. It was then centrifuged and the residue was collected and washed thoroughly to remove the traces of  $H_2SO_4$  present. It was then dried in oven at 105°C for 24 hours. The amount so obtained gave the total lignin content in the leaf litter. ✓

Data on cellulose, hemicellulose and lignin content was expressed on dry weight basis of the litters.

**(c) Total sugars and Amino acids**

100 mg dried powdered litter was taken in a test tube and treated with 80% ethanol. Occasionally, when any colour developed, it was treated with a pinch of activated charcoal and centrifuged at 6000 rpm. The solution was filtered through a filter paper (Whatman No. 1). The clear filtrate was boiled on a hot water bath to remove the traces of ethanol. To it distilled water was added to make the volume upto 5 ml.

To 3 ml of the above solution 6 ml of freshly prepared anthrone reagent (0.2% in  $H_2SO_4$ ) was added slowly by the side of the test tube and shaken gently. The solution was then kept for 3 minutes in the boiling water bath and cooled at room temperature and absorbance of the solution was read at 610 nm in a Hitachi spectrophotometer. Standard curve was obtained from transmittance of varying concentration of glucose solution treated exactly as the samples. From the standard curve the values of the total sugars were expressed as  $\mu g/100$  mg dry weight of the samples.

To the rest of 2 ml of the solution, 2.5 ml of citrate buffer (pH 5.5) and 2 ml ninhydrin solution were added. The mixture was kept in boiling water bath for 30 minutes and then cooled at room temperature. A light

purple colour developed in the solution. The absorbance was determined at 540 nm in the Hitachi spectrophotometer. The total amino acids were calculated from the standard curve obtained from transmittance of varying concentration of leucine solution treated exactly as unknown samples. From the standard curve the amount of amino acids in unknown sample was calculated and expressed as  $\mu\text{g}/100 \text{ mg}$  dry weight of the samples.

#### **(iv) Determination of inorganic constituents of the litter**

##### **(a) Nitrogen**

Total nitrogen in the leaf litters was estimated by Micro-Kjeldahl method (Allen, 1974). 100 mg dried litter was grounded and sieved through a sieve (0.2 mm) was taken in a Micro-Kjeldahl flask. To it 2 g  $\text{K}_2\text{SO}_4:\text{HgO}$  (20:1) mixture was added. Now 3 ml of concentrated sulphuric acid was added slowly down the neck while the flask was rotated. The flask was heated on a digestion rack. After the digest became colourless the heating was continued for another 15 minutes. Thereafter, flasks were allowed to cool at room temperature. The digested material was diluted with distilled water and filtered through filter paper (Whatman No. 1). The blank digestion was prepared only with the mixture. Through digestion all the organic nitrogen converts into ammonia which was measured by Indo-

phenol blue method. The nitrogen was calculated by the following formula.

$$\text{Nitrogen (\%)} = \frac{C(\text{mg}) \times \text{solution volume (ml)}}{10 \times \text{aliquot(ml)} \times \text{sample weight(g)}}$$

Where, C = Concentration reading (mg) in the aliquot.

### (b) Phosphorus

Oven dried litter was ground and sieved through a sieve (0.2 mm) was used for phosphorus determination. 0.3 g ground material was digested in a triacid mixture ( $\text{HNO}_3$ . $\text{H}_2\text{SO}_4$  and 60%  $\text{HClO}_4$  in ratio of 10:1:4) for the analysis of phosphorus. After digestion the volume was made to 50 ml and filtered through filter paper (Whatman No. 42). Phosphorus in the digested sample was estimated following the molybdenum blue method of Jackson (1967).

$$\text{Phosphorus (\%)} = \frac{C(\text{mg}) \times \text{solution volume (ml)}}{10 \times \text{aliquot (ml)} \times \text{sample weight (g)}}$$

Where, C = Concentration of phosphorus in the aliquot.

## RESULTS

### Weight loss of litter

Sequential loss of leaf litter of Alnus nepalensis was observed (Figs. 12 and 13). Maximum weight loss occurred during the month of June. A steady decrease in mass

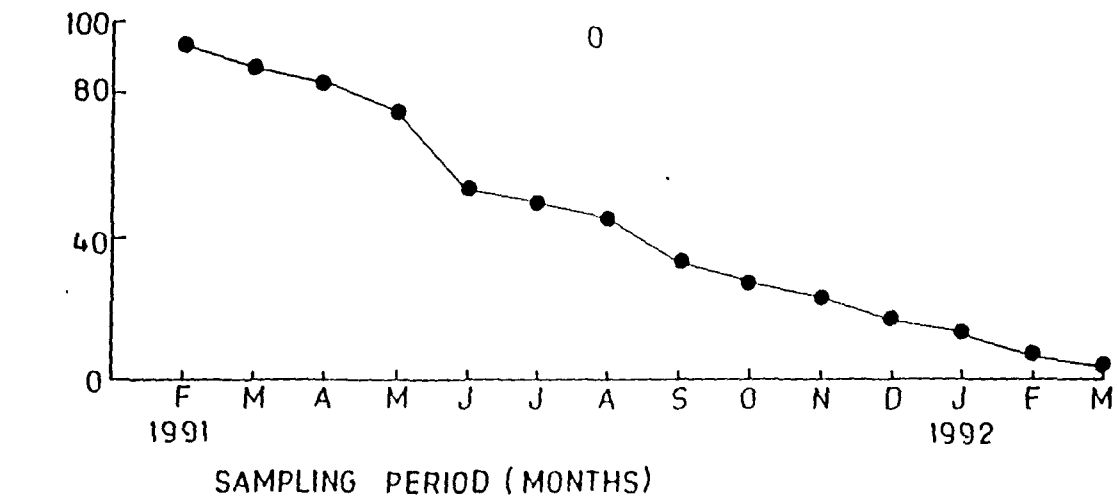
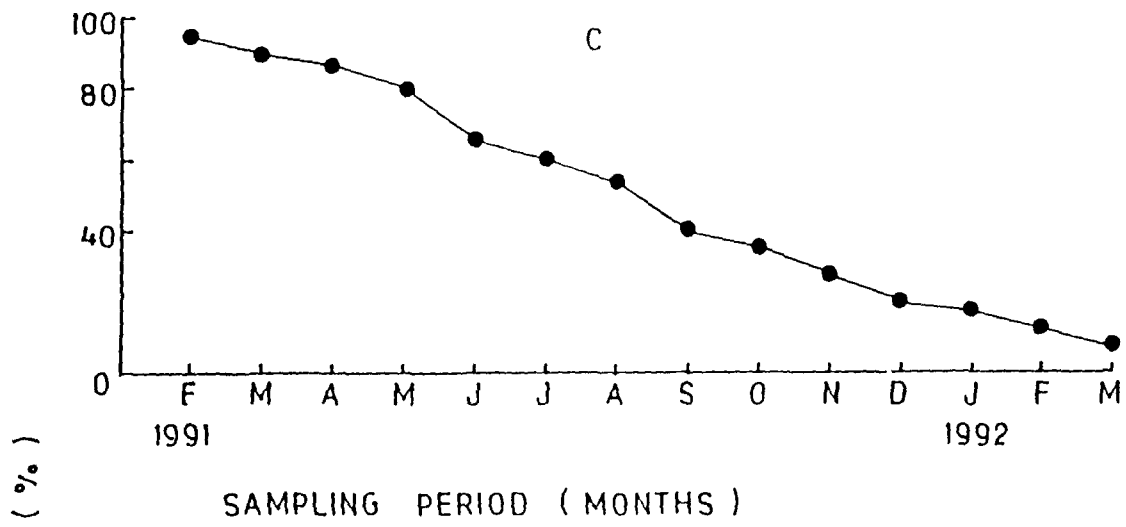


Fig 12. Percentage of original dry weight of leaf litters remaining after different time intervals at two forest stands of Alnus nepalensis. (c = closed, o = open)

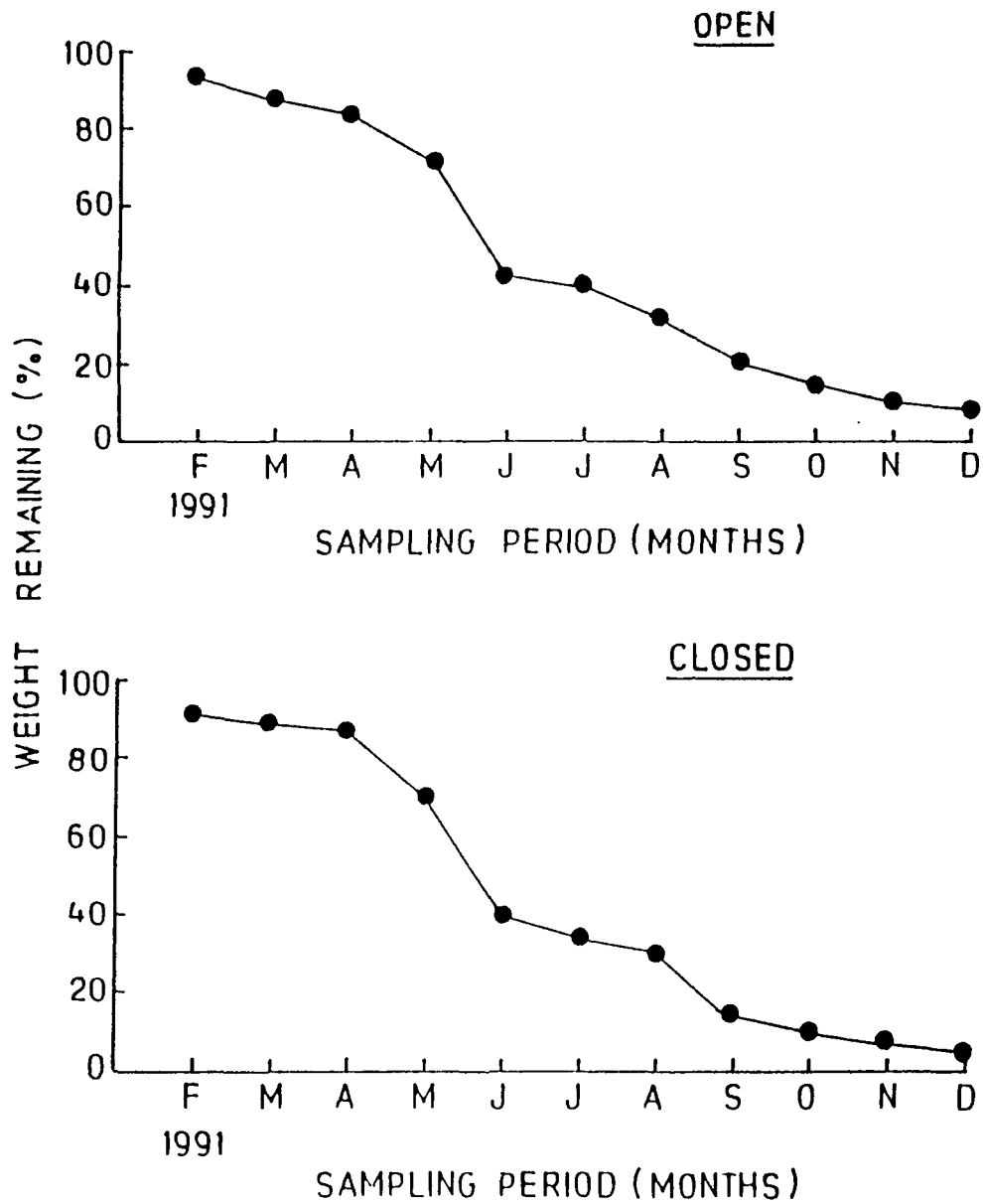


Fig 13. Percentage of original dry weight of buried leaf litters remaining after different time intervals at two forest stands of Alnus nepalensis

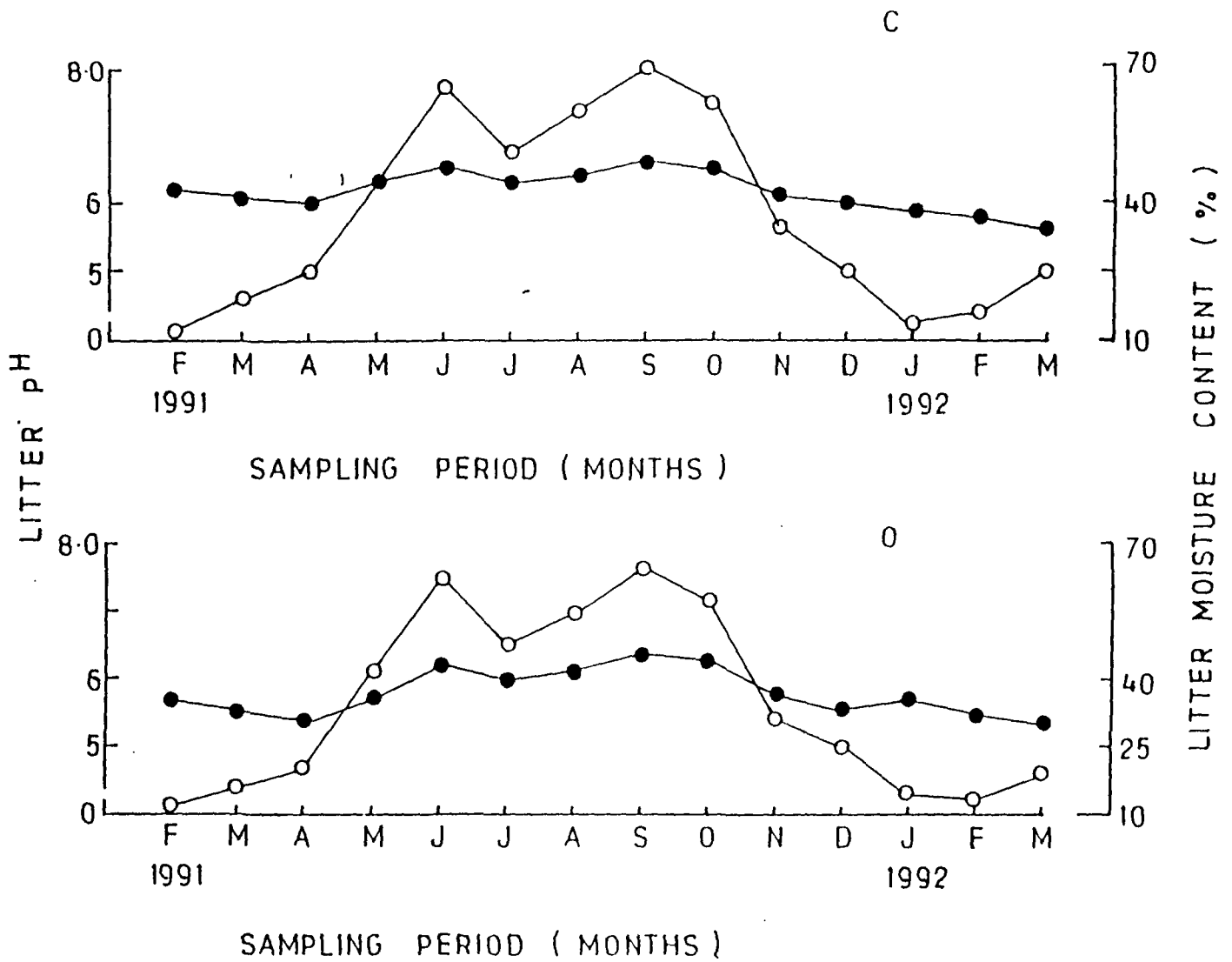


Fig 14. Monthly variation in moisture content (○—○) and P<sup>H</sup> (●—●) of leaf litters at two forest stands of *Alnus nepalensis*. (c = closed, o = open)

