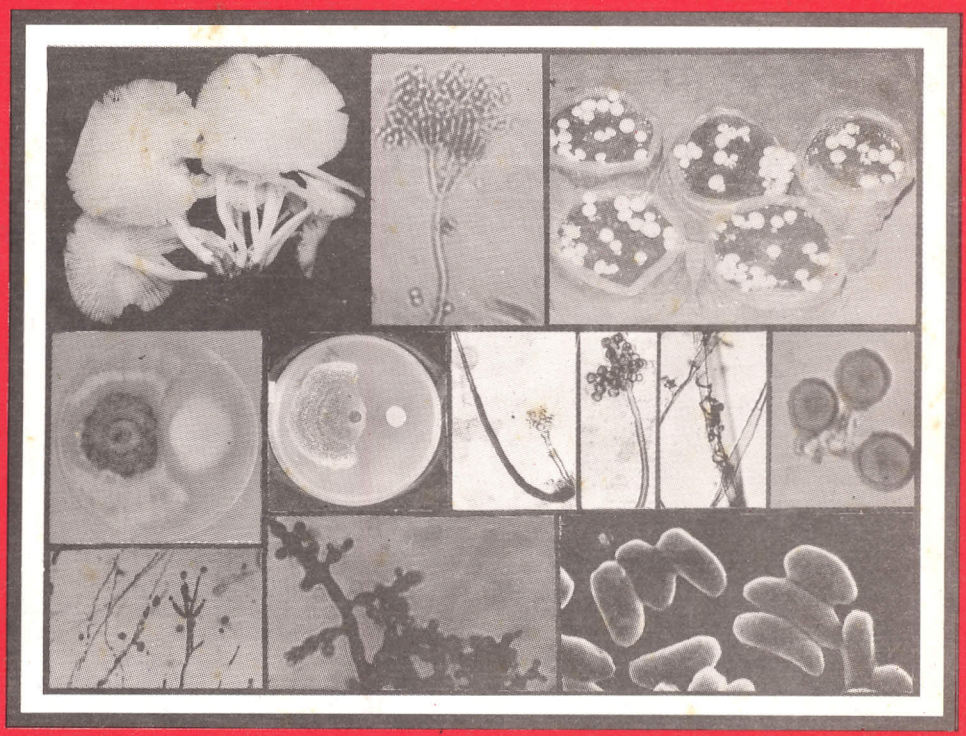
NEW TRENDS IN MICROBIAL ECOLOGY



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New Trends in Microbial Ecology
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Effect of isopod grazing on microbial population and nutrient release in decomposing leaf litter of *Alnus nepalensis* D. Don

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ABSTRACT

The fungal and bacterial standing crops remained almost unchanged in control microcosms without Isopods. All levels of isopods grazing reduces fungal standing crops but increased the bacterial standing crops. Numbers of viable bacteria and fungi were higher in faeces than in the litter and gut content. The spore producing fungi dominated the gut contents rather than less spore producing species or sterile mycelia. However, the gut and faeces of isopods is a suitable environment for the growth of bacteria. The fungi decreased along the intenstinal tract but increased in the faeces. The present series of experiment demonstrated that soil isopods exert a positive influence on comminution and nutrient release from leaf of *Alnus nepalensis*.

Keywords : *Alnus nepalensis*, isopod, feeding fungi and bacteria, nutrients

INTRODUCTION

In natural ecosystems and most managed forests and grasslands, the major fraction of terrestrial net primary production enters the soil litter system uneaten by grazers. Consequently large quantities of organic matter and nutrients enter the soil litter system, which are of great importance to ecosystem dynamics. The productivity of the system 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. Destruction and mineralization of plant litter results from the interaction of biological, biochemical and physical processes. The main vehicles that contribute to these desintegration processes are two groups of organisms, the microflora which comprised mainly bacteria and fungi and the soil fauna which embraces a broad spectrum of species extending over the whole animal kingdom from protozoa to vertebrate.

