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Effect of an isopod grazing (*Burmoniscus* sp.) upon microbes and nutrient release from the decomposing leaf litter of *Alnus nepalensis* D. Don.

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Abstract

Isopods exerted a positive influence on comminution and nutrient release from leaf litter of *Alnus nepalensis*. Fungal standing crop remained constant in ungrazed microcosms whereas it was reduced by all levels of isopod feeding. Bacterial standing crop, however, increased with the numbers of isopods. After seven weeks of grazing bacterial biomass was three times higher than ungrazed ones. Bacteria dropped markedly towards the end of the experiment. K^+, Mg^{++} and Ca^{++} showed significant leaching response to grazing. An increase in the mobilization of K^+ was higher in isopods grazed litter than the ungrazed ones. Mg^{++} concentrations in leachates was higher at all the density gradients of isopods but the differences were nonsignificant in relation to control. The pattern of Ca^{++} loss from grazed and ungrazed litters exhibited a similar trend to K^+ and Mg^{++} leaching. Leaching of this ion was less important than other anions.

Keywords: Isopod, microbes, nutrient, mineralization, *Alnus nepalensis.*

*Effet d'un isopode (*Burmoniscus *sp.) sur le stock microbien et la libération d'éléments dans une litière d'*Alnus nepalensis *D. Don.*

Résumé

Les isopodes exercent une influence positive sur la décomposition des litières d'*Alnus nepalensis* et sur la libération des éléments. Le stock fongique demeure constant dans les microcosmes sans isopodes alors qu'il diminue en leur présence, indépendamment de leur nombre. A l'inverse, les bactéries augmentent avec le nombre d'isopodes mis en place dans les microcosmes. Après sept semaines d'expérimentation, la biomasse bactérienne était trois fois plus élevée dans les microcosmes avec isopodes. Par la suite, cette biomasse diminua nettement jusqu'à la fin de l'expérimentation. K^+, Mg^{++} et Ca^{++} ont été significativement lessivés en présence des isopodes. L'augmentation de la mobilisation de K^+ a été plus forte dans les litières consommées par les isopodes. Les concentrations en Mg^{++} ont été également plus fortes dans les lessivats recueillis après action des isopodes, quel que soit leur nombre, mais sans différence significative avec le témoin. Le lessivage de Ca^{++} suit un modèle similaire à celui de K^+ et Mg^{++}, mais en quantité moins importante.

Mots-clés : Isopode, microorganismes, éléments chimiques, minéralisation, *Alnus nepalensis*

INTRODUCTION

In decaying leaf litter, soil arthropods browsing on senescent microbial populations has been considered as a mechanism by which soil animals may enhance decomposition processes by both the mobilization of nutrients and by annulment of biostatistics (Wallwork, 1967; Hanlon, 1981; Coleman *et al.*, 1983; Tian *et al.*, 1992). Fauna affects rate of mass loss from decomposing substrates, directly by ingestion and indirectly by grazing on microbes. The effects of substrate comminution are major phenomena in forested ecosystems, where a large fraction of mass loss can be attributed directly or indirectly to the

presence of fauna. Microorganisms and saprophytic invertebrates are the main agents of litter catabolism in enhancing nutrient regeneration and nutrient flux rates in terrestrial ecosystems (Anderson and Macfadyen, 1976; Swift *et al.*, 1979; Seastedt, 1984; Mitchel and Nakas, 1986; Crossley *et al.*, 1989; Ponge, 1991).

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 immmobilization 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 the differences in the rate of nutrient turnover.

A microcosm system was used to investigate the effects of density changes in isopod on microbial standing crop and cation release over several months in grazed and ungrazed litter. The aim of the present study 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 microcosms chambers were the ones as described by Anderson and Ineson (1982). Leaf litter of *Alnus nepalensis* was collected shortly after leaf fall from the natural forest site situated at Upper Shillong, 5.5 km away from Shillong, the capital of Meghalaya, (altitude 1500 m MSL, latitude 25°34′N; longitude 91°56′E). Thereafter, it was air dried at room temperature and brushed to remove the 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 of 2 g placed in experimental chambers (Anderson and Ineson, 1982).

The litter in each chamber was rehydrated by the addition of 100 ml of distilled water for 24 h, 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 microbes during an initial three week period permitting establishment of a microflora.