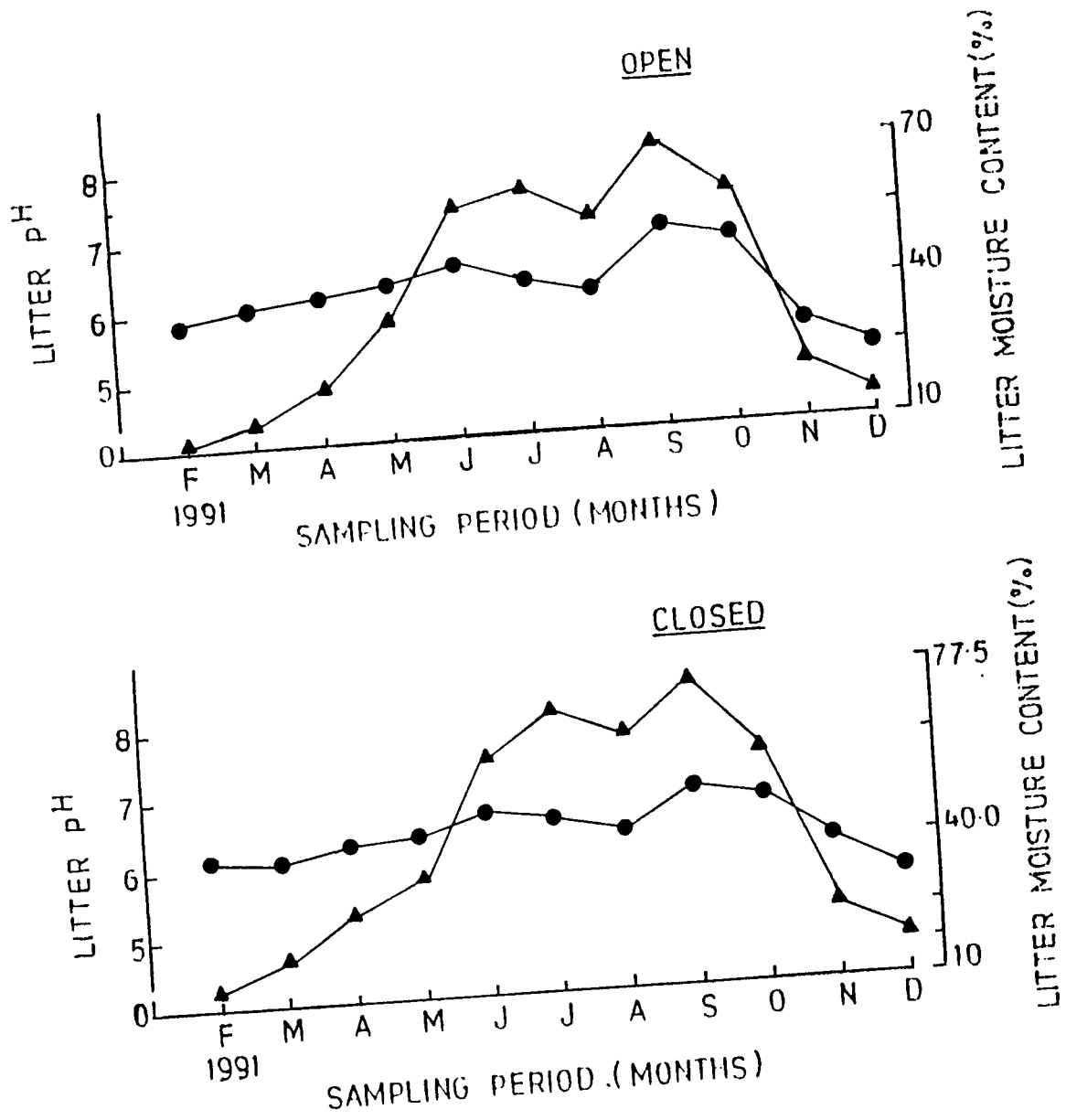


Fig 15. Monthly variation in moisture content (▲—▲) and P<sup>H</sup> (●—●) of buried leaf litters at two forest stands of Alnus nepalensis.

Table 20 - Decay constant (K), half life and 95% life values for decomposition of Alnus nepalensis leaf litters under field condition.

Plant species	K (per year)		Half life (year)		95% life	
	Open	Closed	Open	Closed	Open	Closed
<u>Alnus nepalensis</u>	1.198	0.940	0.578	0.737	2.504	3.190

Table 21 - Decay constant (K), half life and 95% life values for decomposition of buried leaf litters of Alnus nepalensis under field condition.

Plant species	K (per year)		Half life (year)		95% life	
	Open	Closed	Open	Closed	Open	Closed
<u>Alnus nepalensis</u>	1.197	1.421	0.578	0.487	2.506	2.111

loss was noticed after June, which increased from September to October as compared to the preceding months and a further repression was observed during winter months.

The mass loss of alder leaf litter in coarse mesh bags (3 mm) was slightly higher than that from the normal mesh bags (Figs. 12 and 13). The weight remaining in coarse mesh bag at the end of 11 months was 8% and 5% and 4% and 8% in case of normal mesh bag at the end of 14 months in the open and closed forest stands respectively.

Among the two forest stands, the decomposition of leaf alder litter was faster in low mesh size nylon bags (1 mm) under open forest stand condition ( $K = 1.198$ ) than closed ones ( $K = 0.940$ ), whereas disappearance of litter was faster in closed forest stand ( $K = 1.421$ ) than open forest stand ( $K = 1.197$ ) in coarse mesh bags (3 mm) (Tables 20 and 21).

#### pH and Moisture content of the leaf litter

Leaf litter of open forest stand was slightly acidic compare<sup>d</sup> to closed forest stand. The pH ranged from 5.0 to 6.90 in open stand and from 5.5 to 6.95 in case of closed forest stand. In the beginning, the litter was less acidic in both the sites which subsequently became more acidic in winter and at the end of decomposition (Figs. 14 and 15).

Moisture content of litter ranged from 11.5% to 70% in open forest stand and 10.5% to 75% in case of closed forest stand (Figs. 14 and 15). It was minimum during winter months which increased with the onset of rain. However, the moisture content of litter was higher in closed forest compared to open forest ones. However, seasonal pattern was similar on both the stands. The correlation between weight loss of litter and its moisture content was not significant (Tables 22 and 23).

#### **Organic constituents**

Both the soluble (total sugars and amino acids) and insoluble (cellulose and hemicellulose) organic constituents in the leaf litter of A. nepalensis forest stands decomposed rapidly in the beginning and slow towards the end of the process of decomposition. Leaf litter of closed forest stand contained more amount of soluble compounds than of open forest stand. Maximum changes in total sugars and amino acids were observed during rainy months (Figs. 16 and 17). It was observed that the rate of degradation of soluble components was highest followed by hemicellulose, cellulose and lignin. The decomposition of cellulose and hemicellulose followed a similar pattern in both the forest stands whereas lignin contents increased in the beginning and degradation of lignin started towards the

latter part of decomposition (Figs. 18 and 19). At both the forest stands, the absolute weight loss of A. nepalensis leaf litter showed a significant negative correlation with the weight remaining of different organic constituents like cellulose, hemicellulose, sugars and amino acids. However, lignin content was correlated positively with absolute weight loss (Tables 22 and 23).

### **Inorganic constituents**

#### **Nitrogen**

The initial nitrogen content ranged from 0.5 to 1.90% at open forest stand and 0.70 to 2.01% in case of closed stand. Initially, the nitrogen content increased up to the month of June followed by a subsequent decrease till the end of the decomposition in both the cases (Figs. 20 and 21).

#### **Phosphorus**

In both the stands the level of available phosphorus exhibited a similar trend during the present investigation. The initial phosphorus content ranged from .020 to 0.90% in open forest stand and .025 to 0.075% in closed stand (Figs. 20 and 21). Phosphorus was initially retained in the leaf litters for few months and then gradually released along with the decomposition of litter. The absolute weight loss of both the litters was correlated negatively with nitrogen and phosphorus (Tables 22 and 23).

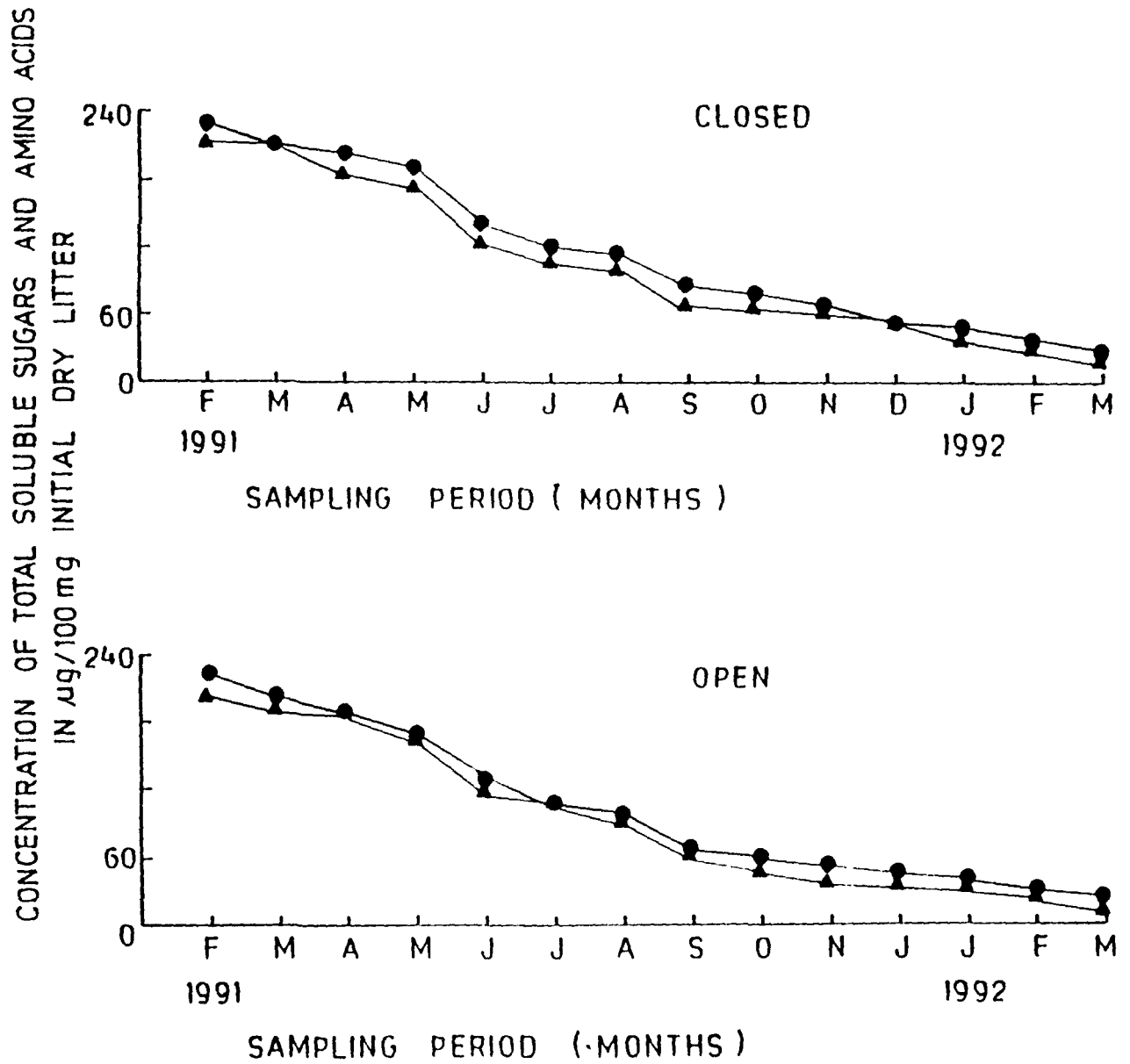


Fig 16. Monthly variation in total sugars (●—●) and amino acids (▲—▲) of leaf litters at two forest stands of Alnus nepalensis.

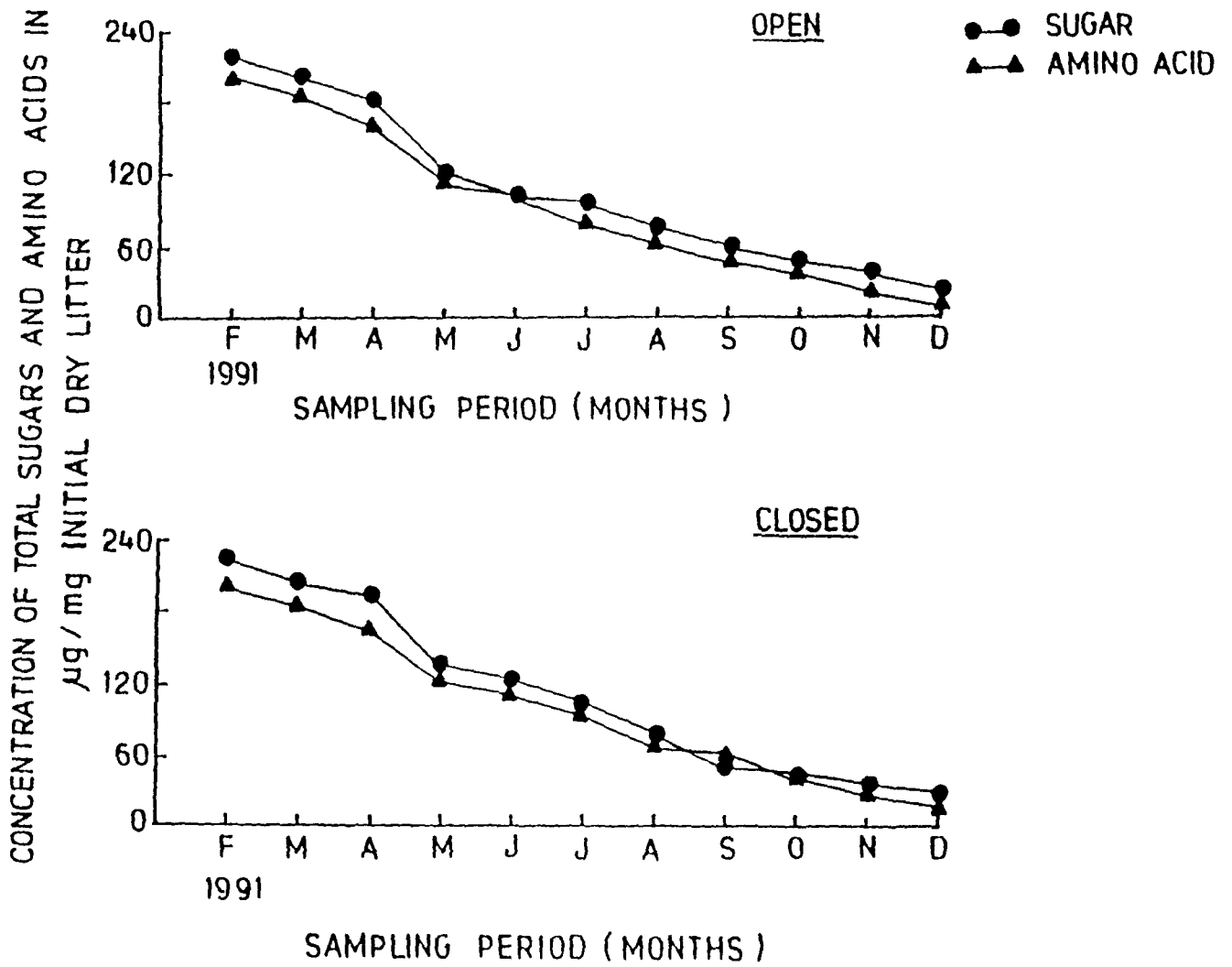


Fig 17. Monthly variation in total sugars (●—●) and amino acids (▲—▲) of buried leaf litters at two forest stands of Alnus nepalensis