A widely accepted principle of soil biology is that members of the soil fauna enhance microbial activity rather than make a direct contribution to decomposition

processes by their own metabolism (Hassal, *et al.* 1987). Interaction between microflora and soil fauna plays an important roles in the decomposition process (Visser, 1986. Coleman and Crossley, 1996). For example, soil invertebrates may graze directly on bacteria and fungi (Hedlund *et al.* 1991, Binet and Trohen, 1992) or indirectly affect them by feeding on litter, producing faecal pellets (Scheu and Wolters, 1991) and by releasing nutrients (Anderson *et al,* 1989, Förster *at al,* 1995). The grazing activities of fauna may have other significant interactive effects for instance in improving the release of nutrients immobilised in microbial tissues. The complimentary process of increased mobilisation of nutrients resulting from the grazing activities of fauna on microorganisms is equally important to the functioning of the decomposers sub-system.

The involvement of isopods in decomposition processes has been studied, mainly in micro-ecosystems, and the outcome of these studies has been variable. It is widely discussed in the literature as to how such interactions affect the litter decomposition and also of the possible effect on the growth and activities of soil microbes. The impact of animal feeding on microorganism and mineralization in the soil is poorly understood, but it is well known that grazing can change the activity of microorganisms. There is however much evidence from laboratory studies to suggest that microbial activities are stimulated by interactions with the soil fauna (Van Wensem *et al.* 1993) The complimentary question of how such feeding affect fungi and bacteria has so far been neglected and received little attention from soil microbiologists. The recent emphasis on nutrient cycling as a means of quantifying ecosystem processes, indicates the need for study of the role of soil animal and microflora in litter decomposition. However, studies on subtropical soil fauna of North Eastern Region of India have yet been descriptive or focused on energy fluxes with little or no emphasis on the indirect role of fauna. These considerations tempted us to design a series of experiments to examine the effects of isopods grazing on microbial population and nutrients release from the decomposing leaf litter of *Alnus nepalensis.*

MATERIALS AND METHODS

Materials and Experimental design

Leaf litter of *Alnus nepalensis* D. Don was collected shortly after leaf fall from the natural site situated at Upper Shillong, 6.5 km away from Shillong, (altitude 1500m MSL, latitude 25° 34'N; longitude 91° 56'E). Thereafter, it was air dried at 20°C and brushed to remove the faecal material and debris. Midribs were removed and the remaining laminae were cut into small fragments (2-5mm) which were mixed well. Two gram aliquot was placed in experimental chambers similar to microcosm techniques described by Anderson and Ineson (1982). It consisted of an outer perspex tube which was modified to provide a sloping base for draining and leaching port. The litter sample sat within an inner removable container which rested upon alkathene beads which acted as an inert supporting medium of low surface area the sample being held in place by fine nylon mesh to support leaf litter

and allow faeces to fall clear of feeding fauna. Leaching of the microcosms was achieved by flooding the experimental material through the leaching port using a large syringe.

The litter in each microcosm was rehydrated by addition of 100 ml distilled water for 24h. This treatment leached out soluble tannins and readily metabolized materials mobilized by the drying and wetting regime. Then the fresh leaf litter was macerated in distilled water to produce a suspension which was used to inoculate the soaking litter and then incubated at 10°C. The chambers were leached weekly with 60ml distilled water, leachates being retained for chemical analysis. Isopods were not added to the microcosm chambers during an initial three week period, thus permitting the establishment of microflora.

Experimental animals and feeding

Burmoniscus specimens (sp. nova; Isopoda; Philosciidae) were collected from the field of alder forest and stored at 18°C in plastic sandwish boxes filled with litter until needed. Before being introduced into the experimental chamber, the isopod specimens were starved for 72h in order to void their guts of prior contents. The small amount of food that could remain the gut was egested upon resumption of feeding. Subsequently, 0, 2, 5, 10, 15 isopod specimens were introduced into experimental chamber separately in replicates and the experiment was carried out for 12 weeks. Three replicates were sampled on each occasion. Subsequent experiments were carried out using groups 25 isopods to determine the distribution of fungi and bacteria in their guts.

