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The influence of isopod grazing on microbial dynamics in decomposing leaf litter of *Alnus nepalensis* D. Don

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Abstract

Fungal and bacterial standing crop remained constant in ungrazed microcosms whereas the fungal standing crop was reduced by all levels of isopod feeding. Bacterial standing crop, however, increased with the number of isopods. Observation of the gut contents and faeces of isopods indicated that they actively grazed fungal hyphae and that extensive bacterial growth occurred in the gut. The faeces of isopods contained more fungi and bacteria than gut contents and leaf litter. Dilution plates showed no significant change in bacterial and fungal counts between the midgut and the hindgut. Higher number of spore-producing fungi dominated the gut contents rather that either less spore-producing species or sterile mycelia.

Keywords: Isopods, microbes, grazing, *Alnus nepalensis*.

*Influence de la prédation par des isopodes sur la dynamique microbienne dans la litière de feuilles d'*Alnus nepalensis *D. Don en décomposition.*

Résumé

Les biomasses fongiques et bactériennes restent constantes dans des microcosmes sans faune alors que la biomasse fongique est réduite à tous les niveaux de prédation par les isopodes ; la biomasse bactérienne, par contre, augmente avec le nombre des isopodes. L'observation du contenu du tube digestif et des fèces d'isopodes indique que ces animaux ingèrent activement des hyphes mycéliens et qu'un développement bactérien important intervient dans l'intestin. Les fèces des isopodes contiennent plus de champignons et de bactéries que le contenu intestinal et la litière de feuilles. Des dilutions-numérations n'ont pas montré de modifications significatives des effectifs bactériens et fongiques entre l'intestin moyen et l'intestin postérieur. Des effectifs élevés de champignons produisant des spores dominent, dans le contenu intestinal, les espèces à faible production de spores ou les mycéliums stériles.

Mots-clés : Isopodes, microorganismes, prédation, *Alnus nepalensis*.

INTRODUCTION

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 (Hasan *et al.*, 1987). Soil fauna affect the decomposition of plant litter by comminuting, assimilating and egesting the remainder which may be invaded by other microorganisms than uningested litter (Visser, 1986). There is much evidence from laboratory studies that microbial activities are stimulated by soil fauna. However, studies on subtropical soil fauna of North Eastern India have yet been descriptive or focused on energy fluxes with little or no emphasis on the indirect role of fauna.

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Clearly, different types of feeding habits might be expected to influence decomposition rates in different ways and an understanding of animal diets is important in assessing the role of fauna in decomposition processes. Thus the aim of the present study was to examine the influence of isopod grazing on microbial dynamics during alder leaf litter (*Alnus nepalensis*) decomposition by analysing the food, gut contents and faeces of woodlice.

MATERIALS AND METHODS

Litter of *Alnus nepalensis* D. Don was collected shortly after leaf fall in an alder forest at Upper Shillong 5.5 km in the west of Shillong (altitude 1500 m MSL, latitude 25°34'N; longitude 91°56'E) on the road to Cherrapunjee, wettest place in the world. Fallen leaves were air dried at 20°C and brushed free of faecal material and debris. Petioles were removed and the lamina was cut into small pieces of 0.5–1.0 cm. These fragments were well mixed and an aliquot of 1.5 g was placed in microcosm chambers as described by Anderson & Ineson (1982). A single microcosm consisted of an outer perspex tube which was modified to provide a sloping base for drainage and leaching port. The litter sample sat within an inner removable container. The inner contains 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 animal. Leaching of the microcosms was achieved by flooding the experimental material through the leaching port using a large syringe. In each chamber the litter was rehydrated overnight in 100 ml distilled water. This treatment leached out soluble tannins and readily metabolizable substances 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 15°C. Isopods were not added to the microcosms during the initial three week period, thus permitting the establishment of microflora.

Burmoniscus specimens (sp. nova; Isopoda; Philosciidae) were collected from the alder forest and stored at 15°C in plastic sandwich boxes filled with litter until needed. Before being introduced into the microcosm, the animals were starved for 72 h in order to void their guts. The small amount of food that could remain in the gut was egested upon resumption of feeding. Subsequently, 0, 2, 5, 10 and 15 specimens were introduced in each chamber and thereafter the experiment ran for 35 days. Microbial standing crop estimates were made after 5, 10, 15 and 40 days. Three replicates were destructively sampled on each occasion.

Microbial standing crop estimates were determined from direct counts of fungi and bacteria, using the membrane filter technique as has been described earlier (Kayang *et al.*, 1994). 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 a described below.

Samples of litter were removed from the experimental microcosms 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 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 from the microcosms were surface sterilized 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 opened ventrally and flooded with insect Ringer's solution (Griffiths & Tauber, 1940). The gut was partitioned *in situ* by a double ligature placed between the midgut and hindgut and an additional single ligatures 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 & 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 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. Three replicates were used for each dilution. The plates were incubated at 20°C for 5-10 days for fungi and 3 days for bacteria and microbial colonies were counted. Identification of the fungi was done by following Gilman (1957), Barnett & Hunter (1972) and Domsch *et al.* (1980). No attempt was made to identify bacteria.