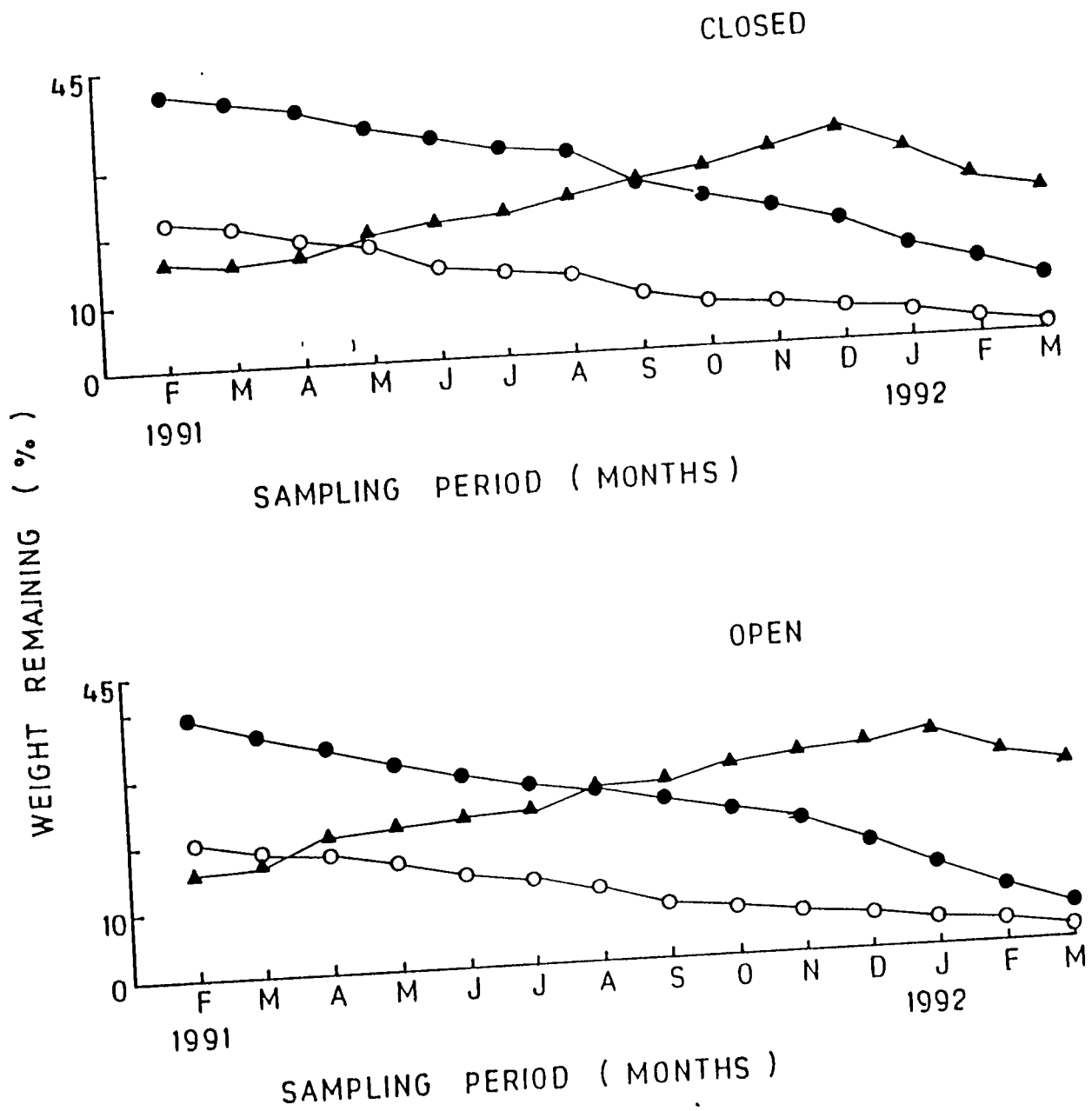


Fig 18. Monthly variation in cellulose (●—●), hemicellulose (○—○) and lignin (▲—▲) content of leaf litters at two forest stands of Alnus nepalensis

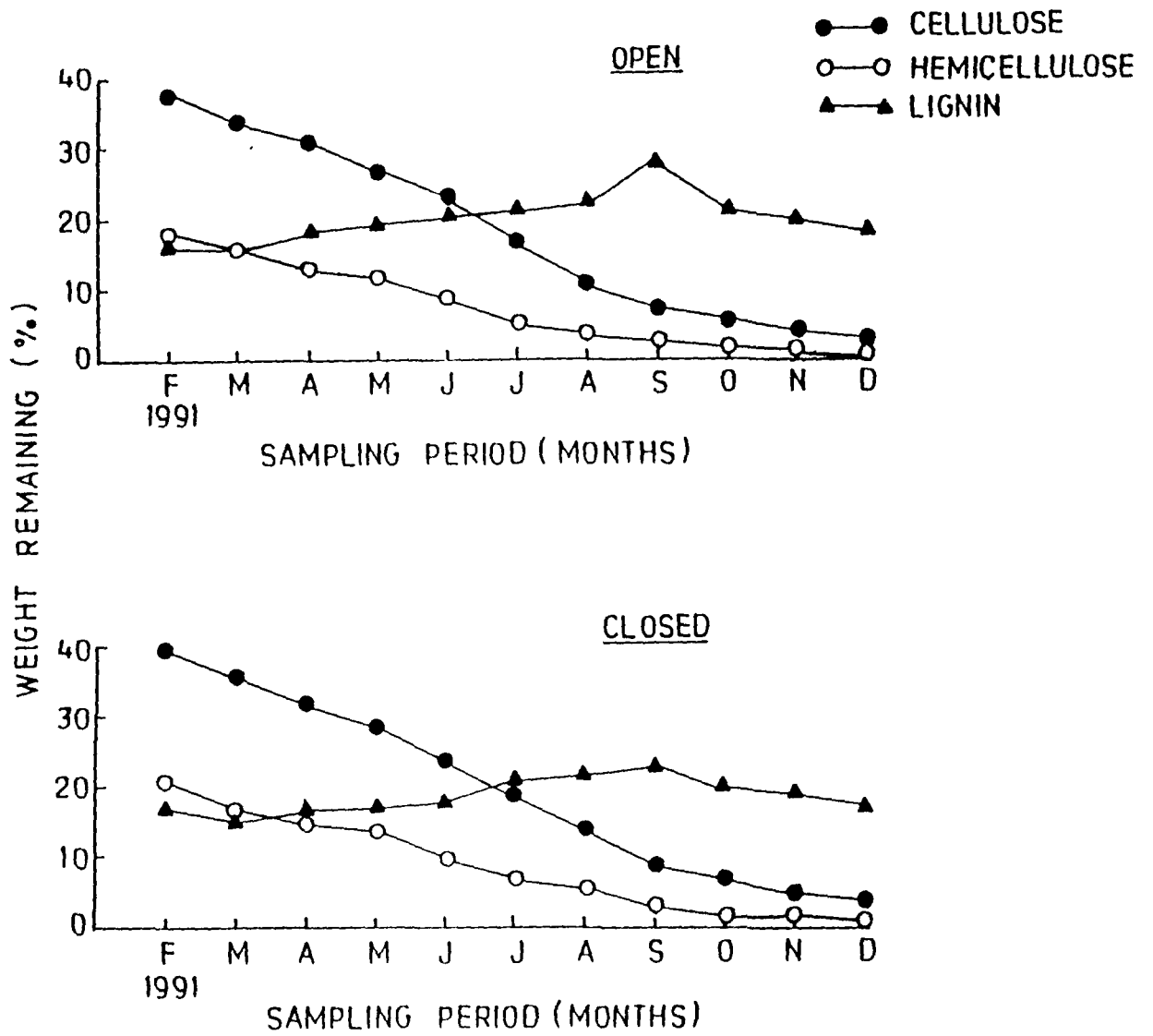


Fig 19. Monthly variation in cellulose, hemicellulose and lignin content of buried leaf litters at two forest stands of Alnus nepalensis

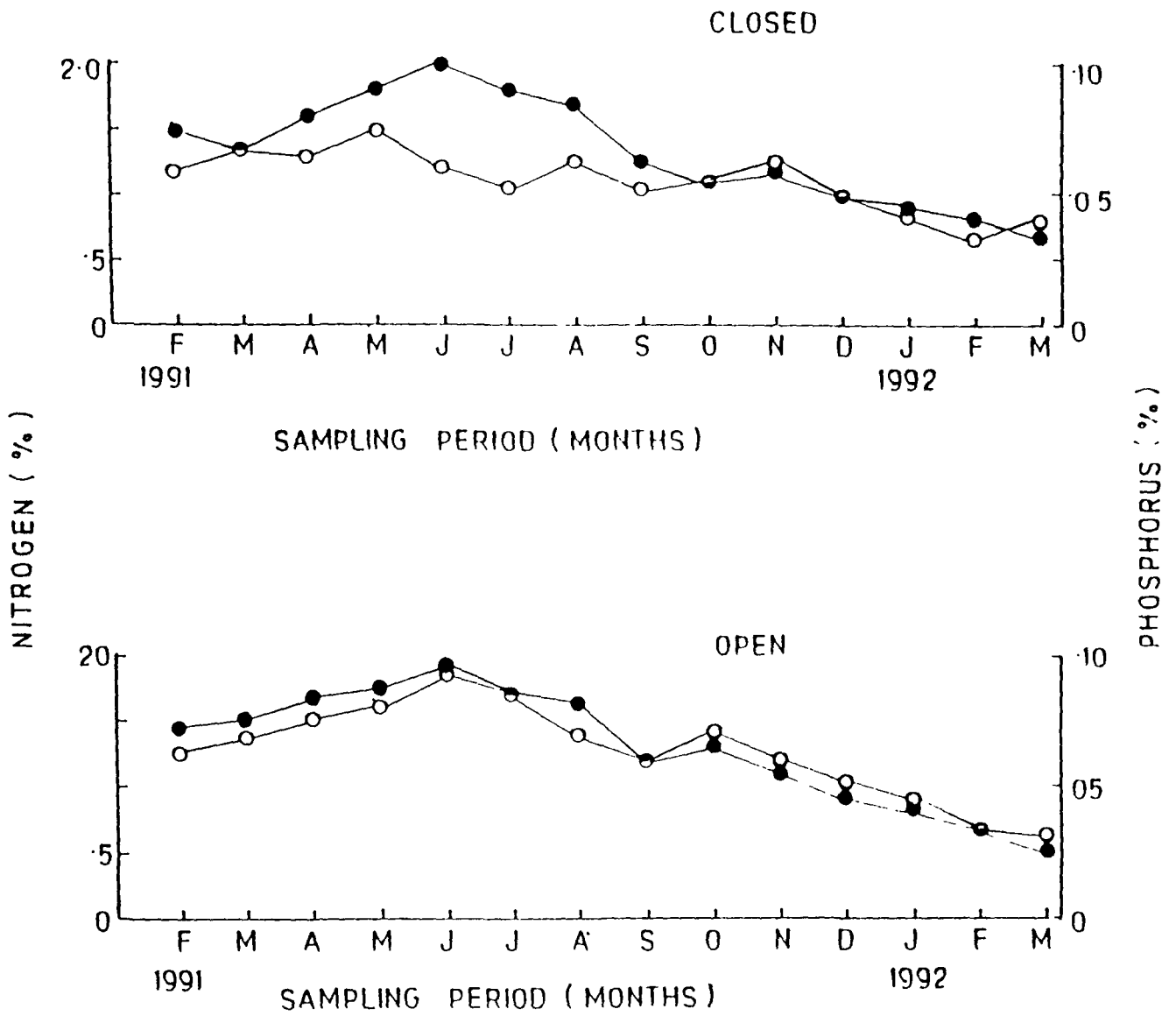


Fig 20. Monthly variation in total nitrogen (●—●) and phosphorus (○—○) content of leaf litters at two forest stands of Alnus nepalensis.

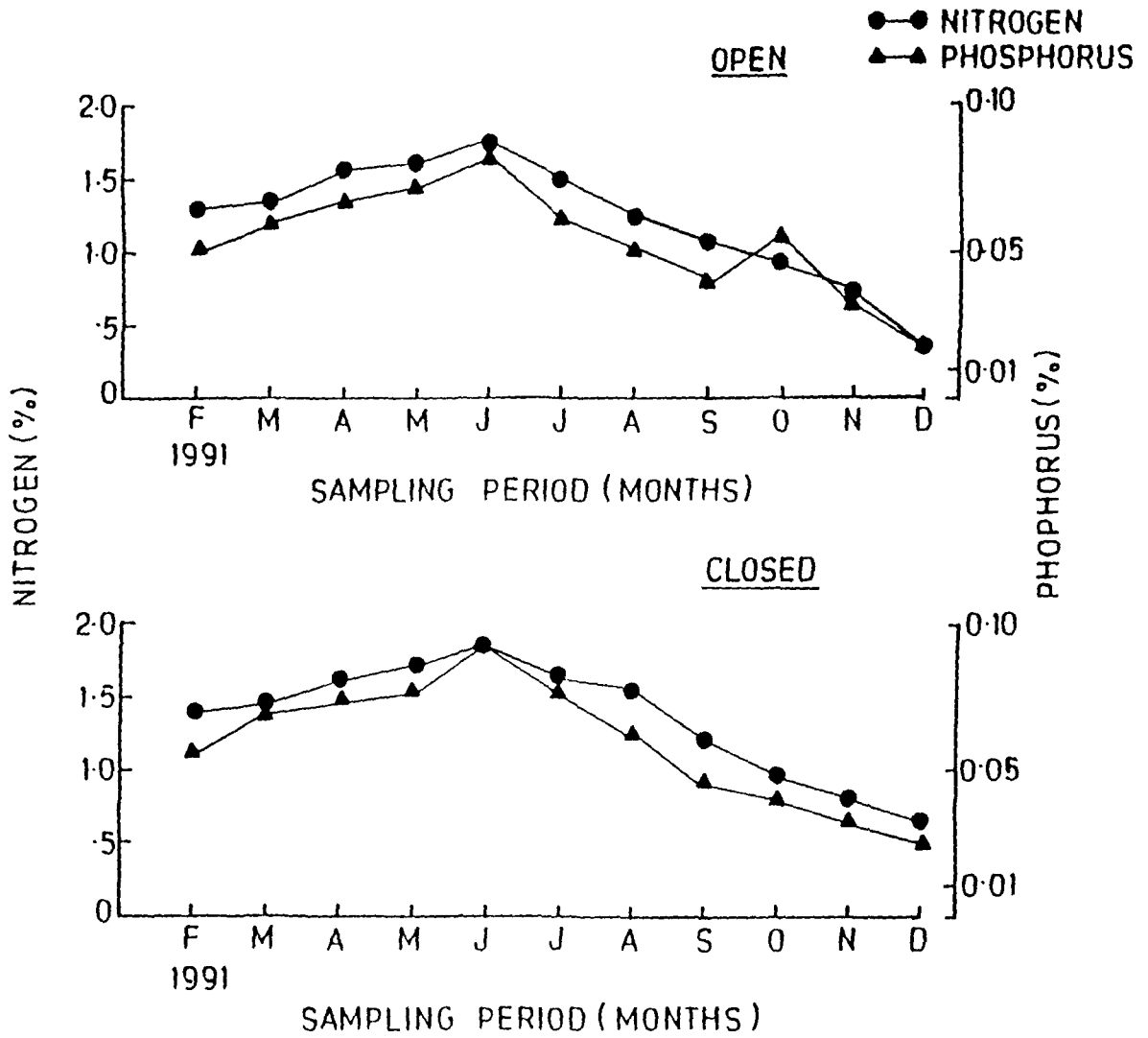


Fig 21. Monthly variation in total nitrogen and phosphorus content of buried leaf litters at two forest stands of Alnus nepalensis.