Determination of microbial standing crop

Litter derived from the sampled experimental set up was examined for fungal and bacterial standing crop, using the membrane filter technique (Hanssen *et al,* 1974). A sample of 1.0 g leaf litter was homogenized in 10 ml sterile distilled water and 1 ml of the sub-samples of the homogenate were stained for 30 mins with 1 ml (0.1%) aqueous Phenylaniline blue. The stained material was flushed through a 25 mm cellulose acetate membrane (pore size 0.22 μm) and mounted for microscopic examination. Bacterial numbers and fungal hyphal length were converted to standing crop estimates (Parkinson *et al,* 1971). Replicates were made for each sample and ten fields of view were examined per filter.

Determination of viable microorganisms in litter, guts and faeces of isopods

Litter samples from the experimental microcosms were removed and taken for dry mass determination at 105°C. Simultaneously, approximately 0.5 g wet mass 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 2-5 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

counting of viable bacteria and fungi. The faecal material collected from the bases of the microcosm was also processed in the same way.

Isopods were removed from the experimental chambers and surface sterilized by gentle agitation in sodium hypochlorite solution (1.6% available chlorine) for 1 min. They were blotted dry on sterile paper and the body cavity was opened 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 the hindgut and an additional single ligature was placed around the oesophagus and the rectum. Midgut and hindgut were dissected free and agitated gently in 3-4 changes of Ringer's solution to remove contaminable (Anderson and Bignell, 1980). The section were then transferred to 4 ml of 0.1% pepton water in a conical flask and shaken vigorously by hand for 5 min to disintegrate the gut wall and disperse the contents. A sample of the homogenate was filtered through a tared weighed and dried Millipore membrane filter (pore size 0.45 μm), gut tissue and ligatures being removed and the oven dried mass of litter fragments in that section of the gut was determined. Dilution series in peptone water were prepared from the gut and faecal samples. Fungi were isolated on Rose Bengal Agar (Martin, 1950) containing streptomycin to suppress bacterial growth. Bacteria were isolated on nutrient Agar medium (Difco Manual, 1953) using Nystatin (100 g ml-1) to suppress fungi. Replicates were used in each dilution. The plates were incubated at 20°C for 5-10 days for fungi and 3 days for bacteria and microbial colony forming unit were counted. Identification of the fungi was done by following Gilman (1957), Barnett and Hunter (1972) and Domsch *et al.* (1980). No attempt was made to identify bacteria.

Analyses of Nutrients Concentration in Leachates

The leachates were collected from the experimental chambers at weekly intervals. The leachates were analysed for potassium, magnesium and calcium. The leachates were stored at 5°C until analysis was completed. The latter was normally done within a week of sampling. Analysis of K, Mg and Ca was carried out on atomic absorption spectrophotometer using the methods of Allens *et al.* (1974). Results were converted to mg of element released per microcosm per week.

RESULTS

Fungal and Bacterial standing crop

In control microcosms, the fungal and bacterial standing crop remained almost unchanged.

All levels of isopods grazing reduced fungal standing crop and increased bacterial standing crop. All along the experiment, the amount of the fungi in the grazed microcosm series dropped markedly. Bacterial standing crop, however, increased with the number of isopods (Fig-1).