RESULTS

Fungal and bacterial standing crop remained constant in ungrazed microcosms whereas fungal standing crop was reduced by all levels of isopod feeding and the more the animals are numerous. All along the experiment, the amount of fungal population in the grazed microcosm series dropped markedly

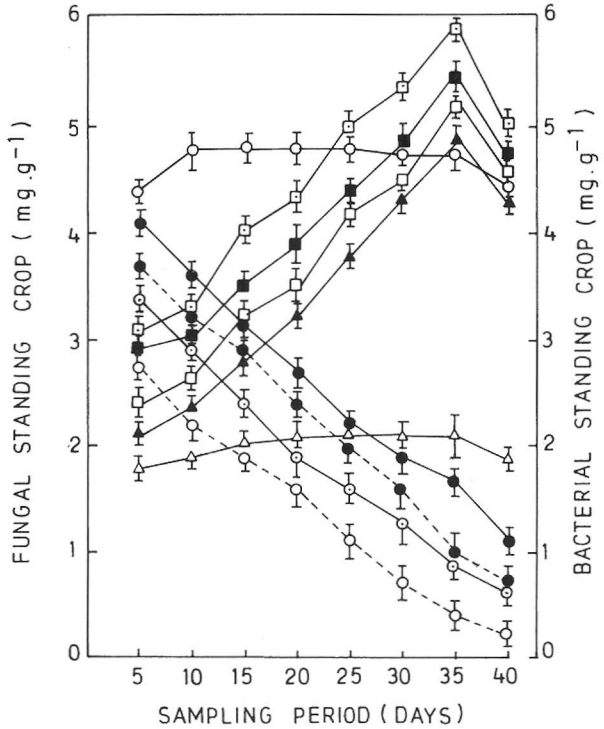

Figure 1. – Variation in fungal (○–○ = control, ●–● = 2, ●...● = 5, ⊙ – ⊙ = 10 and ○...○ = 15) and bacterial (△ – △ = control, ▲ – ▲ = 2, □ – □ = 5, ■ – ■ = 10 and ⊡ – ⊡ = 15) standing crops of fragmented leaf litter of *Alnus nepalensis* D. Don grazed by different numbers of isopod. Vertical lines show limits of one SE on either side of mean.

(*fig.* 1). On the other hand, the trend was reverse for the bacterial standing crop. After 35 days microcosm chamber with 15 isopods contained 5 times more bacteria than control. However, it dropped a little towards the end of the experiment (*fig.* 1).

Distribution of total fungi and bacterial in leaf litter, midgut, hindgut and faeces of isopods is given in *figure* 2. Extensive bacterial growth occured in the guts of isopods, while fungal densities decreased along the intestinal tract then increased in the faeces. Fungal community composition in the litter was comprised by 22 species (*table* 1). The fungal species composition associated with the gut contents of isopods (*table* 1) were *Mucor hiemalis, M. racemosus, Rhizopus stolonifer, Alternaria alternata, Aspergillus flavus, A. niger, Cladosporium cladosporioides, Fusarium moniliforme, F. oxysporum, Penicillium chrysogenum, P. citrinum, P. funiculosum, Trichoderma viride* and *T. harzianum.* Fungal species like *M. hiemalis, A. flavus, A. niger, F. moniliforme, P. chrysogenum. P. citrinum* and *T. viride* were abundant in faecal homogenates. A general decrease in the number of fungal species has been observed from litter to midgut then to hindgut (*table* 1). Species that subsisted through the intestine were *M. hiemalis, A. flavus, P. chrysogenum, P. citrinum, T. harzianum* and *T. viride*. All of them were present in the faeces. The faeces were recolonised by some species that had disappeared during the transit time, such as *F. solani, Verticillium chlamydospores, Geotrichum candidum* and white sterile mycelia.

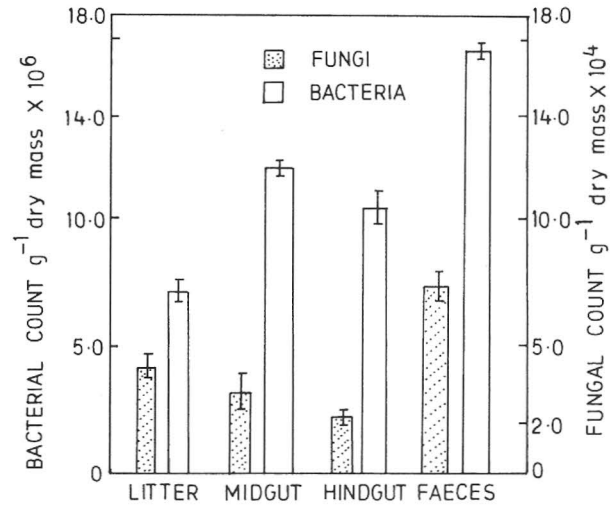

Figure 2. – Distribution of fungal and bacterial population in litter, midgut, hindgut and faeces of isopods. Vertical lines show limits of one SE on either side of mean.