Table 22 - Correlation coefficient (r) for cellulose, hemicellulose, lignin, total sugars, total amino acids, total nitrogen, phosphorus, fungal population, bacterial population and absolute weight loss in Alnus nepalensis litters at two forest stands.

Sources of variation	D.F.	Absolute weight loss	
		Open	Closed
Cellulose	12	- 0.957***	- 0.983***
Hemicellulose	12	- 0.993***	- 0.994***
Lignin	12	0.935***	0.815***
Total sugars	12	- 0.993***	- 0.990***
Total amino acids	12	- 0.993***	- 0.986***
Total nitrogen	12	- 0.769***	- 0.760***
Phosphorus	12	- 0.697***	- 0.777***
Fungal population	12	- 0.151	- 0.213
Bacterial population	12	- 0.111	- 0.067
Moisture content	12	0.045	- 0.038

\*, \*\*, \*\*\*,  $p < 0.1$ , 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 23 - Correlation coefficient (r) for cellulose, hemicellulose, lignin, total sugars, total amino acids, total nitrogen, phosphorus, fungal population, bacterial population and absolute weight loss in buried litters of Alnus nepalensis.

Sources of variation	D.F.	Absolute weight loss	
		Open	Closed
Cellulose	9	- 0.981***	- 0.980***
Hemicellulose	9	- 0.985***	- 0.981***
Lignin	9	0.630**	0.664**
Total sugars	9	- 0.976***	- 0.983***
Total amino acids	9	- 0.980***	- 0.970***
Total nitrogen	9	- 0.658**	- 0.618**
Phosphorus	9	- 0.562*	- 0.605**
Fungal population	9	0.200	0.113
Bacterial population	9	0.341	0.467
Moisture content	9	0.465	0.478

\*, \*\*, \*\*\*,  $p < 0.1, 0.05$  and  $0.01$  respectively.

Without asterisk statistically insignificant at these levels.

## DISCUSSION

From the results obtained, apparently there was not much variation in the rate of leaf litter decomposition on both the forest stands. Moreover, the rate of litter decomposition in fine mesh bag has been found to be slightly faster in the open forest stand compared to that of closed forest stand. This was attributed to the high microbial population encountered in open forest stand, whereas in case of coarse mesh bags, higher mass loss from closed forest stand than from the open stand, may be attributable to the initial higher N content, narrower C/N ratio litter texture and greater arthropod abundance in the litter bags (Anderson, 1973).

Greater mass loss from coarse mesh bags containing alder leaf litter compared to that from fine mesh bags has also been reported by Gupta and Singh (1981) and Reddy and Alfred (1989). It could be due to the influence of mesh size on the access of different sizes of soil animals and greater abundance of certain taxa determining the decomposition rate. Lesser decomposition in the fine mesh bags may be due to the inability of the soil microfauna to invade the bags. The importance of soil animals in decomposing organic debris by employing varying mesh size litter bags or by using chemicals (Anderson, 1973; Wood,

1976). More weight is lost when all groups of soil animals and microorganisms take part in the decomposition processes. The higher weight losses in the present study in coarse mesh bags (3 mm pore size) compared to fine mesh bags (1 mm pore) can be accounted in the light of the above discussion. The difference in decomposition rate may be assigned to physical nature of plant material (Bhatt et al., 1985), difference in pH, moisture content and substrate quality, which ultimately govern the distribution of microflora (Swift et al., 1979 and Howard and Howard, 1980).

The slow rate of litter decomposition at the initial stages may be due to unfavourable abiotic conditions and low microbial population. The increased rate of litter decomposition in both the cases during June - October may be due to the favourable temperature and water potentials (Stott et al., 1986 and Tiwari, 1988) which might have favoured the luxurious growth of microbes (Nagy and Macauley, 1982; Moore, 1986 and Tiwari, 1988). The concomitant peaks of weight loss, microbial activity and population obtained during rainy season were attributed to the high moisture content of litter and warm conditions prevailing during the same season. This trend may be due to higher initial content of water soluble materials and

simple substrates, the breakdown of the litter by decomposers, especially microflora and the removal of leaf particles by animals through comminution e.g., by Isopods (Anderson *et al.*, 1983 and Swift *et al.*, 1981).

During winter the rate of litter decomposition decreased which may be due to the low temperature and microbial population which resulted in low microbial activity. Shukla (1976) and Harper and Lynch (1981) also found a slow rate of decomposition at low temperature in the field condition. A repression in the litter decomposition rates during winter months may be caused by a combination of factors like temperature and dry environmental conditions and the predominance of slow decomposing litter components such as lignin (Meentemeyer, 1978). William and Gray (1974) observed that during periods of droughts, litter decomposition rates are drastically retarded and the number of saprophagous animals in the litter reduced.

The organic constituents followed almost a similar pattern of distribution in litters in both types of mesh bag of both the forest stands. Generally, the amount of cellulose, hemicellulose, lignin, total sugars and amino acids was maximum in the beginning and minimum at the end of the decomposing process. It has been observed that the rate of hemicellulose breakdown was the fastest followed

by the cellulose and lignin. This trend was similar in both types of mesh bag of both the forest stands. The amount of lignin increased in the beginning and degraded towards the later part of decomposition can be assigned to the active degradation of cellulose and hemicellulose and other simpler forms in the beginning which can be easily utilized by the microbes (Berg et al., 1984). This may also be due to the resistant nature of ligno cellulosic complexes (Summerell and Burges, 1989). The preferential use of sugars, amino acids, cellulose and hemicellulose in the beginning indicates their role in the microbial growth (Harper and Lynch, 1981). Summerell and Burges (1989) have also reported a decrease in the proportion of cellulose and hemicellulose during decomposition which increase in lignin concentration similar to the present investigation.

Rapid degradation of total sugars, amino acids, cellulose and hemicellulose in the beginning of decomposition process was attributed to their simpler chemical nature and improved utilization by the microbes (Harper and Lynch, 1981). This can also be attributed to their highly soluble nature which must have enhanced their leaching along with rain water.

The increase in nitrogen content in the beginning

in both the forest stands may be due to a demand for nitrogen by heterotrophs in which the nitrogen gets immobilized during decomposition (Lousier and Parkinson, 1978). The decrease in nitrogen content during later part of rainy months may be attributed to the leaching of labile nutrients. Similar observations were reported by Kashatriya (1990). Studies on plant material decomposition have followed the change in N concentration with time (Bocock, 1963, 1964; Latter and Cragg, 1967; Singh, 1969; Wood, 1974; Lambert and Lang, 1980). Shukla and Singh (1984) reported that low moisture condition was not suitable for the leaching of nutrients from the decomposing litters. They further suggested that during the onset of rains the litters were exposed to the attack of the microorganisms. The soluble carbohydrates, protective layers of wax and cutin were washed away during rainy season and this resulted into increased leaching of the minerals (N, P and K) recorded during the present investigation as most of these minerals leached into the soil during rainy seasons.

Higher phosphorus content was obtained at early stage of decomposition. Drop in phosphorus content was noted with the progress of decomposition process. Staaf (1980) ascribed it to preferential retention of P and N while other nutrients are being rapidly lost during decomposition.

The activity of microorganisms and isopods in leaf litter decomposition seemed to be influenced by the micro climatic conditions and the rate of decomposition mainly depended upon the moisture and temperature requirements of the microorganisms involved therein. Similarly litter and soil moisture affected the mass loss and isopod populations.

Thus decomposition of leaf litter is controlled by a multiplicity of factors including those resulting from changes in the forest floor temperature and moisture regimes, substrate quality, and microbial and soil animal populations (Williams and Gray, 1974, Maclean and Wein, 1978).

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CHAPTER V  
MICROBIAL ENZYME ACTIVITY DURING DECOMPOSITION  
OF ALDER LITTER

Rate of litter decomposition on the forest floor is known to be under the influence of moisture and temperature requirements of the microorganisms involved and upon the nature of the substratum. The abiotic part of the forest floor consists of partially degraded litter, mainly of plant origin compounds such as lignin, cellulose, carbohydrates and protein which are bio-derivatives of the litter bio-organic compounds (Kononova, 1966, Schnitzer, 1978). Cellulose, lignin and hemicellulose are covalently linked and physically intercalated polymers and extensive degradation requires concerted action by a variety of enzymes (Marsden and Gray, 1986).

Process of leaching and microbial utilization of labile components results in relatively rapid mass loss and refractory litter components largely lignocellulose are slowly degraded (Sinsabaugh and Linkins, 1987). The degradation is mediated by the activity of extracellular and oxidative microbial enzymes (Burns, 1983). Bacteria and fungi may release these enzymes directly into the environment or retain them within their cell walls and periplasmic spaces. In the latter case, the enzymes may enter the environment upon cell lysis (Saddler, 1986; Rhee et al., 1987).

The microbial degradation of cellulose, hemicellulose and other oligosaccharides may be brought about by those enzymes directly involved in initial chemical breakdown (Ross and Speir, 1979). Cellulose a polymer of glucose is an important constituent of leaf litter (Hueck, 1960) and therefore is a potential energy source for the microbes (Kshattriya et al., 1992). Cellulose complex plays an important role in the hydrolysis of cellulose and continuous supply of energy source for the organisms in forest soil. There are three classes of cellulases  $\beta$ - 1, 4 exoglucanases, exocellulases, which can bind to crystalline cellulose and cleave molecules;  $\beta$  - 1, 4 endoglucanases, endocellulase, which randomly cleave glucosidic linkages along non-crystalline domains, and  $\beta$  - glucosidases which release glucose from celloligosaccharides and aryl  $\beta$  - glucosides. Cellulolytic enzymes are known to be produced by bacteria (Yamane et al., 1970 and Morales et al., 1984), actinomycetes (Stulzenberger, 1972), fungi (Clarke and Stone, 1965) and some invertebrates (Okada et al., 1966).

Sucrose a disaccharide of glucose and fructose is abundant in nature. It is a major intermediate product of photosynthesis. It is solubilized by invertase which can be either acidic or alkaline. It is important to understand the mechanism of sucrose degradation because it

✓ the major soluble storage carbohydrate and major form from which carbon is translocated (Edelman, 1971).

Starch a polymer of glucose is second only to cellulose as the most common polysaccharides in the plant realm. Starch is found in all parts of the plant, the leaves, stem, shoot and storage organs such as tubers, rhizomes and seeds. The enzyme which hydrolyses the starch is called amylase which cleaves the  $\beta$  (1-4) bonds of starch.

Although invertase and amylase play an important role in the hydrolysis of sucrose and starch respectively, they have not been studied in details (Spalding, 1977, 1980).

The cellulase enzyme which mediates the hydrolysis of insoluble litter constituents (cellulose) is generally insoluble while amylase and invertase mediating the hydrolysis of more soluble constituents (sugars) are mostly insoluble. *is the soluble*

Amylase, cellulase and invertase are some of the important enzymes in forest floor which are partially responsible for the rate and course of decomposition of plant litter. Thus, the present study was carried out to investigate the efficiency of these enzymes in decomposition of leaf litter at two forest stands of Alnus nepalensis.

## MATERIALS AND METHODS

Two sites i.e., open forest and closed forest were selected for the present investigation (described in Chapter III). 10 g leaf litter of Alnus nepalensis was weighed and kept in the nylon bags separately (mesh size - 1 mm and 3 mm, bag size 20 cm x 20 cm) (Bocock et al., 1960) on the forest floor and other bags (mesh size 3 mm) buried at 2-5 cm depth in soil of respective forest stands. Three bags in both types of mesh bag of each leaf litter were collected at monthly intervals from each forest stand in ice box for the estimation of cellulase, amylase and invertase enzymes to understand the microbial degradation of cellulase, starch and sucrose.

### Extraction of enzymes

For extraction and assaying of cellulase, amylase and invertase enzymes, Spalding's methods (1977) were followed. The litter bags were washed in 90 mm sieve with ice cold distilled water to remove the adhered soil particles. Extra water from the litter was removed with filter paper. 10 g of litter was transferred separately into the Waring blender and ground with 100 ml of acetate buffer (pH 5.5) for 1 minute. Homogenate was then centrifuged at 9400 g at 2°C for 20 minutes in a Sorvall Rc 5 refrigerated centrifuge. The supernatants were filtered through

Whatman filter paper (No. 1). The extracts were stored at 2°C until assaying was completed.

### Assay of enzyme

For enzyme assay, 1 ml of substrate solution was taken in a test tube along with 2 ml of enzyme extract. Carboxymethyl cellulose, sodium salt (Sigma chemical USA) (3%), soluble starch (Sigma) (6%) sucrose (Merck (6%) were used as substrates for cellulase, amylase and invertase respectively. These substrates were prepared in the same buffer which was used for homogenising the litter material. The test tubes with enzyme extract and substrate were incubated at  $37 \pm 1^\circ\text{C}$  for 2 hours. After 3 ml of dinitrosalicylic acid was added to each tube and kept in boiling water for 5 minutes. To each tube 1 ml of sodium potassium tartarate (40%) was added. They were cooled at room temperature and the absorbance of the solution was taken at 575 nm in a Hitachi 220 spectrophotometer. Microbial enzyme activities were expressed in terms of reducing sugars/g oven dried litter/hour.

### RESULTS

Amylase and cellulase activities were less at the beginning of litter decomposition in both the forest stands. However, they showed a steady increase with the progress of litter decay. Both cellulase and amylase

activities increased consistently from May onwards and attained a peak in June in litters of both the forest stands which ultimately decreased from July to September but again increased slightly in October exhibiting a second peak except in case of cellulase activity in fine mesh bag (1 mm) of open forest stand where a second peak was observed in August (Figs. 22 and 23). Cellulase activity showed a significant positive correlation with fungal and bacterial populations, moisture content, total nitrogen and cellulose of the litters of A. nepalensis (Tables 24 and 25). Whereas in case of coarse mesh bag (3 mm), cellulase activity correlated only with fungal population, total nitrogen and cellulose in both the forest stands.

The amylase activity in both types of mesh bag of both forest stands showed a significant positive correlation with fungal and bacterial populations, moisture content, pH and total nitrogen (Tables 24 and 25).