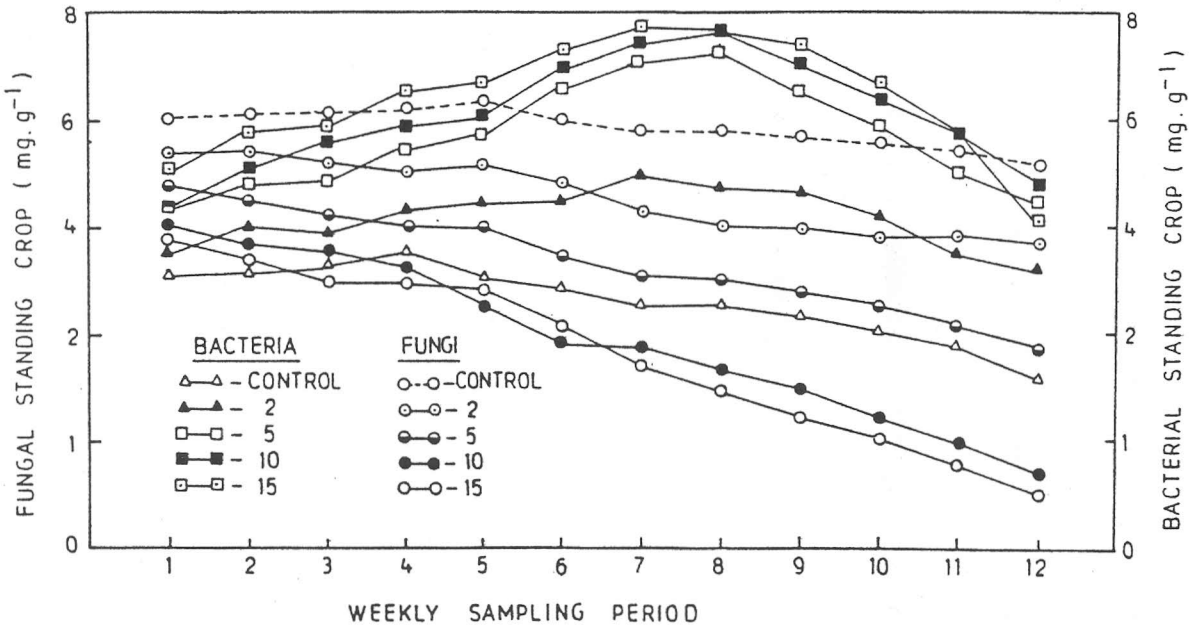

Fig. 1 Weekly variation in fungal and bacterial standing crop of fragmented leaf litters of *Alnus nepalensis* grazed by different numbers of isopods.