The isopods, *Burmoniscus* (sp. nova; Philosciidae) (Dudgeon *et al.*, 1990) were collected from the field and stored at 15°C in plastic sandwich boxes filled with litter until needed. Thereafter, 0, 2, 5, 10 or 15 isopods were added to the experimental chambers separately in five replicates each and the experiment was carried out for 12 weeks.

DETERMINATION OF MICROBIAL STANDING CROP

Litter derived from the sampled microcosms was 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 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 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.

CHEMICAL ANALYSIS OF LEACHATES

The leachates were collected from the microcosms at weekly intervals. The leachates were analysed for K^+, Mg^{++} and Ca^{++}. The leachates were stored at 5°C until analysis was completed the latter 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 (Zar, 1974).

RESULTS

Microbial standing crop

Fungal and bacterial standing crop remained constant in ungrazed microcosms whereas the fungal standing crop was reduced by all levels of isopod feeding. By the end of the experiment, the amount of fungal population in the grazed microcosm series dropped markedly (*fig.* 1). Bacterial standing crop, however, increased with the increased grazing by the isopods and after seven weeks grazing by 10 and 15 isopods enhanced 3 times bacterial biomass than controls. However, it droped markedly towards the end of the experiments (*fig.* 1).

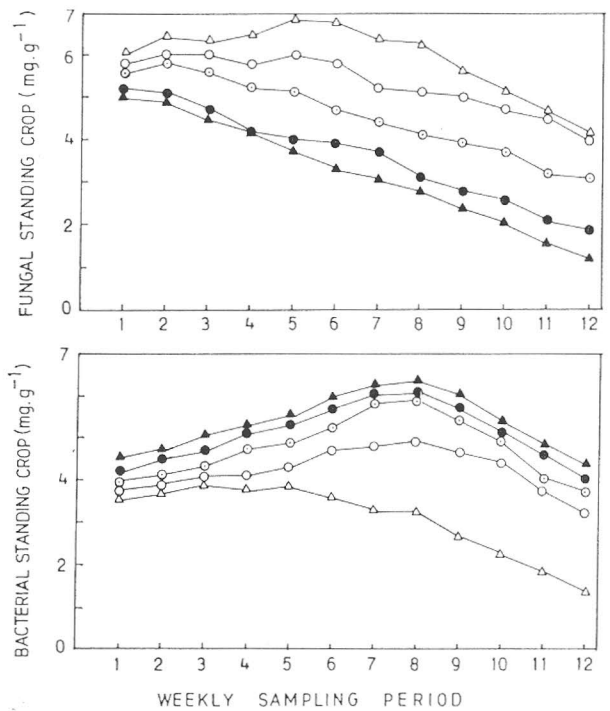

Figure 1. – Weekly variation in fungal and bacterial standing crop of fragmented leaf litters (*Alnus nepalensis*) grazed by different numbers of isopods (△——△, control, ○——○, 2, ⊙——⊙, 5, ●——●, 10, ▲——▲, 15).

Nutrients release from the leaf litter

The effects of isopod density on nutrient release from alder leaf litter are shown in Figure 2. 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.* 2). An increase in the mobilization of K^+ was higher in isopods grazed litter than the ungrazed ones. Release of K^+ ions due to litter grazing by 10 and 15 isopods was apparent 7 weeks and was related to the isopod population in microcosm chambers. Mg^{++} concentrations in leachates were higher in almost all the density gradients of isopods but the differences in its quantity were nonsignificant in relation to control sets. Release of nutrients *i.e.*, K^+, Mg^{++} and Ca^{++} was significantly ($p<0.01$) enhanced by density gradient of the isopods at different time period (*table* 1). Increase in Mg^{++} ions leachates in isopod fed litter was low compared to K^+ ions. The pattern of Ca^{++} loss from grazed and ungrazed litters exhibited a similar trend to K^+ and Mg^{++} leaching. The loss of Ca^{++} ions was however less important than other anions from the litter (*fig.* 2)

DISCUSSION

Bacterial growth was stimulated by macroarthropod feeding activities at different levels of isopods.

Table 1. – Analysis of variance to test the effects of isopod grazing on the mineralization of elements (K, Mg and Ca) from leaf litter.