Invertase activity exhibited its maximal peak in April in both types of mesh bag litter of both the forest stands (Figs. 22 and 23) after which it gradually decreased towards the successive months of litter decay. The amount of total sugars showed a significant positive correlation ( $P < 0.05$ ) with invertase activity. The activity was also correlated significantly with total nitrogen

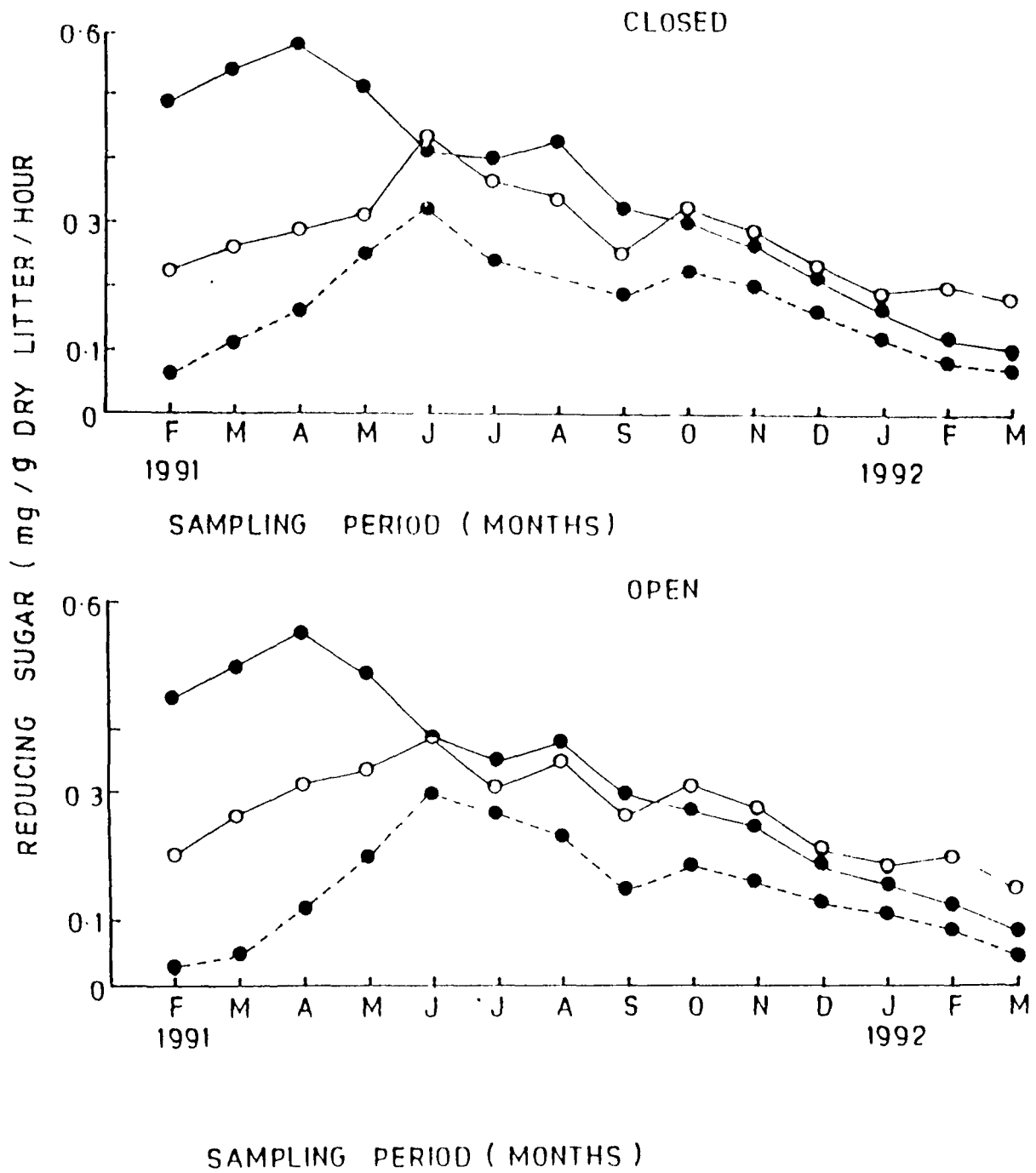


Fig 22. Monthly variation in invertase (●—●), cellulase (O—O) and amylase (●---●) activities of leaf litters at two forest stands of Alnus nepalensis.

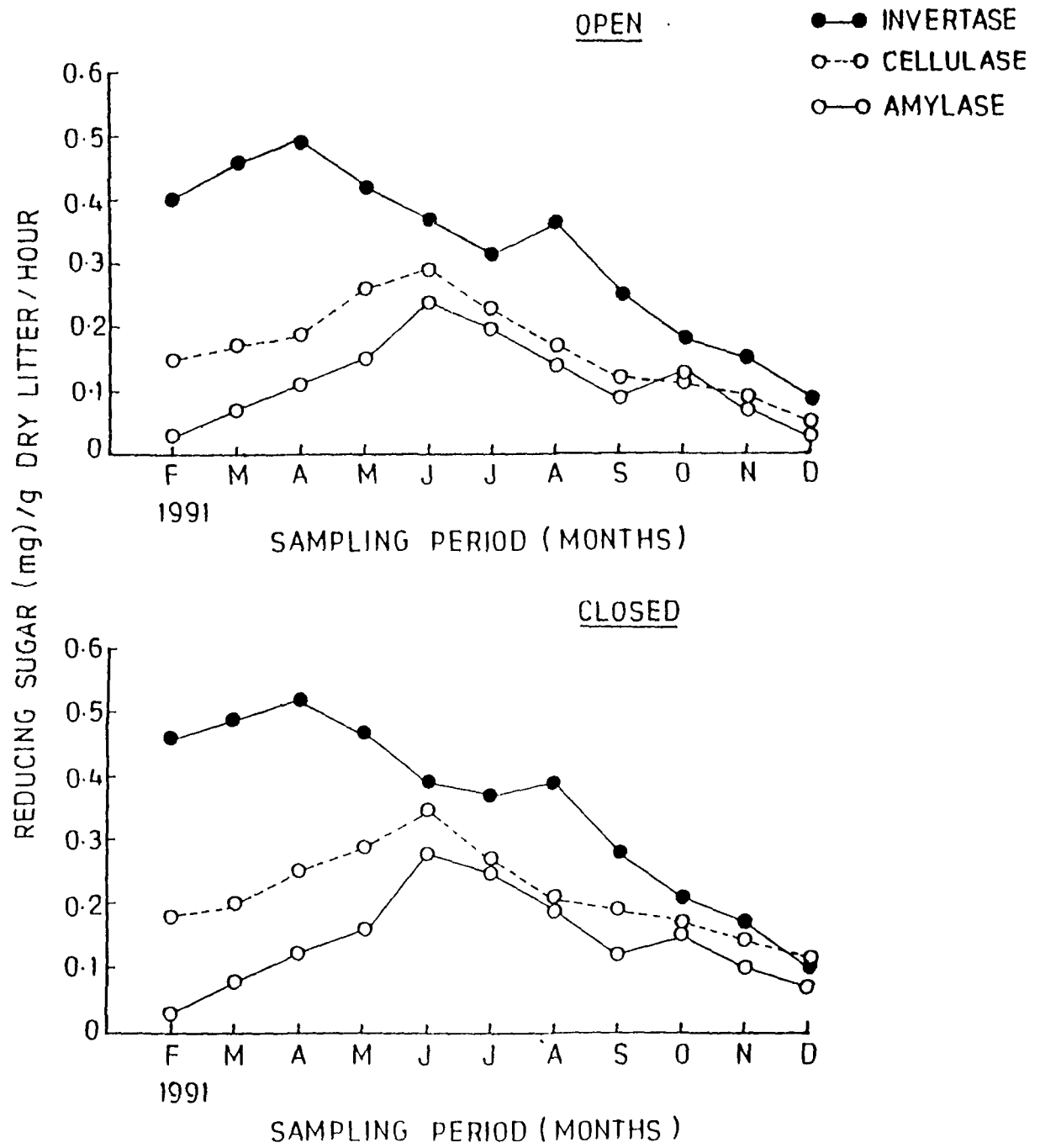


Fig 23. Monthly variation in invertase, cellulase and amylase of buried leaf litters at two forest stands of Alnus nepalensis.

Table 24 - Correlation coefficient (r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of Alnus nepalensis litter at two forest stands.

Sources of variation	D.F.	Amylase		Cellulase		Invertase	
		Open	Closed	Open	Closed	Open	Closed
Fungal population	12	0.913***	0.868***	0.841***	0.872***	0.317	0.304
Bacterial population	12	0.894***	0.787***	0.814***	0.743***	0.274	0.171
Moisture content	12	0.822***	0.816***	0.743***	0.728***	0.143	0.164
pH	12	0.698***	0.735***	0.582**	0.716***	0.031	0.469*
Total nitrogen	12	0.614**	0.710***	0.871***	0.844***	0.872***	0.802***
Weight loss	12	0.020	-0.195	-0.412	-0.445	-0.959***	-0.969***
Cellulose	12	-	-	0.548**	0.520*	-	-
Total sugars	12	-	-	-	-	0.928***	0.951***

\*, \*\*, \*\*\*, p < 0.1, 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

Table 25 - Correlation coefficient (r) for fungal population, bacterial population, moisture content, pH, total nitrogen, weight loss, cellulose, total sugars, amylase, cellulase and invertase activities of buried litters of Alnus nepalensis.

Sources of variation	D.F.	Amylase		Cellulase		Invertase	
		Open	Closed	Open	Closed	Open	Closed
Fungal population	9	0.939***	0.963***	0.703**	0.812***	0.134	0.218
Bacterial population	9	0.709**	0.782***	0.422	0.421	0.026	-0.070
Moisture content	9	0.692**	0.756***	0.332	0.401	-0.136	-0.095
pH	9	0.557*	0.541*	0.487	0.511	0.310	0.322
Total nitrogen	9	0.693**	0.577*	0.946***	0.914***	0.862***	0.865***
Weight loss	9	0.044	0.239	-0.488	-0.374	-0.898***	-0.904***
Cellulose	9	-	-	0.565*	0.482	-	-
Total sugars	9	-	-	-	-	0.859***	0.899***

\* , \*\* , \*\*\* ,  $p < 0.1$ , 0.05 and 0.01 respectively.

Without asterisk statistically insignificant at these levels.

of the litter. However, it showed a significant negative correlation with weight loss (Tables 24 and 25) of alder litters in both the forest stands.

## DISCUSSION

Cellulase activity on decomposing leaf litter of A. nepalensis was maximum in May-June <sup>by now</sup> might be attributed to the maximum build up of the microbial population harboured during that period. The improved activity was assigned to its significant positive correlation with fungal and bacterial numbers (Kshattriya, 1990). The positive correlation between cellulase enzyme and moisture content has also been inferred by Speir and Ross (1981) and Kshattriya et al, (1992). Production of cellulase is markedly influenced by moisture content of litter (Gresel et al., 1983 and Ross, 1981). Kshattriya (1990) has observed a negative correlation between cellulase enzyme and cellulose content. However, the present investigation showed a positive correlation between cellulase activity with cellulose content. But in principle, the rate of cellulose degradation in leaf litter should be related to the level of cellulase activity. However, the relationship is complex because at least three types of hydrolytic enzymes are required along with additional oxidative ones as well (Ljundahl and Eriksson, 1985). Linkins et al.

(1990b) found that an index of cellulase interaction, the product of exocellulase and endocellulase activities, was correlated with the rate of disappearance of cellulose from decomposing litter, both within and between species. This relationship suggested that interspecific differences in cellulase activity might be functionally related to differences in decomposition rates.

Slow amylase activity at the beginning of litter decomposition increased steadily with the progress of litter decay and which again decreased towards the end of the process. The changes in amylase activity during litter decomposition signified to the changes in the numbers of microorganisms. This confirms the probable microbial origin of the enzyme (Ross and Roberts, 1973). The results of Ross (1981) and Kshattriya (1990) have supported the findings and have mainly related the activity to the fungal population colonizing the litter.

Invertase activity showed an increasing trend in the initial stage of litter decomposition and subsequently decreased towards the end of the process. The low invertase activity towards the end of the decomposition may have resulted from comparatively its low synthesis by microbes either regulated by environmental changes or species composition (Ross, 1981). Ross (1981) also

observed more invertase activity in fresh leaves and less in an organic horizon.

The results of the present investigation have suggested that the quality of litter and microbes in the forest floor seemed to be the chief determinants of the enzymes activity gradient. Soluble polysaccharides, cellulose and lignin were important driving force of the enzyme activities.

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CHAPTER VI

INFLUENCE OF ISOPODS ON MICROBES POPULATION IN LEAF  
LITTER DECOMPOSITION UNDER LABORATORY CONDITION

## INTRODUCTION

Natural ecosystems are inhabited by large number of arthropods, along with other small animals, which depend primarily on a microflora food source. The role of this segment of the soil microbiota in organic matter decomposition and nutrient cycling is well established and now there is growing evidence that these microphagous animal populations can modify microbial activities in the rhizosphere sufficiently to influence a number of plant growth-related phenomena including root disease. ✓

Animals affect the decomposition of dead plant material by eating and assimilating part of it and by egesting the remainder which may be preferentially invaded by microorganisms. They may also physically disturb the litter and alter drainage and aeration. Soil invertebrates have indirect <sup>!e</sup> affects on decomposition by affecting the processes as conversion of litter to faeces, fragmentation of litter mixing of litter and soil, regulation of microflora and reducing the immobilisation of nutrients through senescent fungal tissue (Edwards et al., 1970 and Crossley, 1977).

Visser (1985) pointed out that soil fauna can

influence soil microorganism in three main ways: (i) by communication, mixing and channelling of organic matter and soil, (ii) by grazing on the microflora, (iii) by dispersal of components of the microflora. Animal feeding activities are thought to increase the surface area of plant material exposed to tissues and maintain fungal and bacterial populations in an active growth phase. In addition there is evidence that the animals can affect gross qualitative changes in the litter microflora.

Previous studies have shown that decomposition rate of an organic substrate is enhanced after passage through an invertebrate gut (Parle, 1962b; Witkamp, 1966). The role of the woodlice fauna in litter decomposition has not been clearly elucidated. Most investigators consider these organisms to be decomposers, their major contribution being the mechanical breakdown of litter (Van der Drift, 1949; Gere, 1956; Paris, 1963; Reichle, 1965). It has long been known that woodlice eat prodigious quantities of food as indicated by the large number of faecal pellets produced (Murlin, 1902).

Thus the aim of this study was to examine the influence of isopods on microbes population and occurrence of microorganisms in the guts, food and faeces of isopods, clearly, different types of feeding habits might be expected

to influence decomposition rates in different ways and an understanding of the feeding attributes of fauna is important in assessing its role in decomposition. ✓

#### MATERIALS AND METHODS

Alnus nepalensis leaves were collected shortly after leaf fall from the litter layer of a study site, air dried at 20°C and brushed free of faecal material and debris. Midribs were removed and the remaining lamina cut into small fragments of 0.5-1.0 cm. The fragments were mixed well and aliquots 1.5 g placed in microcosm chambers described by Anderson and Ineson (1932). The litter in each chamber was rehydrated overnight in 100 ml distilled water. This treatment served to leach out soluble tannins and readily metabolizable materials, which can alter fungal activity (Harrison, 1971). The leaf litter was drained and inoculated with a coarsely sieved suspension of macerated fresh leaf litter and then incubated at 15°C. Isopods were not added to the microcosms during the initial three week period permitting establishment of microflora.

Isopods were collected from the sites used to provide experimental materials. Isopods were collected by hand and stored at 15°C in plastic sandwich boxes filled with litter until needed. Before being introduced into

microcosms, Isopods were starved for 72 hr, to void their guts of prior contents. The small amount of food that remained in the gut was egested upon resumption of feeding. 0, 2, 5, 10 and 15 isopods were added to the microcosm chamber and the experiment ran for 35 days after the addition of isopods. Standing crop estimates were made after 5, 10, 20 and 35 days. Three replicates were destructively sampled on each occasions.

Litter derived from the sampled microcosms were examined for fungal and bacterial standing crop, using the membrane filter technique (Hanssen et al., 1974). A sample of 0.5 g leaf litter was homogenized in 10 ml of sterile distilled water and 1 ml sub-samples of the homogenate were stained for 30 min. with 1 ml 0.1% (W/V) aqueous phenylalanine blue. The stained material was flushed through a 25 mm cellulose acetate membrane (pore size 0.22  $\mu\text{m}$ ) and mounted for microscopic examination. Bacterial numbers and hyphal lengths were converted to standing crop estimates (Parkinson et al., 1971). Three replicates were made for each sample and ten fields of view were examined per filter.

Subsequent experiments were carried out using groups of 15 animals to determine the distribution of fungi and bacteria in isopod guts. Litter, gut and faecal

samples were collected for analysis as described below.

Samples of litter were removed from the experimental microcosms and taken for dry weight determination at 105°C. Simultaneously, approximately 0.5 g wet weight of litter was removed and placed in a sterile conical flask containing 4 ml of 0.1 % peptone water and shaken vigorously by hand for 5 min. to disintegrate the sample materials. The resulting homogenate was coarsely filtered through a sterile 2 mm pore mesh nylon filter with the filtrate being used to prepare a dilution series for subsequent determination of viable counts of bacteria and fungi. Faecal material which had collected in the bases of the microcosms was handled in the same way.