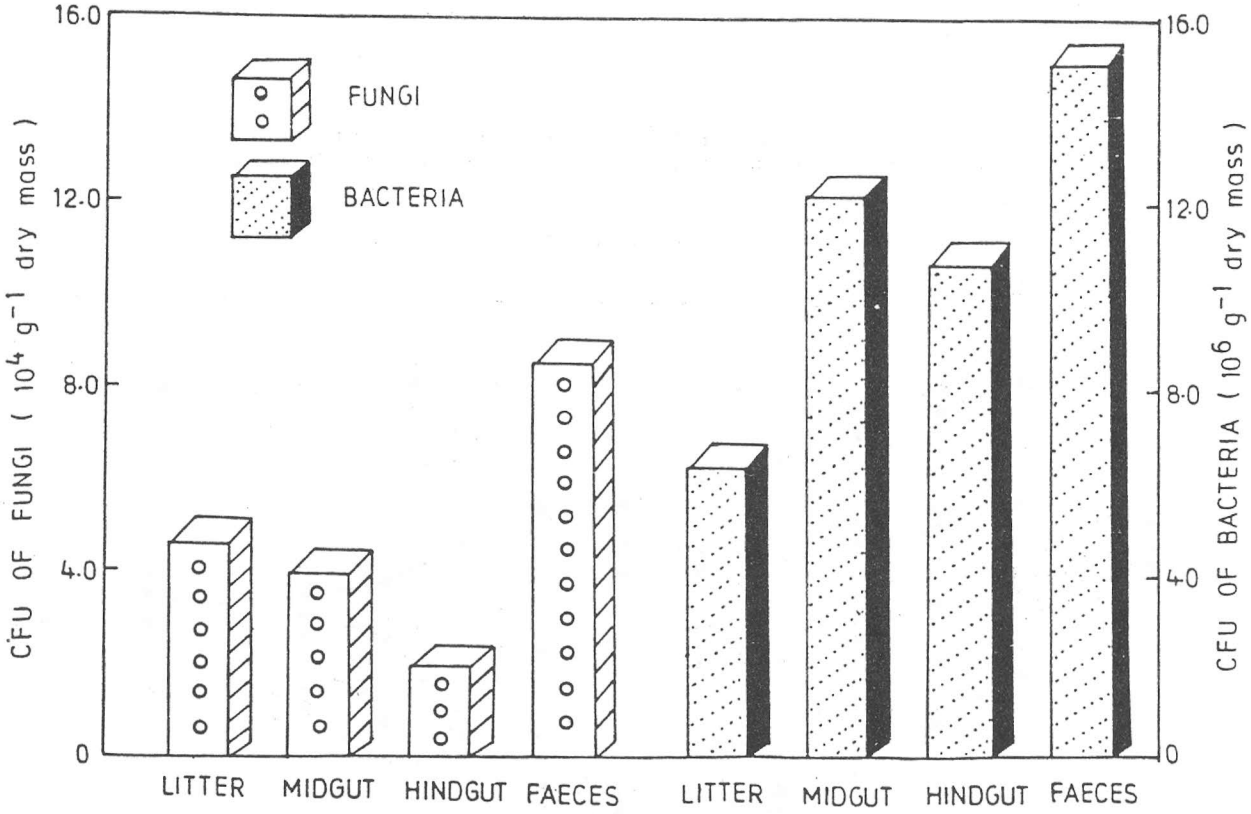

Fig. 2. Distribution of colony forming unit (CFU) of fungi and bacteria in litter, midgut, hindgut and faeces of isopods.