DISCUSSION

In the ungrazed microcosms fungal and bacterial standing crop remained constant whereas in the grazed microcosm series, the fungal standing crop was reduced by all levels of isopod feeding. Bacterial growth, however, was stimulated by isopod feeding at different densities as was observed by Hanlon & Anderson (1980) and Kayang *et al.* (1994). Fungi are sensitive to isopod feeding (Reyes & Tiedje, 1976; Coughtrey *et al.* 1980; Gunnarsson, 1987; Stöckli, 1990) and animal intestine is a favourable environment for bacteria (Stefaniak & Seniczak, 1976; Ullrich *et al.*, 1991). Parkinson *et al.* (1979) and Hanlon & Anderson (1979) observed that at some densities gazing rates may exceed the production of fungal hyphae but Anderson & Ineson (1983) have shown that the balance of these processes is determined by the physical structure of the substrate and the available nutrient supply. Grazing in such microcosms may be species selective in that some fungal species of the decomposing litter remained unaffected or may even be stimulated but the net effect was a reduction in total fungal standing crop.

Lysis of some bacteria occurs in the gut of isopods (Reyes & Tiedje, 1976; Findlay *et al.*, 1984; Gunnarsson & Tunlid, 1986) even though the total number of bacteria often increases during gut transit (Brown *et al.*, 1978; Coughtrey *et al.*, 1980; Ineson & Anderson, 1985). The increase in numbers of bacteria was related to favourable environment and nutrients

Table 1. – Fungi isolated from the litter, gut contents and faeces of isopods (*Burmoniscus* sp).

Fungal species	Litter	Gut Contents		Faeces
		Midgut	Hindgut	
Absidia cylindrospora (Hageni)	+	–	–	–
A. glauca (Hageni)	+	–	–	–
Mucor hiemalis (Wehmer)	+	+	+	+
M. racemosus (Fres)	+	+	–	–
Rhizopus oryzae (Went & Prinsen)	+	–	–	–
R. stolonifer (Ehrenb)	+	+	–	–
Pythium sp.	+	–	–	–
Alternaria alternata (Fr Keissler)	+	+	–	–
Aspergillus flavus (Link)	+	+	+	+
A. niger (V. Tiegh)	+	+	+	+
Cladosporium cladosporioides (Fresenius de Vries)	+	+	+	+
Fusarium moniliforme (Sheld)	+	+	+	+
F. oxysporum (Scchlechtendahl)	+	+	+	+
F. solani (Mart) Sacc.	+	–	–	+
Penicillium chrysogenum (Thom)	+	+	+	+
P. citrinum (Thom)	+	+	+	+
P. funiculosum (Thom)	+	+	–	–
Trichoderma harzianum (Rifai)	+	+	+	+
T. viride (Pers)	+	+	+	+
Verticillium chlamydosporum (Goddard)	+	–	–	+
Geotrichum candidum (Link)	+	–	–	+
Aureobasidium pullulans (de Barry Amand)	+	–	–	–
White sterile mycelia	+	–	–	+
Black sterile mycelia	+	–	–	–

+ = Present, – = Absent.

provided for the multiplication of bacteria that were ingested with the food (Hassal *et al.*, 1987; Van Wensem *et al.*, 1993).

The bacterial and fungal counts in isopod faeces were higher than the leaf litter and may thus form micro-habitats of intense microbial activity. Drift & Witkamp (1959) and Hassal *et al.* (1987) have also shown that the faecal pellets of soil animals were a more favourable habitat for soil microorganisms than whole leaf litter. Reyes & Tiedje (1976) have demonstrated that isopods secrete in their gut some enzymes responsible for the digestion of bacterial cells belonging to certain group of species, allowing a few specific bacterial cells to multiply, the net effect being an increase in counts in faeces with respect to the ingested food. Szabo *et al.* (1969) and Griffiths & Wood (1985) 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.

In the present investigation a high number of spore-producing fungi dominated the gut contents. This indicated that spores of certain fungi were resistant to the enzymatic digestion compared to mycelial forms and this influenced the species composition of microbial communities. Such a selective pressure allowed to dominate *P. chrysogenum, P. citrinum, T. viride, T. harzianum, F. moniliforme, F. oxysporum, C. cladosporioides, A. flavus, A. niger* and *M. hiemalis* with their saprophytic and pathogenic ability.

Our investigation suggested that soil isopods affect decomposition indirectly because the material they egest as faeces is a more suitable substrate for microbial growth than the undigested litter and because they cause a shift in species composition of fungal communities.

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