Source of variation	d.f.	MS	F
Time effects	11	0.026072	6.46**
Element effects	2	0.305656	75.72**
K effects	4	0.027594	49.25**
Mg effects	4	0.002145	14.51**
Ca effects	4	0.001981	17.67**
Animal effects	4	0.035613	8.82**
Time × Element	22	0.008759	2.17**
Animals × Elements	44	0.006403	1.59**
Elements × Animals	8	0.005978	1.48

** Significant at $P<0.01$.

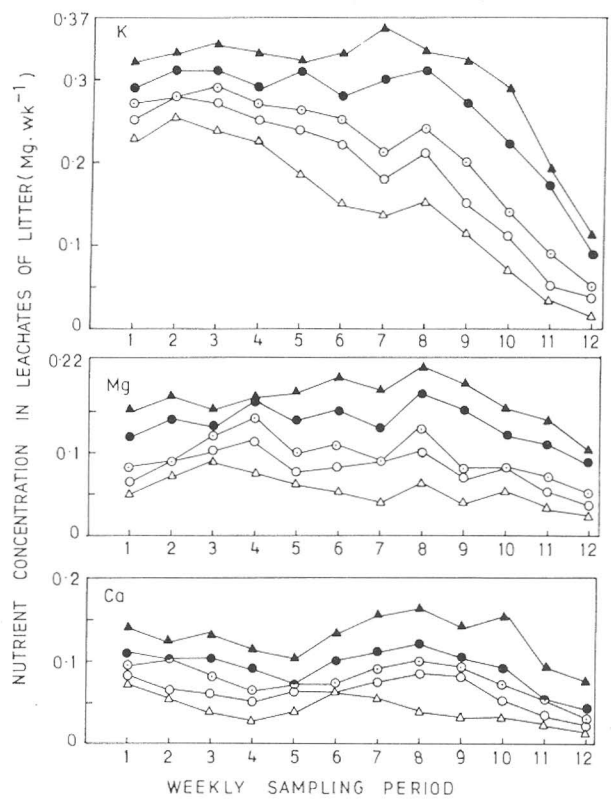

Figure 2. – Weekly variation in nutrient release of Potassium (K) Calcium (Ca) and Magnesium (Mg) added by numbers of isopods (△——△, control, ○——○, 2, ⊙——⊙, 5, ●——●, 10, ▲——▲, 15) in leaf litter of *Alnus nepalensis*.

The fungal standing crop decreased markedly with the increase in number of isopods (Hanlon and Anderson, 1979, 1980). This phenomenon may be caused by effects of grazing of the fungi, gut secretions or the remobilization of nutrients, but the relative contributions of these processes have not been quantified (Ineson *et al.*, 1982). Parkinson *et al.* (1979) and Hanlon and Anderson (1979) also observed that grazing rates may exceed the production of fungal hyphae but Anderson and Ineson (1983) have shown that the balance of these processes is determined by the physical structure of the substrate and the

available nutrient supply. Change in fungal standing crops was regulated by isopods grazing as the fungi are sensitive to this feedings and thus render favourable environment for the growth of bacteria (Szabo *et al.*, 1969); Stefaniak and Seniczak, 1976). The results of the study suggests that specific grazing may be occurring in temperate microcosms but in subtropical conditions the net effect was a reduction in total fungal standing crop.

Isopod feeding activities accelerated leaching of K^+, Mg^{++} and Ca^{++} from the leaf litter of *A. nepalensis*. Over the 7 week period significantly greater amounts of K^+, Ca^{++} and Mg^{++} were leached from the grazed microcosms than ungrazed one. These differences occurred earlier with K^+ (7 weeks) than Ca^{++} and Mg^{++} (8 weeks) which gradually decreased with time. The dynamics of nutrients particularly nitrogen are complicated since they appear in different forms and are subject to various transformation such as leaching, immobilization and mineralization. Mg^{++} and K^{++} are usually available in excess of saprotroph requirements. These elements are highly soluble inorganic ions which leached rapidly from decaying litter. The loss rates generally decreased with time as the number of microbes increased in litter tissues (Gosz *et al.*, 1973). The pattern of anions 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).