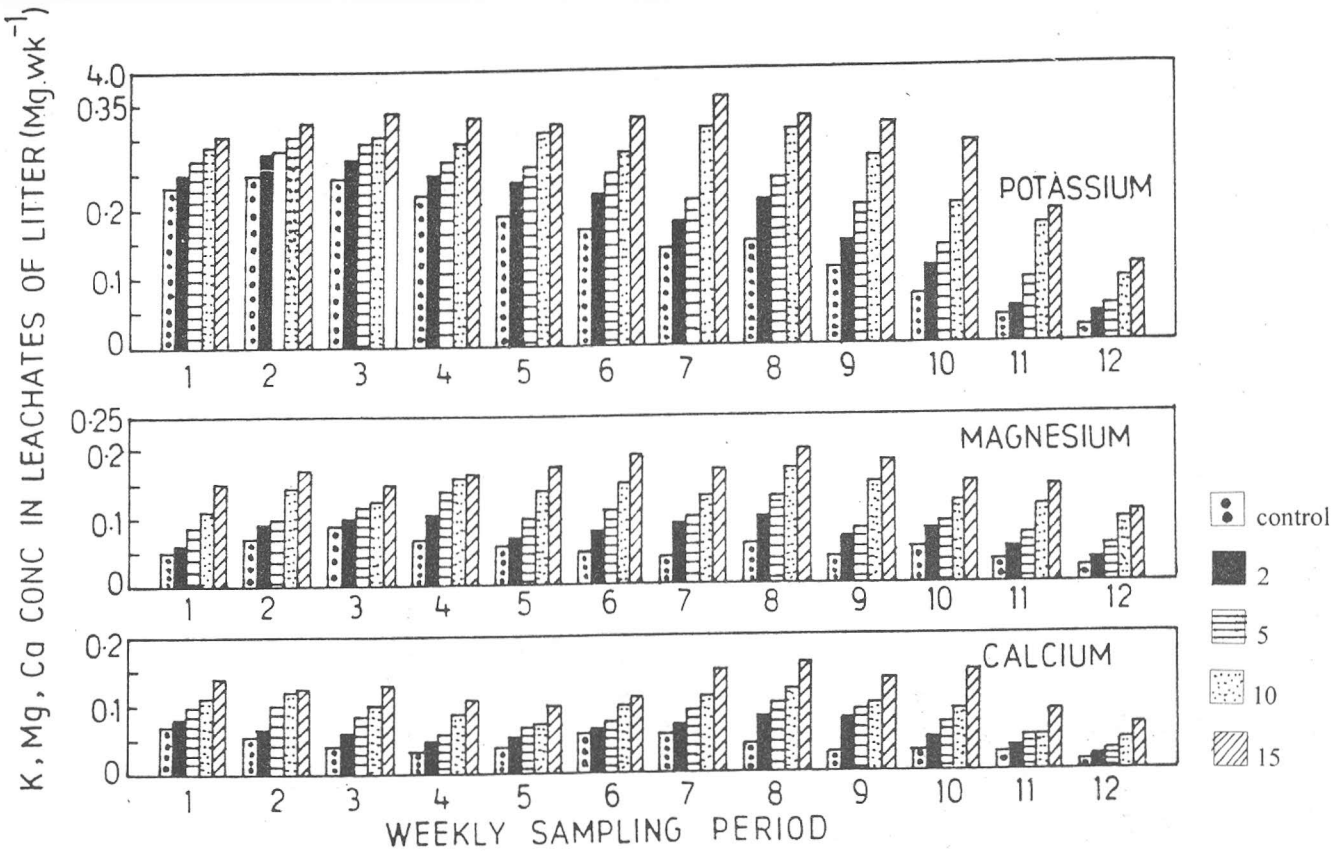

Fig. 3 Weekly variation in nutrients release of potassium (K), Magnesium (MG) and calcium (Ca) added by numbers of isopods in leaf litter of *Alnus nepalensis*.

Table : 1. Fungi isolated from the litter, gut contents and faeces of isopods

Fungi isolated	Litter	Midgut	Hindgut	Faeces
Absidia cylindrospora (Hageni)	+	-	-	-
A. glauca (Hageni)	+	-	-	-
Mucor heimalis (Wehmer)	+	+	+	+
M. racemosus (Fres)	+	-	-	-
Rhizopus oryzae (Went & Prinsen)	+-	-	-	-
R. stolonifer (Ehrenb)	+	+	-	-
Phoma glomerata (Wollenweber & Hochafel)	+	+	-	-
Pythium sp	+	-	-	-
Alternaria alternata (Fr Keissler)	+	+	-	-
Aspergillus flavus (Link)	+	+	+	+
A. niger (V. Teigh)	+	+	+	+
A. candidus (Link)	+	-	-	+
A. fumigatus (Fresenius)	+	+	-	+
Cladosporium cladosporioides (Fres de Vries)	+	+	+	+
Fusarium moniliforme (Sheld)	+	+	+	+
F. oxysporum (Scchlechtendahl)	+	-	-	+
F. solani (Mart) Sacc	+	-	-	+
Humicola grisae (Traaen)	+	-	-	+
Penicillium chrysogenum (Thom)	+	+	+	+
P. citrinum (Thom)	+	+	+	+
P. funiculosum (Thos)	+	+	-	-
Trichoderma harzianum (Rifai)	+	+	+	+
T. viride (Pers)	+	+	+	+
Verticillium chlamydosporum (Goddard)	+	-	-	-
Paecilomyees verioti (Bainer)	+	-	-	+
Geotrichum candidum (Link)	+	+	-	-
White sterile mycelia	+	-	-	+
Black sterile mycelia	+	-	-	-

+ = Present, - = Absent.