Isopods from the microcosms were surface sterilised by gentle agitation in sodium hypochlorite (1.6% available chlorine) for 1 min. The animals were carefully blotted dry on sterile paper and the body cavity was open ventrally and flooded with insect Ringer's solution (Griffiths and Tauber, 1940). The gut was partitioned in situ by a double ligature placed between the midgut and hindgut, and an additional single ligatures were placed around the oesophagus and rectum. The midgut and hindgut were dissected free and agitated gently in 3-4 changes of Ringer's solution to remove contaminants (Anderson and Bignell, 1980). The

sections were then transferred to 4 ml of 0.1 % peptone water in a conical flask and shaken vigorously by hand for 5 min to desintegrate the gut wall and disperse the contents. A sample of the homogenate produced was filtered through a tared weighed and dried millipore membrane filter (pore size 0.45  $\mu\text{m}$ ), gut tissue and ligatures being removed, and the oven weight of litter in that section of the gut determined.

Dilution series in peptone water were prepared from isopod guts and faecal samples. Fungi were isolated from the gut and faecal samples on Rose Bengal agar (Martin, 1950) containing streptomycin to suppress bacterial growth and bacteria was done on nutrient agar medium (Difco manual, 1953) using Nystatin ( $100 \text{ ug ml}^{-1}$ ) to suppress fungi. Three replicates were made at each dilution. The plates were incubated at  $10^\circ\text{C}$  for 5-10 days for fungi and 3 days for bacteria and microbial colonies were counted.

## RESULTS

Analysis of changes in bacterial and fungal populations grazed by different numbers of isopod are shown in Fig. 24. The fungal standing crop in the ungrazed microcosms remained constant. In the grazed microcosms series, the fungal standing crop was reduced by all levels of isopods feeding. By the end of the experiment the amount

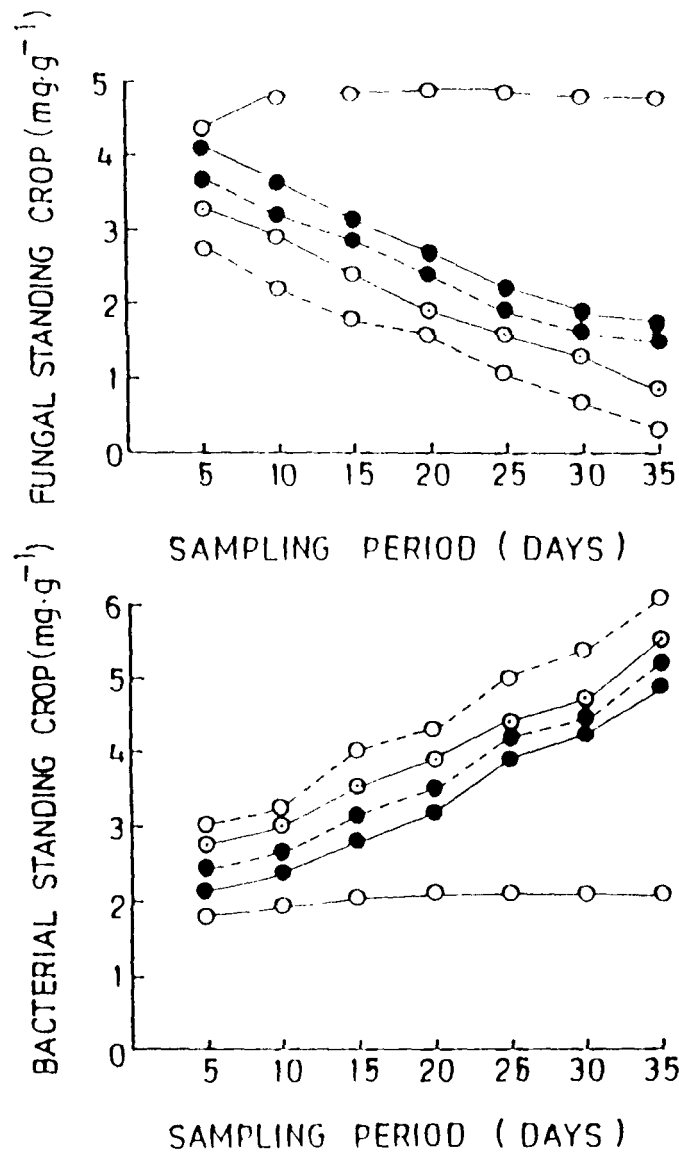


Fig 24. Variation in fungal and bacterial standing crops of fragmented leaf litters (*Alnus nepalensis*) grazed by different numbers of isopod ( 0—0 - control, ●—● = 2, ●---● = 5, ○—○ = 10 and 0-----0 = 15)

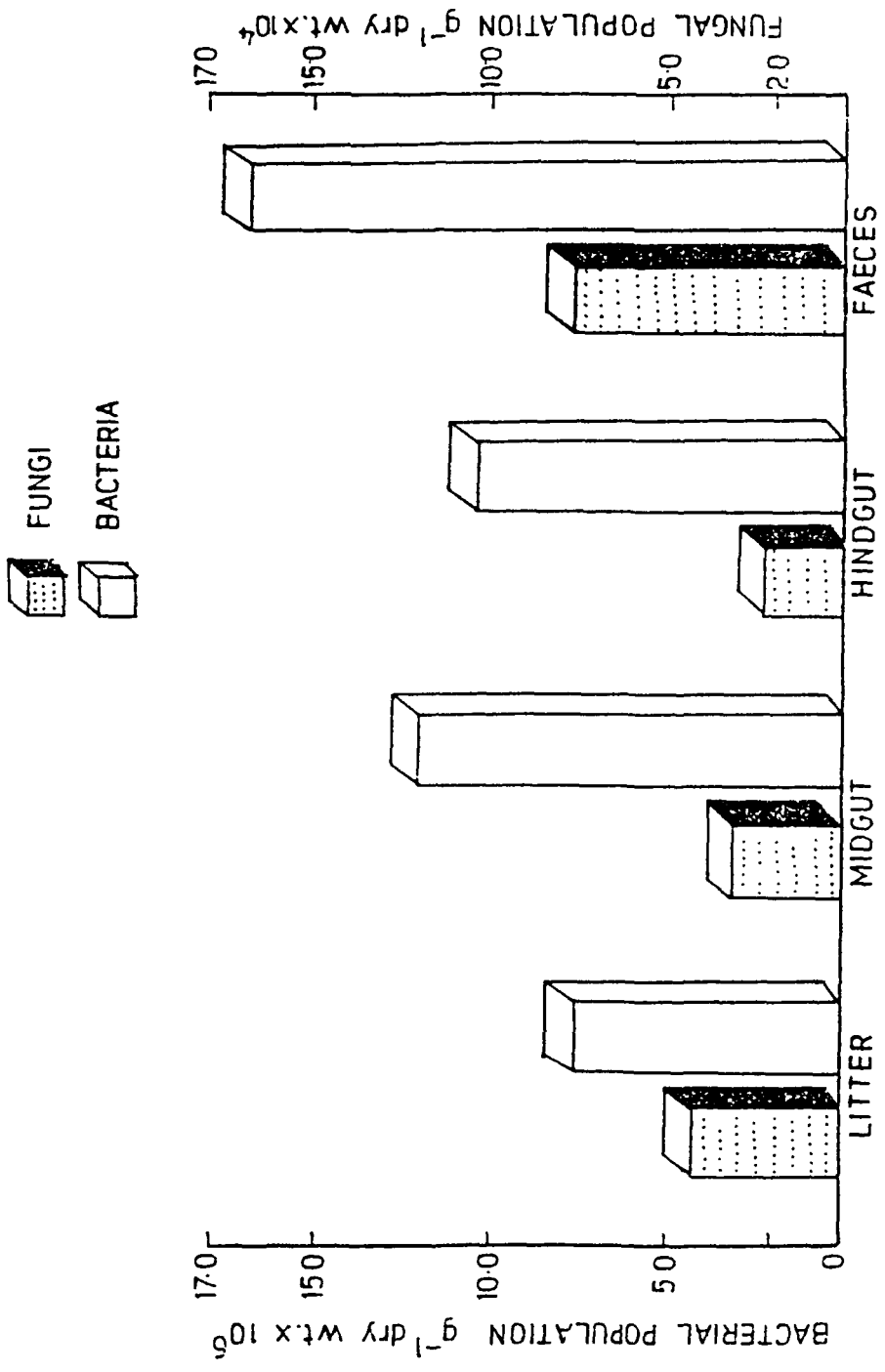


Fig 25. Distribution of fungal and bacterial population in litter, midgut, hindgut and faeces of isopods.

of fungal population in the grazed microcosms series dropped markedly (Fig. 24). Bacterial standing crop, however, increased with the numbers of isopods and after 35 days microcosm chamber with 15 numbers of isopods contained 5 times more bacteria than controls.

Observations of the gut contents of isopod indicated that they were actively grazing the fungal hyphae. Fungi isolated from the faecal homogenates, gut contents were Mucor alternans, M. hiemalis, Acremonium indicum, Aspergillus flavus, A. niger, Cladosporium cladosporoides, C. herbarum, Fusarium moniliforme, Humicola grisea, Penicillium chrysogenum, P. funiculosum, Trichoderma viride and white sterile mycelia. Extensive bacterial growth occurs in the guts of isopods. Bacterial and fungal counts in isopod faeces from the microcosms were higher than the leaf litter (Fig. 25). Dilution plate techniques showed no significant change in bacterial and fungal populations from the midgut to the hindgut.

## DISCUSSION

In the ungrazed microcosms fungal and bacterial development remained constant whereas in the grazed microcosm series, the fungal standing crop was reduced by all levels of isopod feeding. Bacterial standing crop, however, increased with the numbers of isopod similar to the results

of Hanlon and Anderson (1979, 1980). Bacteria were stimulated by macro arthropod feeding activities at different levels of isopod feeding. the fungal standing crop decreased markedly with the increase in number of isopods. Parkinson et al. (1979) and Hanlon and Anderson (1979) also observed that grazing rates may exceed the production of fungal hyphae but Leonard (in Anderson and Ineson, 1983) has shown that the balance of these processes is determined by the physical structure of the substrate and the available nutrient supply. Parkinson et al. (1979) showed that such grazing may be species selective in that certain members of the fungal flora of litters, remained unaffected or may even be stimulated at high animal levels. Although such specific grazing effects may be occurring in such microcosms, the net effect was a reduction in total fungal standing crop.

Observation of the gut contents and faeces of isopods indicated that they actively grazed fungal hyphae and extensive bacterial growth occurs in their gut contents. The bacterial and fungal counts in isopod faeces from the microcosms were higher than the leaf litter. Drift and Witkamp (1959) found that the faecal pellets of soil arthropods were a more favourable habitat for litter microorganisms than whole leaf litter. Several workers have

recorded greater microbial activity or higher members of decomposer microorganisms in the freshly produced faeces of soil animals than in comparable food material (Van der Drift and Witkamp, 1959 and Parle, 1963). The increases in numbers of bacteria occurring on gut passage of isopod is in accordance with the observation of Nicholson et al. (1966). Reyes and Tiedje (1976) demonstrated that the gut of isopods is a favourable environment for bacterial growth and although some digestion of bacterial cells occurred, the net effect was an increase in counts in faeces with respect to the food. Anderson and Bignell (1980) concluded from circumstantial evidence that the growth response by bacteria in the gut of G. marginata was by the ingested litter microflora and not indigenous gut symbionts. Szabo et al (1969) have shown that the gut environment can be highly selective and the proliferation of a single species can assist both in metabolism and in the elimination of other microflora ingested with the food. If, however, the gut environment provided a general stimulus to microbial growth and viability a correlation would be expected between microbial numbers in litter and the gut populations. ✓

Hissett and Gray (1976) have calculated that the growth rate of bacteria in natural soils is slow with

generation times of several days. The gut of soil animals, however, represents a more favourable environment for microbial growth (Parle, 1963, Szabo et al, 1969; Stefaniak and Seniczak, 1976; Reyes and Tiedje, 1976). Consumption and gut passage are also likely to affect the species complement of microorganisms in leaf litter whether as a result of dynamic feeding activities or the static affects of a single gut passage. Reyes and Tiedje (1976) showed that the digestion of Flavobacterium and Pseudomonas was inversely related to the feeding rates of the animals. The nutritional quality of the food may affect bacterial growth in the animal and both palatability and the feeding rate could affect differential digestion of microbial species and the rate of removal from the gut (Hanlon and Anderson, 1980). Thus the environment offered by the gut and faecal pellets may stimulate microbial metabolism and lead to greater viability on the plating medium.

On the basis of evidence and similar arguments put forward by Macfayden (1961, 1963), it can be concluded that the overall rate of decomposition of plant debris is increased when the material is converted to faeces.

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CHAPTER VII

EFFECTS OF ISOPODS AND MICROBES ON  
THE NUTRIENT RELEASE FROM THE LEAF  
LITTER UNDER LABORATORY CONDITION

## INTRODUCTION

In most terrestrial ecosystems, the major fraction of terrestrial net primary production enters the soil-litter of organic matter and nutrients enter the soil litter system and are of great importance of ecosystem dynamics. System productivity depends not so much on the total supply of mineral elements in the soil but on the rate of circulation of matter, the destruction and mineralization of production, and the assimilation of products passing into the soil litter layers (Ghilarov, 1970). Destruction and mineralization of litter results from interaction of biological, biochemical and physical processes. Organisms that contribute most to these processes are microflora and many invertebrate groups.

Soil arthropods browsing on senescent microbial populations in decaying leaf litter has been considered a mechanism by which soil animals may enhance decomposition processes by both the mobilization of nutrient and by annulment of biostatistics (Engleman, 1961; Macfadyen, 1963). Fauna affect rates of mass loss from decomposing substrates, directly by ingestion and indirectly by grazing on microbes. The effects of substrate commi-

nution are major phenomenon in ingested ecosystems, where a large fraction of mass loss can be attributed directly or indirectly to the presence of fauna.

Microorganism are the main agents of litter catabolism, but the role of saprophytic invertebrates in enhancing energy and nutrient flux rates through microbial populations has been demonstrated in both aquatic and terrestrial systems (Anderson and Macfadyen, 1976).

In forests and grassland, the importance of faunal activities is well recognised, they influence decomposition systems, rates of nutrient regeneration, soil structure and so forth (Swift et al., 1979; Seastedt, 1984; Mitchel and Nakas, 1986). The importance of animal microbial interactions for energy and nutrient flux rates in aquatic systems has been demonstrated in the field (Nixon et al., 1976) and in the laboratory microcosms (Hargrave, 1970, 1976; Fenchel and Harrison, 1975; McDiffett and Jordan, 1978).

A major proportion of potentially limiting nutrients (N, P, K) in the soil pool may be maintained within the fungal population (Ausmus et al., 1976). The immobilization of plant litter nutrients by microorganisms may cause the accumulation of nutrients in senescent microbial tissue

which may be responsible for the stagnation of nutrient turnover in some soil systems and may slow down the rates of mineral cycling in all compartments of the ecosystem (Witkamp et al., 1966). The intensity of grazing on senescent fungal colonies by soil animals may, therefore, determine the rate of mineral and nutrient cycling within the system. The degree of animal activity may also be responsible for differences in the rate of nutrient turnover in different humus form. The greater surface area resulting from faunal feeding increases substrate area for microfloral activity and for nutrient leaching.