Our experiment demonstrated that soil isopods 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. It has been demonstrated that recycling of fecal pellets is necessary to optimize the overall uptake of nitrogen e.g., nitrogen, by isopods and that microbial activity makes the required nutrient more readily available (Hassal and Rushton, 1982; Rushton and Hassall, 1983; Gunnarsson *et al.*, 1986; Gunnarsson, 1987). The greater litter surface area resulted from faunal grazing increased substrate area for microfloral activity and for nutrient leaching (Douce and Crossley, 1982). Fungal mycelium 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 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.* 1985) who 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 decrease in fungal biomass and greater increase in the bacterial standing crop of decomposing oak litter inoculated with mircroarthropods. 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 contributed to the turnover of bacterial and fungal tissue (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.

The study revealed that ions immobilized in fungal tissue 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). However, a long term significance of these processes during litter decomposition and nutrient cycling remains to be determined under natural conditions.

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REFERENCES

Allen S., Grimshaw M., Parkinson J. and Quarmby C. (1974). – *Chemical analysis of ecological materials.* Blackwell Scientific Publication, Oxford.

Anderson J. M. and Ineson P. (1982). – A soil microcosm system and its application to measurement of respiration and nutrient leaching. *Soil Biology and Biochemistry*, **14**, 415-416.

Anderson J. M. and Ineson P. (1983). – Interactions between soil arthropods and microbial populations in a carbon, nitrogen and mineral nutrient fluxes from decomposing leaf litter. *In*: *Nitrogen as an Ecological factor* (Lee J. A., McNeil S. and Rorison I. H., Eds.) Blackwell, Oxford.

Anderson J. M., Ineson P. and Huish S. A. (1983). – Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodlands. *Soil Biology and Biochemistry*, **15**, 463-467.

Anderson J. M. and Macfadyen A. (1976). – *The role of terrestrial and aquatic organism in decomposition processes*. Blackwell, Oxford.

Anderson R. Y., Coleman D. C. and Cole C. V. (1981). – Effects of saprophytic grazing on net mineralization. *In* Clark F. C. and Rosswall T., Eds. *Terrestrial Nitrogen Cycles, Ecological Bulletin*, NFR, **33**, 201-216.

Ausmus B. S., Edward N. T. and Witkamp M. (1976). – *Microbial immobilization of carbon, nitrogen, phosphorus and potassium implication for forest ecosystem process. In:* The role of Terrestrial and Aquatic Organisms on decomposition process, Anderson J. M. and Macfadyen A., Eds. Blackwell, Oxford, 397-416.

Coleman D. C., Reid C. P. P. and Cole C. V. (1983). – Biological strategies of nutrient cycling in soil systems. *Advances in Ecological Research*, **13**, 1-55.

Cromack K. Jr., Todd R. L. and Monk C. D. (1975). – Patterns of basidiomycetes nutrient accumulation in conifer and deciduous forest litter. *Soil Biology and Biochemistry*, **7**, 265-268.

Crossley D. A. Jr., Coleman D. C. and Hendrix P. F. (1989). – The importance of the fauna in agricultural soils: research approaches and perspectives. *Agriculture, Ecosystem and Environment*, **27**, 47-55.

Douce G. K. and Crossley D. A. (1982). – The effect of soil fauna on litter mass loss and nutrient loss dynamics in Artic Tundra at Barrow, Alaska. *Ecology*, **62(2)**, 523-537.

Dudgeon D., Ma H. T. T. and Lam P. K. S. (1990). – Differential palatability of leaf litter to four sympatric isopods in a Hong Kong forest. *Oecologia*, **84**, 398-403.

Gosz J. R., Likens G. E. and Bormann F. H. (1973). – Nutrient release from decomposing leaf and branch litter in the Hubbard Brook Forest, New Hampshire. Ecological Monographs, **43**, 173-191.

Gunnarsson T. (1987). – Selective feeding on a maple leaf by *Oniscus asellus* (Isopoda). *Pedobiologia*, **30**, 161-166.

Gunnarsson T. and Tunlid A. (1986). – Recycling of fecal pellets in isopods: Microorganisms and nitrogen compounds as potential food for *Oniscus asellus*. L. *Soil Biology and Biochemistry*, **18(6)**, 595-600.