Counting of platable microorganisms in litter, guts and faeces of Isopods

The faeces of isopods contained more colony forming unit of fungi and bacteria than the guts content and leaf litter. Extensive bacterial growth occurred in the guts whereas fungi decreased along the intestinal tract then increased in the faeces. A general decrease in the number of fungal species has been observed from litter to midgut (Fig-2). Altogether 26 fungal species were isolated from the litter microcosms, 25 species from the gut content (Midgut = 15, Hindgut = 10) and 16 species from the faeces of *Burmonicus* sp nova. Species that subsist in the intestinal tract were *Mucor hiemalis, Aspergillus flavus, A. niger, Cladosporium cladosporidioides, Fusarium moniliforme, Penicillium chrysogenum, P. citrinum Trichoderma viride, T. koningii,* and *T. harzianum. A*ll of them were present in the faeces. The faeces of isopods were recognised by some species that had disappeared during the transit time, such as Aspergillus candidus, *A. fumigatus, Fusarium oxysporum, F. solani, Humicola* sp, *Geotrichum candidum* and white sterile mycelia (Table-1).

Nutrients release from the leaf litter

The effects of different numbers of isopods on nutrient leaching from alder leaf litter are shown in Fig-3. Losses of potassium from control showed similar patter to the grazed microcosms but the rate of leaching increased in the later sets than the earlier ones. Potassium losses from the litter were considerably enhanced by the presence of isopods and by the end of experiments, losses were over three times higher than controls. The effect was more pronounced at the later stage of the experiment than the earlier ones. (Fig 3). Magnesium concentrations in leachates were higher in nearly all the treatments but the increase were small in relation to control levels. Increase in Mg ions was low compared to K ions. The pattern of Ca loss from grazed and ungrazed litter exhibited a similar trend to K and Mg leaching. The loss of Ca ions was however less important than other anions from the litter. Calcium concentrations showed small increase with treatment with isopods in comparison with K and Mg. Release of nutrients *i.e.* K, Mg and Ca was significantly ($p > 0.01$) enhanced by the isopod grazing at different time period of the experimental chamber.

DISCUSSION

All levels of isopods grazing reduced fungi and increased bacterial standing crop as observed earlier (Hanlon and Anderson, 1980; Kayang *et al.* 1994). This phenomenon was regulated by isopods grazing as fungi are sensitive to such feeding thus render favourable environment for the multiplication for bacteria (Gunnarsson, 1987; Stöckli, 1990). The feeding activity in such micro-ecosystems may be species specific that some fungi or the decomposing litter remained unaffected but there is an overall reduction in the total fungal counts. The generally low counts of fungi in animal guts suggests that the animal were destroying fungi during digestion,

furthermore the particularly low count in litter suggests active grazing on the part of isopod. However, the relative contributions of these processes have not been quantified. The isopod faeces contain significantly more microorganisms than the ingested litter and may thus form microhabitats of intense microbial activity corroborating the results of Hassal *et al.* (1987). Drift and Witkamp (1959) and Lavelle *et al.* (1994) have also shown that the faecal pellets of soil fauna were a more favourable habitat for soil microorganisms than whole leaf litter. The increase in the number of bacteria occurring in the guts of isopods is in accordance with the observations of Ullrich *et al.* (1992). The increase in bacterial population was related to the favourable micro-environment and nutrients provided for the multiplication of bacteria that were ingested along with the litter (Hassal *et al.* 1987; Van Wesem, 1993) or that it contains a high density reservoir of microorganisms which invade the food as it is passing through. Another reason for the increase in the population of bacteria may be due to chemical changes in ingested materials such as in pH or C : N ratio (Martin *et al.* 1987). A general decrease in the number of fungal species has been observed from litter to midgut then to the hindgut. These results suggested that the gut of isopods can be selective and the proliferation of a single species can assist both in metabolism and in the elimination of other microbes ingested with the food. High spore producing fungi dominated the intestinal tracts of isopods rather than either less spore producing species of sterile mycelia. This indicated that certain species were more resistant to the isopod digestive enzymes as compared to mycelial forms and this largely affected the species composition of microbial conmmunities. Such a selective pressure allowed to dominate *P. chrysogenum, P. citrinum, T. viride, T. harzianum, F. moniliforme, F. oxysporum, C. cladosporioides, Aspergillus. flavus, A. niger* and *Mucor hiemalis* with their saprophytic and pathogenic ability (Kayang *et al.* 1997).