In the experiment described here a microcosm system was used to follow changes in isopod numbers, microbial standing crop and cation release over several months in grazed and ungrazed litter. The aim was to assess the functional significance of isopod grazing in nutrient release from decomposing leaf litter. ✓

#### MATERIALS AND METHODS

To study the effect of isopods and microbes on mineralization of nutrients from the litter microcosm chamber were developed as described by Anderson and Ineson (1982). Leaf litter of Alnus nepalensis was collected shortly after leaf fall from the forest site under investigation. After collection, surface litter was air dried

at room temperature and brushed free of faecal material and debris. Midribs were removed and the remaining laminae cut into small fragments (2-4 mm). The fragments were mixed well and aliquots 2 g placed in experimental chambers.

The litter in each chamber was rehydrated by the addition of 100 ml of distilled water for 24 hr, which served to leach out soluble tannins and readily-metabolized materials mobilized by the drying and wetting regime. Fresh leaf litter was macerated in distilled water to produce a suspension which was used to inoculate the soaking litter and then incubated at 15°C. The chambers were leached weekly with 60 ml of distilled water, leachates being retained for chemical analysis. Animals were not added to the microcosms during an initial three week period permitting establishment of a microflora.

Burmoniscus species of soil isopods were collected from the sites used to provide experimental materials and stored at 15°C in plastic sandwich boxes filled with litter until needed. Thereafter, 0, 2, 5, 10 and 15 isopod species were added to the experimental chambers and the experiment was carried out for 12 weeks.

#### **Chemical analysis of leachates**

The leachates were collected from the microcosms

at weekly interval. The leachates were analysed for  $K^+$ ,  $Mg^{++}$  and  $Ca^{++}$ . The leachates were stored at  $5^{\circ}C$  until analysis was completed which was normally done within a week of sampling. Analysis for  $K^+$ ,  $Mg^{++}$  and  $Ca^{++}$  was carried out on atomic absorption spectrophotometer using the methods of Allen et al. (1974).

Results were converted to mg of element released per microcosm per week and statistical analysis was carried out using analysis of variance to test the effects of time and elements on the population of isopod grazing from leaf litter.

## RESULTS

The effects of isopod density on nutrient release from alder leaf litter are shown in Figure 26. Of the cations analysed only  $K^+$  showed significant leaching response to grazing. Losses of  $K^+$  from controls showed similar pattern to the density gradient of isopods but an increase in leaching rates was observed in the later sets than the earlier ones (Fig. 26). The increase in the mobilization of  $K^+$  was significantly higher in isopods grazed litter than the ungrazed ones. The enhancement of  $K^+$  release resulting from grazing of 10 and 15 isopods only became apparent 7 weeks after addition of isopods and was related to the isopod population in microcosm chambers.

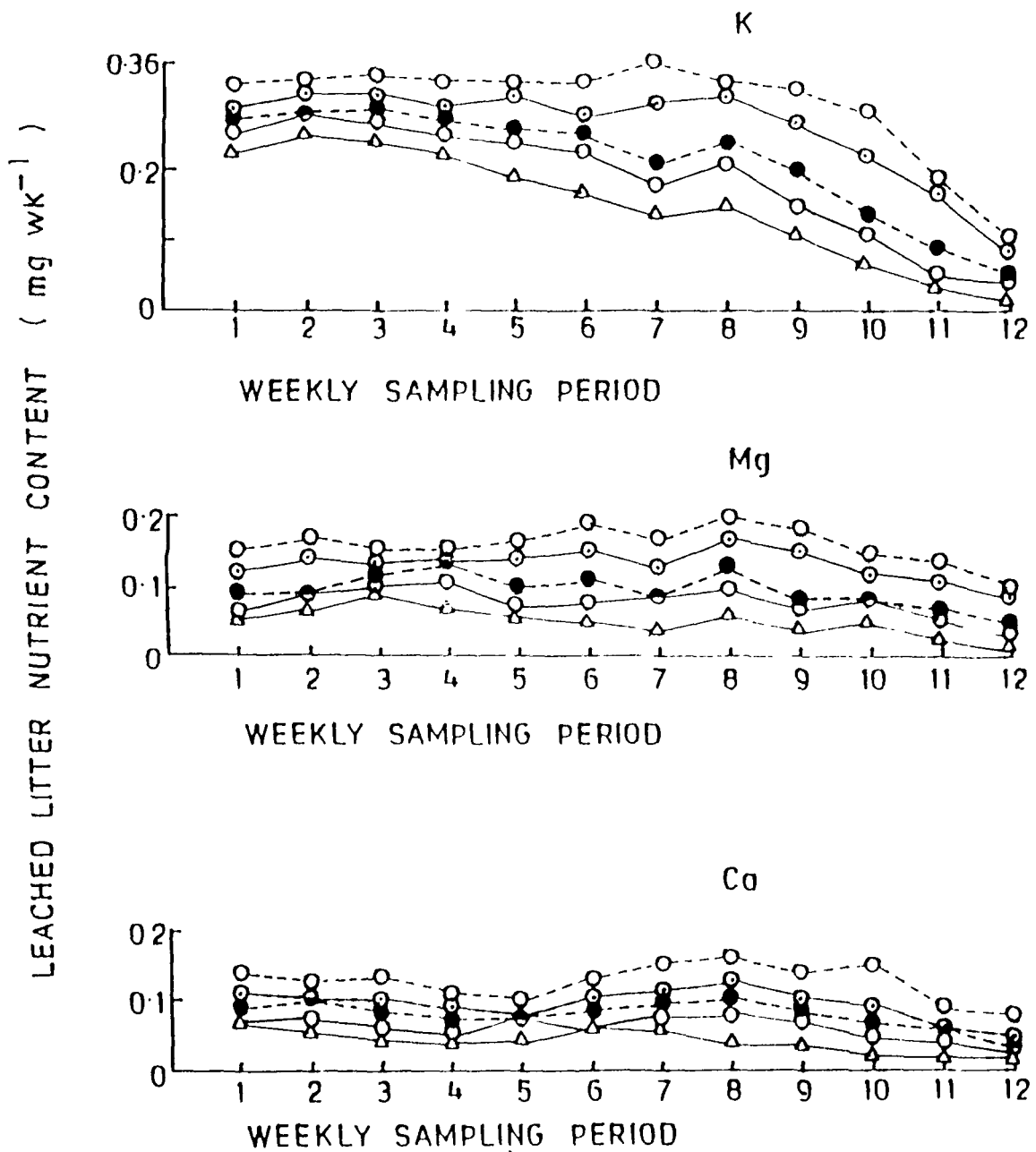


Fig 26.

Weekly variation in nutrient release of potassium (K), Magnesium (Mg) and calcium (Ca) added by different numbers of isopod ( $\Delta$ — $\Delta$  = control,  $\circ$ — $\circ$  = 2,  $\bullet$ — $\bullet$  = 5,  $\ominus$ — $\ominus$  = 10,  $\circ$ — $\circ$  = 15) in leaf litter of *Alnus nepalensis*.

Table 26 - Analysis of variance to test the effects of time and elements (K, Mg and Ca) on the populations of isopods grazing from leaf litter.

Source of variation	d.f.	MS	F
Time effects	11	0.026072	6.46**
Element effects	2	0.305656	75.72**
Animal effects	4	0.035613	8.82**
Time x Element	22	0.008759	2.17**
Animals x Elements	44	0.006403	1.59**
Elements x animals	8	0.005978	1.48

\*\* = Significant at  $p < 0.01$

$Mg^{++}$  concentration in leachates was higher in almost all the treatments with different density of isopods but the difference was insignificant in relation to control levels. Increase in  $Mg^{++}$  concentration in leachates of treatment was low compared to  $K^+$ . Similarly, there was little enhancement of  $Ca^{++}$ . The pattern of  $Ca^{++}$  loss from ungrazed litter exhibited a similar trend to  $K^+$  and  $Mg^{++}$  leaching. The loss of  $Ca^{++}$  ions was however less from the litter (Fig. 26).

The amount of cation release ( $K^+$ ,  $Mg^{++}$  and  $Ca^{++}$ ) from decomposing alder litter was enhanced with the addition of high numbers of isopods in all treatment (Table 26).

## DISCUSSION

Isopod feeding activities had accelerated leaching of  $K^+$ ,  $Mg^{++}$  and  $Ca^{++}$  from the leaf litter of A. nepalensis. Over the 7 week period significantly greater amount of  $K^+$ ,  $Ca^{++}$  and  $Mg^{++}$  was leached from the grazed microcosms than from ungrazed ones (Fig. 26). These differences occurred earlier with  $K^+$  (7 weeks) than  $Ca^{++}$  and  $Mg^{++}$  (8 weeks) which gradually decreased with time. The dynamic of nutrients is complicated since they appear in different forms and are subject to various transformations such as leaching, immobilization and mineralization.  $Mg^{++}$

and  $K^+$  are usually available in excess of saprotrophs requirements. These elements are highly soluble inorganic ions which leached rapidly from decaying litter. The loss rates generally decrease with time as concentration in the litter approach those of microbial tissues (Gosz et al. 1973). The pattern of  $Ca^{++}$  loss from microcosms showed a similar trend to  $K^+$  and  $Mg^{++}$ . However, initial leaching losses were lower (Fig. 26).  $Ca^{++}$  unlike  $K^+$  and  $Mg^{++}$  is a structurally important ion in higher plants and therefore subjected to less leaching (Ineson et al., 1982).

Our experiment demonstrated that soil isopod exerts a positive influence on nutrient release from leaf litter of A. nepalensis (Table 26). The grazing of fungal hyphae or leaf material or both by the isopods resulted to an increased nutrients loss from the litter. The greater litter surface area resulting from faunal grazing increases substrate area for microfloral activity and for nutrient leaching (Douce et al., 1982). Fungal mycelium in forest floor can contain a major proportion of nitrogen, potassium and other cations in the soil pool (Cromack et al., 1975) and thus the sensitivity of fungal hyphae to microarthropod grazing may well have considerable significance for nutrient mobilization (Hanlon and Anderson, 1979; Parkinson et al., 1979). Our observation agrees in general with the

results of Ineson et al., (1982), Anderson et al., (1983) and Huish et al., (1983) who<sup>s</sup> observed increasing leaching of ammonium and other cations from decomposing oak and beech litter in the presence of several representative types of fauna that feed the litter. Hanlon and Anderson (1980) reported marked decreases in fungal biomass and greater increase in the bacterial standing crop of decomposing oak litter inoculated with microarthropods. This has been attributed to grazing from microsites influencing microbiological activity (Anderson et al., 1983) which in turn could significantly alter nutrient dynamics.

Microbial lysis and autolysis undoubtedly contribute to the turnover of bacterial and fungal tissues (Mitchell and Alexander, 1963; Shield et al., 1973) but the result of microcosm experiment suggested that soil fauna feeding activities are quantitatively more important. The feeding activities of soil fauna are also widely stated to stimulate microbial growth and activity through grazing of senescent tissues.

These studies supported the hypothesis that soil fauna strongly influence the nutrient dynamics of the forest floor with insignificant changes in soil metabolism (Anderson et al., 1981; Anderson and Ineson, 1983; Coleman et al., 1983).

The present investigation revealed that the grazing of microorganisms or leaf material or both by the isopods had either stimulatory or inhibitory effects on microbial population as a result of differential action on fungi and bacteria. The long term significance of these processes for leaf litter decomposition and nutrient cycling remains to be determined but they are likely to be real phenomena under natural conditions.

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**GENERAL DISCUSSION**

The composition of air spora differed in both the forest stands. The majority of the forms isolated from the air belonged to deuteromycetes which accounted for approximately 80 per cent of the total mycoflora. Species of Cladosporium, Alternaria, Penicillium and Aspergillus were the dominant forms in the air at both the forest sites which was attributed to their ability to produce and discharge large number of spores. Fungi belonging to Phycomycetes, Ascomycetes and sterile mycelial forms were poorly represented in the air which may probably be due to their poor survival in the air. A fall in number of Cladosporium propagules during hot months was correlated to high temperature and low humidity as has been reported by Frey and Durie (1960) and Taylor and MacFadden (1962). However, these observations were in contrast to the findings of Hyde and William (1953). Kramer et al. (1959), Morrow (1947), Ripe (1962) and Targow and Plunkett (1951). Population of C. herbarum in air was maximum during winter season indicting the resistance of the fungus to low temperature. ✓

Dominance of species of Alternaria, Aspergillus and Penicillium in closed forest than open ones was attri-

buted to the inhibition of spore dissimulation due to physical barrier due to high density of trees in the earlier site than the later ones. Sreeramulu and Seshavataram (1962) have also noticed high number of spores of Penicillium spp. in paddy field than in open field. A. niger and A. flavus occurred frequently from the air of both the forest sites. Alternaria alternata was dominant during warm months as has also been reported by Sinha (1971), and Dickinson (1967, 1981). Curvalaria sp. showed a rapid rise at the beginning of warm and moist season and suggested that it is favoured by warmer conditions. Resistance to high temperature is also assigned due to melanin pigment and its host range growing in that period (Sreeramulu and Seshavataram, 1962).

Fusarium sp. was most common during a period of heavy rain in September and October (Figs. 3c and d) and was favoured by closed forest site. Helminthosporium was rare and showed its peak in August and September in the open forest site and in April-June in closed site when temperature and relative humidity was high. Nigrospora was favoured by dry period in the open site and it exhibited a peak before the onset of heavy rain. The increased number of spores of the fungus was correlated to the availability of suitable hosts and climatic factors (Cammack,

1955), Trichoderma was dominant during hot and wet season in both the sites corresponding to the increase in the population on the leaves which might have been released into the air.

The total air fungal population increased with increase of phylloplane fungi in both the forest sites. The number of fungal species fluctuated at different sampling periods due to changes in climatic conditions (Mishra and Srivastava, 1971a; Mishra and Tewari, 1976a; Kumar and Gupta, 1980). The fungal spores produced on the leaf surface might be getting released into the air which attributed to the richness of air spora of the locality. The distribution of air fungi was also affected by the climatic condition especially during rainy season (Dickinson, 1967). The fall in number of fungi during the hot and wet months was supported by observation of Taylor and McFadden (1962). The increase in fungal population in the air after rains may be due to their release from the leaf surfaces. Temperature changes in atmosphere had considerable influence on spore viability in general and larger spores in particular (Gregory, 1961; McDonald, 1962) which could account for reduced numbers excluding Penicillium and Aspergillus during hot and wet months. Factors such as humidity and wind velocity were contributed to the diurnal periodicities

of fungal spores in the air (Mishra and Tiwari, 1976a and Kumar and Gupta, 1980).

Invisible qualitative changes in leaf surface mycoflora of alder forests was mainly attributed to insignificant dissimilarity in nutrient status of leaf resources. The representatives of Phycomycetes and Ascomycetes were weak colonizers whereas Deuteromycetes were strong colonizers, showing better adaptation and higher competitive saprophytic ability (Rai, 1973 and Garg and Sharma, 1985). Maximum number of fungal species was isolated by moist chamber and dilution plates methods followed by impression plate and washed leaves plating techniques and least by direct observation method (Fig. 5). ✓

A steady increase in fungal population on the leaf surface of alder was correlated to the availability of nutrients (Kumar and Gupta, 1976 and Sinha and Dayal, 1983). The least fungal population at the bud or folded stage, soon after flushing, was perhaps due to the presence of thick waxy cuticle. The number of phylloplane mycoflora of matured leaves were higher than their corresponding senescent leaves possibly due to lower relative humidity and moisture content during winter (Sinha and Dayal, 1983). ✓

The dominance of Fusarium moniliforme, Trichoderma

viride, Penicillium spp. and Verticillium alboatrum on matured leaf indicates their preparatory role for the ensuing senescent stage. These species have been reported to colonize senescing plants parts (Lamb and Brown, 1970; Bainbridge and Dickinson, 1970 and Ericker, 1976). Dickinson (1976) has also advocated the increase in activity of saprophytes on senescent leaves primarily due to change in leaf physiology. In addition, prevalence of significant superficial colonist of fungi on different age of the leaves reflected the abundance of the species in the air during different sampling periods (Gregory and Hirst, 1957).