Hanlon R. D.G. and Anderson J. M. (1979). – The effects of collembola grazing on microbial activity in decomposing leaf litter. *Oecologia*, **38**, 93-99.

Hanlon R. D. G. and Anderson J. M. (1980). – Influence of macroarthropod feeding activities on microflora in decomposing oak leaves. *Soil Biology and Biochemistry*, **12**, 225-261.

Hanlon R. D. G. (1981). – Influence of grazing by collembola on the activity of senescent fungal colonies grown on media of different nutrient concentration. *Oikos*, **36**, 362-367.

Hanssen J. F., Thingstat T. F. and Goksoys J. (1974). – Evaluation of hyphal lengths and fungal biomass in soil by a membrane filter technique. *Oikos*, **15**, 102-107.

Hassall M. and Rushton S. P. (1982). – The role of coprophagy in the feeding strategies of terrestrial isopods. *Oecologia*, **53**, 374-381.

Huish S., Leonard M. A. and Anderson J. M. (1985). – Wetting and drying effects on animal/microbial mediated nitrogen mineralization and mineral element losses from deciduous forest litter and raw humus. *Pedobiologia*, **28**, 177-183.

Ineson P., Leonard M. A. and Anderson J. M. (1982). – Effects of Collembolan grazing upon nitrogen and cation leaching from decomposing leaf litter. *Soil Biology and Biochemistry*, **14**, 601-605.

Mitchell R. and Alexander M. (1963). – Lysis of soil fungi by bacteria. *Canadian Journal of Microbiology*, **9**, 169-177.

Mitchel M. J. and Nakas J. P. (1986). – *Microflora and faunal interactions in natural and agro-ecosystems*. Nijhoff/W. Junk, Dordrecht, pp. 505.

Parkinson D., Gray T. R. G., Holding A. J. and Nagel De Boois H. M. (1971). – Heterotrophic microflora in methods of study in quantitative Soil Ecology Phillipson J., Ed., 34-50, I. B. P. Handbook 18, Blackwell, Oxford.

Parkinson D., Visser S. and Whittaker J. B. (1979). – Effects of collembolan grazing in fungal colonization of leaf litter. *Soil Biology and Biochemistry*, **11**, 529-535.

Ponge J. (1991). – Succession of fungi and fauna during decomposition of needles in a small area of Scots pine litter. *Plant and Soil*, **138**, 99-113.

Rushton S. P. and Hassal M. (1983). – The effects of food quality on the life history parameters of the terrestrial isopod (*Armadillidium vulgare* (Latreille)). *Oecologia*, **57**, 257-261.

Seastedt T. R. (1984). – The role of microarthropods in decomposition and mineralization process. *Annual Review of Entomology*, **29**, 25-46.

Shields J. A., Paul E. A., Lowe W. E. and Parkinson D. (1973). Turnover of microbial tissue in soil under field conditions. *Soil Biology and Biochemistry*, **5**, 753-764.

Stefaniak O. and Seniczak S. (1976). – The microflora of the alimentary canal of *Achipteria coleoptrata* (Acarina, Oribatei). *Pedobiologia*, **16**, 185-194.

Swift M. J., Heal O. W. and Anderson J. M. (1979). – *In*: Decomposition in Terrestrial Ecosystems. University of California Press, Los Angeles, California, USA.

Szabo I., Marton M. and Buti I. (1969). – Intestinal microflora of the larvae of St. Mark's Fly IV studies on the intestinal bacterial flora of a larval population. *Acta microbiologica Academic Scientiarum hungaricae*, **16**, 381-397.

Tian G., Kang B. T. and Brussard L. (1992). – Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions – Decomposition and nutrient release. *Soil Biology and Biochemistry*, **24(10)**, 1051-1060.

Wallwork J. A. (1967). – Acari. *In*: Burges N. A. and Raw F. Eds., *Soil Biology*, Academic Press, London, 363-395.

Witkamp M., Frank M. L. and Shoopman J. L. (1966). – Accumulation and biota in a pioneer ecosystem of Kudza vine at Copperhill, Tennessee. *Journal of Applied Ecology*, **3**, 383-391.

Zar J. H. (1974). – *Biostatistical Analysis*. Prentice Hall Inc. London.