All base cations like K, Mg and Ca were accelerated by the feeding activities of isopods. Over the 7 weeks period significantly greater amounts of K, Ca and Mg were leached from the isopod inoculated chambers than from the controls. These differences occurred earlier with K (7 weeks) than Ca and Mg (8 weeks) which gradually decreased with time. The dynamics of nutrients are complicated since they appear in different forms and subject to various transformations such as leacing, immobilization and mineralization. These elements are highly soluble inorganic ions which leached rapidly from decaying litter. The loss rates are generally decreased with time as the number of microorganisms increased in litter tissues (Gosz *et al.* 1973). The pattern of nutrients loss from alder leaf litter showed a similar trend. However, initial leaching losses were lower. Ca unlike K and Mg is subjected to less leaching (Ineson *et al.* 1982). The present series of experiments demonstrate that isopod exerted a positive influence on comminution and nutrient release from leaf litter of *A. nepalensis.* The grazing of fungal hyphae or leaf material or both by the isopods resulted in an increased nutrient loss from the litter. Gunnarsson (1987) has shown that recycling of faecal pellets is necessary to optimize the overall uptake of nutrients by isopods and that microbial activity makes the required nutrient more readily available. Others have concluded that the treated litter surface area resulted from grazing increased

substrate area for microbial activity and for nutrient leaching (Douce and Crossley, 1982; Hendrix *et al.* 1990).

Fungal mycellium in forest 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 animal grazing may well have considerable signifance 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) m Huish *et al.* (1985). and De Ruiter *et al.* (1993) who observed increased leaching of ammonium and other cations from decomposing litter in the present of several representative types of fauna that feed the litter. The marked decrease in fungal biomass and greater increase in the bacterial standing crop of decomposing leaf litter inoculated with fauna has been attributed to grazing from microsites influencing microbiological activity (Anderson *et al.* 1983; Linden *et al.* 1994) which in turn could significantly alter nutrient dynamics. Microbial lysis and autolysis undoubtedly contributed to the turnover of bacterial growth and fungal mass (Mitchell and Alexander, 1963; Shields *et al.* 1983) but the result of microcosm experiment suggested that soil fauna feeding activities are quantitatively more important which stimulated bacterial growth and activity through grazing of senescent tissues (Kayang *et al.* 1996). The study revealed that ions immobilized in fungal hyphae during litter degradation are mineralized due to isopods grazing supporting the hypothesis that soil fauna strongly influenced the nutrient dynamics of the forest floor (Anderson *et al.* 1981; Anderson and Ineson, 1983; Coleman *et al.* 1983; Wolter, 1991). However, a long term significance of these processes, during litter decomposition and nutrient cycling, remains to be determined under natural condition.

Our investigation have shown that the grazing activity of isopods on microbial population, associated with leaf litter, affects the decomposition indirectly as the isopod egesta is a more suitable substrate for microbial growth than the uneaten litter (Kayang *et al.* 1996). Quantitative and and qualitative removal of fungi or by altering the fungal species composition of litter, either through selective grazing or by changing the nutrient status of the material, the isopod may be an important regulator of microbial activity associated with alder litter in the Sub-tropical forest ecosystems of North Eastern India. This study has also shown that some fungi can survive passage through the digestive tract of leaf eating invertebrate to remains to be seen how these results compare with those other habitants and with different organisms and how, if at all this phenomenon influences the species composition of leaf colonizing fungi in soil. The understanding of this relationship is a formidable task but it is necessary for an understanding of the soil litter system.

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