Low counts of fungi and bacteria in the initial stages of leaf litter decomposition may be due to the unavailability of soluble nutrients which were still in a complex form (Kshattriya, 1990). The improvement in litter moisture level, moderation of temperature and higher relative humidity (Fig. 1) favoured the build up of the microbial population in June-September (Fig. 2) particularly bacterial population which was correlated to rate of decomposition indicating the mobilization of the nutrients from the litter required for their growth (Das, 1980 and Kshattriya, 1990). This indicates that the prevailing temperature and moisture conditions were

favourable to allow bacteria and fungi to grow and multiply in rich decomposing litter. The decline in population of fungi and bacteria after September signified the adverse effect of low temperature and moisture during winter as has also been observed by others (Witkamp, 1963; Holm and Jensen, 1972; Das, 1980; Deka and Mishra, 1982 and Kshattriya, 1990).

The high fungal and bacterial population at open forest stand compared to the closed ones may be due to the dominance of temperature influence over moisture effect (Shanks, 1954 and Witkamp, 1963). Under the existing environmental conditions of forest soils, fungi viz., deuteromycetes and ascomycetes constituted the primary decomposer community on leaf litters suggesting that they could utilize locked up nutrients in litters while the appearance of phycomycetes as late successional species could only utilize the simpler nutrients either as fungal products or soluble forms. The difference in fungal species composition may be due to the fact that a variety of organic substances were released which favoured the groups of certain fungi while adversely affected the others (Garrett, 1951 and Alexander, 1961).

There was seasonal changes in the isopod densities in the soil cores of 0-2 cm, buried litter bags at 2-5 cm

and 5-10 cm depths. The isopod population showed a general trend of increase in both the forest stands with the advent of the warm and rainy season and tended to decrease with the approach of dry and cold season (Mukherjee and Singh, 1970 and Price, 1973).

The seasonal variation in soil isopods may also be due to their vertical movement in the soil. Such migration as an influence was also noted by Price (1973). The vertical distribution of the population of isopods between the three different depths showed a higher concentration in the buried litter bags layer (Fig. 9). Forces outside the soil community which influence population density were mainly the climatic factors (Drift, 1963). The present study showed a significant positive correlation between isopod population and the air and soil temperature. The high population of soil isopods in rainy season corresponded the excessively high moisture content of the soil and litter. The winter minima of isopods was assigned to dissiccation of the soil combined with low temperature (Choudhuri and Roy, 1967).

The rate of litter decomposition in fine mesh bag was slightly faster in the open forest stand compared to closed forest stand. This was attributed to the high microbial population encountered in open forest stand,

whereas in case of coarse mesh bags, higher mass loss from closed forest stand than from the open stand, may be attributable to the initial higher N content, narrower C/N ratio, litter texture and greater arthropod abundance (Anderson, 1973).

Greater mass loss from coarse mesh bags compared to fine mesh bags has also been reported by Gupta and Singh (1981) and Reddy and Alfred (1989). It could be due to the influence of mesh size on the access of different sizes of soil animals and greater abundance of certain taxa determining the commutation rate. Lesser decomposition in the fine mesh bags may be due to the inability of the soil animals to invade the bags. The difference in decomposition rate may be assigned to physical nature of plant material (Bhatt et al., 1985), difference in pH, moisture content and substrate quality, which ultimately govern the distribution of microflora (Swift et al., 1979 and Howard and Howard, 1980).

The slow rate of litter decomposition at the initial stages may be due to unfavourable abiotic conditions and low microbial population. The concomitant peaks of weight loss, microbial activity and population obtained during rainy season were attributed to the high moisture content of litter and warm conditions prevailing during the season.

This trend may be due to higher initial content of water soluble materials and simple substrates, the breakdown of the litter by decomposers, especially microflora and the removal of leaf particles by animals through comminution e.g., by isopods (Anderson *et al.*, 1983 and Swift *et al.*, 1981). A repression in the litter decomposition of factors like temperature and dry environmental conditions and the predominance of slow decomposing litter components such as lignin (Meentemeyer, 1978). William and Gray (1974) have also observed a drastically retarded decomposition and the number of saprophgous animals during drought period.

The organic constituents of litter were maximum in the beginning and minimum at the end of the decomposition process. This was attributed to their positive correlation with the weight remaining of decomposing litter (Tables 22 and 23). However, lignin was correlated negatively with weight remaining. This may be due to the resistant nature of lignocellulosic complexes (Summerall and Burger, 1989). The preferential use of sugars, amino acids, cellulose and hemicellulose in the beginning indicates their role in the microbial growth (Harper and Lynch, 1981).

The increase in nitrogen content in the beginning

of decomposition in both the forest stands (Figs. 20 and 21) may be due to a demand by heterotrophs in which nitrogen gets immobilized (Lousier and Parkinson, 1978). The decrease in nitrogen content at later part of rainy months may also be attributed to the leaching of labile nutrients (Kshattriya, 1990). An increase in the initial litter phosphorus content was also observed or which was due to its immobilization in microbial tissue (Kshattriya, 1990).

Cellulase activity on decomposing leaf litter was maximum in May-June which might be attributed to the maximum build up of the microbial population harboured during that period. The improved cellulase activity was assigned to its significant positive correlation with fungal and bacterial numbers (Kshattriya, 1990). The positive correlation between cellulase activity and moisture content has also been inferred by Speir and Ross (1981) and Kshattriya *et al.* (1992) and concluded that the moisture content favoured the synthesis of cellulase. Amylase activity was less at the beginning of litter decomposition but showed a steady increase with the progress of litter decay after which it decreased towards the end of the process. The changes in amylase activity during litter decomposition signified to the changes in the numbers

of microorganisms. The significant positive correlation of amylase and cellulase with fungal and bacterial populations confirmed their microbial origin (Miele and Linkins, 1978). Invertase activity was more in the beginning and subsequently decreased towards the end of the decomposition may have resulted from comparatively its low synthesis by microbes either regulated by environmental changes or species composition (Ross, 1981). A significant positive correlation between invertase and remaining amount of sugars has suggested the substrate specific of the enzyme.

Bacterial and fungal population grazed by different density of isopods showed that the fungal and bacterial standing crop in the ungrazed microcosm remained constant whereas in the grazed microcosm series, the fungal standing crop was reduced by all levels of isopod feeding. Bacterial standing crop however, increased with the numbers of isopod similar to the results of Hanlon and Anderson (1979, 1980). Parkinson *et al.*, (1979) and Hanlon and Anderson (1979) have also observed that grazing rates may exceed the production of fungal hyphae and the balance of these processes was determined by the physical structure of the substrate and the available nutrient supply (Anderson and Ineson, 1983).

Observation of the gut contents and faeces of isopods indicated that they actively grazed fungal hyphae

and extensive bacterial growth occurs in their gut contents. The bacterial and fungal counts in isopod faeces from the microcosms were higher than the leaf litter. Van der Drift and Witkamp (1959) found that the fungal faecal pellets of soil arthropods were more favourable habitat for litter macro organisms than whole leaf litter. The gut of soil animals, however, <sup>eg?</sup> represents a more favourable environment for microbial growth (Parle, 1963; Szabo et al., 1969; Stefaniak and Seniczak, 1976; Reyes and Tiedje, 1976). The environment offered by the gut and faecal pellets may stimulate microbial metabolism and lead to greater viability on the plating medium.

Isopod feeding activities had accelerated leaching of  $K^+$ ,  $Mg^{++}$  and  $Ca^{++}$  cations from the leaf litter of A. nepalensis. Over the 7 week period significantly greater amount of  $K^+$ ,  $Ca^{++}$  and  $Mg^{++}$  was leached from the grazed microcosm than from ungrazed ones (Fig. 26). The grazing of fungal hyphae or leaf material or both by the isopods resulted to an increased nutrient loss from the litter. The greater litter surface area resulting from faunal grazing increases substrate area for microfloral activity and nutrient leaching (Douce et al., 1982). Fungal mycelium in forest floor can contain a major proportion of nitrogen, potassium and other cations in the soil pool (Cromack

et al., 1975) and thus the sensitivity of fungal hyphae to arthropod grazing may well have considerable significance for nutrient mobilization (Hanlon and Anderson, 1979; Parkinson et al., 1979). Hanlon and Anderson (1980) reported marked decreases in fungal biomass and greater increase in the bacterial standing crop of decomposing oak litter inoculated with macroarthropods. This has been attributed to grazing from microsites influencing microbiological activity (Anderson et al., 1983) which in turn could significantly alter nutrient dynamics.

The results show that the litter decomposition rates were influenced by litter quality, soil and litter moisture, combined action of leaching of water soluble substances and activity of soil animals and microorganisms.

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## SUMMARY

The study was carried out at two forest stands i.e., open and closed forest stand dominated by alder (Alnus nepalensis) which is located at Upper Shillong 5.5 km away from Shillong, the capital of Meghalaya. The study sites lie between 25°34'N latitude and 91°56'E longitude and at an altitude of 1500 m MSL. The present work was designed to investigate the distribution and successional pattern of microorganisms and soil isopods and their influence on the biodegradation of A. nepalensis leaf litter.

A total number of 30 air fungal species belonging to 21 genera was recorded during the present investigation. The air spora of both the sites belonged to 3 main groups of fungi viz., mucorales, ascomycetes and hyphomycetes. Phycomycetes, ascomycetes and sterile mycelial forms were poorly represented in the air of both the sites. Cladosporium herbarum, Aspergillus spp., Alternaria spp. and Trichoderma spp. were dominant species at open forest site. The abundance of Fusarium, Geotrichum, Penicillium and Trichoderma was favoured by the closed forest site.

Number of fungal species fluctuated at different sampling periods. At both the forest stands, the population

of air fungi exhibited a positive correlation with phylloplane fungi isolated by moist chamber technique whereas the correlations were not significant statistically with the rest of isolation techniques of both the stands. However, the fungal population of both the litters showed a significant positive correlation with air spora of both the sites.

The fungal population of the phylloplane increased gradually from folded to senescent stage and the peak of fungal population was attained at the maturity of the leaves. The least fungal population was obtained at the bud or folded stage soon after flushing. The maximum fungal species were isolated in moist chamber and dilution plates followed by impression plate, washed leaves plating and least by using direct observation methods. The representatives of Phycomycetes and Ascomycetes fungi were found to be weak colonizers, whereas Deuteromycetes were strong colonizers. The phylloplane colonizers of both the forest stands revealed that fungi like Alternaria alternata, Cladosporium herbarum, Penicillium funiculosum and Trichoderma viride dominated the entire phylloplane region at different age of the leaves. The senescent leaves were dominated by Fusarium moniliforme, T. viride, Penicillium spp., Verticillium alboatrum.

The moisture content of leaves at both the forest sites was higher in rainy months and lower in winter and during senescent stage the moisture content abruptly dropped to lowest. At both the forest stands, the fungal population isolated by dilution plate showed a positive correlation with moisture contents. However, the correlation of pH with fungal population of both the leaves was not significant.

The fungal and bacterial populations in litter showed marked seasonal variations at both the forest stands. Quantitatively, the bacterial population was higher than the fungal population. In both types of mesh bag, bacterial and fungal populations were less in the beginning of litter decomposition, but increased with the progress of decomposition. Microbial population was minimum at the end of litter decomposition. Bacterial population exhibited peaks in June and September, while the fungal population showed its peak in June and August. During winter months, the litter harboured minimum microbial population. The litters in both mesh bags of open forest stand harboured more microbial population than the closed ones.

Nineteen fungal species were isolated from leaf litter in both types of mesh bag of both the stands. Penicillium chrysogenum, Fusarium oxysporum, Aspergillus flavus,

A. niger and Trichoderma viride were dominant species in both types of mesh bag of the two forest stands.

Fungal and bacterial population in both the cases showed significant positive correlation with the moisture content and pH of the litter.

Isopod population exhibited seasonal variation in their distribution in different soil depths. They were higher in number in upper most surface layer in spring (March-April) and deeper layer in winter (Nov-Feb) and summer (May-Oct). Three new species of dominant isopod i.e., Burmoniscus n sp. A, B. n. sp. B and B. n. sp. C were identified. The first two were dominant in occurrence in all the three depths. While the third one was represented by a very low number in the litter layer (2-5 cm) and its presence was restricted to a few months only. Population of isopods was highest in July in litter bags whereas in soil cores of lower layer (5-10 cm) a maximum count was obtained in October in both the forest stands. However, the population of isopod was considerably reduced in drier and winter months. It showed significant positive correlation with the air and soil temperature.

The leaf litter of open forest stand was more acidic compared to closed forest stand. Initially, the

litter was less acidic in both the sites which became more acidic in winter and at the end of decomposition. The moisture content of litter ranged from 11.5 to 70% in open forest stand and 10.5 to 75% in case of closed forest stand. The correlation between weight loss of litter and its moisture content was not significant in both the sites.

Quantitatively, organic constituents of litter were maximum in the early stage of decomposition than its final stage. Litter closed forest stand contained more amount of soluble compounds than of open forest stand. The rate of degradation of soluble component was highest followed by hemicellulose, cellulose and lignin. The decomposition of cellulose and hemicellulose exhibited a similar pattern in both the forest whereas lignin contents increased in the beginning and its degradation started towards the later part of decomposition. The absolute weight loss of litter showed a significant negative correlation with the weight remaining of different organic constituents. However, lignin content was correlated positively with absolute weight loss.

Initially, the nitrogen content increased up to the month of June followed by a subsequent decrease till the end of the decomposition in both the cases. Phosphorus

was initially retained for few months in the litter and then gradually released along with the decomposition of litter. The absolute weight loss of both the litters was correlated negatively with nitrogen and phosphorus.

The activities of amylase and cellulase were less at the beginning of litter decay in both the forest stands. However, they showed a steady increase with the progress of litter decay, while a reverse pattern was observed for invertase. Both amylase and cellulase showed a significant positive correlation with fungal and bacterial populations of litter. The amount of total sugars showed a significant positive correlation ( $P < 0.05$ ) with invertase activity. The invertase activity was also correlated significantly with total nitrogen of the litter. However, invertase activity showed a significant negative correlation with weight loss of litter.

The bacterial and fungal populations grazed by different isopods showed that the fungal standing crop in the ungrazed microcosms remained constant whereas in the grazed microcosms it was reduced by all levels of isopods feeding. At the end of the experiment, the amount of fungal population in the grazed microcosms series dropped markedly. Bacterial standing crop, however, increased with the numbers of isopods and after 35 days

microcosm chamber with 15 numbers of isopods contained 5 times more bacteria than controls. Bacterial and fungal count in isopod faeces from the microcosms were higher than the leaf litter. Dilution plate technique showed no significant change in bacterial and fungal populations from the mid gut to the hind gut.

The amount of cation release ( $K^+$ ,  $Mg^{++}$  and  $Ca^{++}$ ) from decomposing alder litter was enhanced with the addition of high numbers of isopods in all the treatments. Losses of  $K^+$  from control set showed similar pattern to the density gradient of isopods but an increase in leaching rates was observed in the latter sets than the earlier ones. The increase in the mobilization of  $K^+$  was significantly higher in isopods grazed litter than the ungrazed ones. The pattern of  $Mg^{++}$  and  $Ca^{++}$  loss from ungrazed litter exhibited a similar trend to  $K^+$ . The loss of ions from grazed litter was however low compare to  $K^+$ .

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\* Original not seen.

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