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Fungal and bacterial enzyme activities in *Alnus nepalensis* D. Don

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Abstract – Fungal and bacterial numbers and their enzymes activities in terms of enzymes, namely cellulase, amylase and invertase were estimated in two forest stands of alder (open and closed forest). The fungal and bacterial population numbers were higher in the closed forest than in the open ones. Consequently, the different enzyme activities were also greater in the closed forest. A correlation coefficient was calculated between fungal population numbers, bacterial population numbers, moisture content, pH, total nitrogen, weight loss, cellulose and total sugars and the enzymes activities. Invertase activity showed a positive correlation ($P < 0.05$) with litter soluble sugars and total nitrogen but negatively with weight loss. Amylase and cellulase activities were correlated significantly with fungi and bacteria and moisture content of litter. Cellulase also correlated significantly but negatively with the cellulose ($P < 0.01$). The results of the investigation indicated that changes in forest canopy has an effect on fungal and bacterial population numbers and microbial enzymes activities. © 2001 Éditions scientifiques et médicales Elsevier SAS

alder / decomposition / enzyme / forest canopy / Fungi and bacteria

1. INTRODUCTION

The plant litter decomposition on forest floor consists of two main stages, the first stage involves leaching and microbial utilization of labile compounds while the second stage brings about degradation of recalcitrant compounds mainly lignocellulose [30].The degradation is mediated by the activity of extracellular and oxidative microbial enzymes [4, 34]. Amylase, cellulase and invertase are some of the important enzymes in soil litter system, which are partially responsible for the rate and course of decomposition of plant litter.

Cellulose, hemicellulose and lignin are the major components of forest litter, comprising 50–80% of the dry mass [39]. Cellulose and hemicellulose are recalcitrant products added to soil through plant remains and must be transformed into soluble substances prior to microbial assimilation through extracellular enzymes [28]. The microbial degradation of cellulose, hemicellulose and other oligosaccharides may be brought about by those enzymes directly involved in initial chemical breakdown [37]. Cellulose is a major structural component of litter and therefore is a vital energy source for the microbes associated with litter degradation [31]. Its hydrolysis into glucose is achieved by cellulase enzyme complexes produced by fungi [18]. Cellulolytic enzymes may be produced by bacteria [20], actinomycetes [38], fungi [5] and some invertebrates [21].

Sucrose is a major soluble carbohydrate in plant tissues and it has been studied because of its widespread distribution in plants and soil [26]. The mechanism of sucrose breakdown is an important process as it forms the major source for carbon allocation. Sucrose is solubilized by invertase, which can be either acidic or alkaline in nature [34] Starch is another common compound within most plant tissues which increases during photosynthesis and decrease as it is enzymatically converted into sugars. Amylase hydrolyses starch and makes its carbon available for translocation.

The cellulase enzyme which mediate the hydrolysis of insoluble litter constituents (cellulose) is generally soluble, while amylase and invertase mediating the hydrolysis of more soluble constituents (sugars) are mostly insoluble [34]. Due to the ubiquity of cellulose, cellulases are the most extensively studied enzyme system in plant litter [7, 14, 15]. Very little information is available on amylase and invertase inspite of their role in the hydrolysis of sugars and starch respectively [1, 8, 40] and they have not been studied in such detail [13].

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The production of extracellular enzymes (cellulase, amylase and invertase) by microorganisms during litter degradation may be influenced by temperature, moisture, pH and the substrate involved [10, 30], but the studies on fungal and bacterial enzymes under subtropical fields are meagre [12, 13]. Although large scale planting with alder tree species began several decades ago, to date only little is known about the litter dynamics and nutrient cycling in forest plantation in Meghalaya and the effect of forest canopies on fungal and bacterial enzyme activities has not been studied. Therefore, the aim of our study was to determine the fungal and bacterial populations and their activities in leaf litter decomposition of *Alnus nepalensis*.

2. EXPERIMENTAL DESIGN

Two forest stands dominated by alder (*Alnus nepalensis* D. Don) were selected for the present study at Upper Shillong 5.5 km away from Shillong, the capital of Meghalaya, India (altitude 1500 m MSL; latitude 25°34'N; longitude 91°56'E). The alder stands were of 35 years old and varied in their tree density. The closed forest stand had 1160 trees ha^{-1} being the undisturbed stand while the open ones was with 380 trees ha^{-1} exposed to disturbances such as cutting twigs and collection of wood for fuel by the local inhabitants. Both the stands faced the eastern side of the hill with undulated slope and were about 500m apart from each other. The sites are closely comparable; the functional differences are attributed to their tree density. The climate of the study area is subtropical monsoonic type largely control and influenced by the seasonal winds, like the south west monsoonic wind and the north east winter ones. On the basis of meteorological data, four distinct season i.e., spring (March to April), summer rainy (May to September), autumn (October to November) and winter (December to February) seasons are recognised. Winter months are cold and dry and the temperature ranged from 4.8 to 8.7°C, low temperature of the winter results into frost during December, January months. During March and April the air temperature gradually warms up and increase to 23.5°C and the weather is relatively dry. From the middle of May to the end of July, the temperature reaches the maximum and receives maximum precipitation of the year. The maximum temperature was 24.6°C and the minimum temperature was 15.5°C. The rain starts in the middle of April becomes intense in June and August–September and continues upto the middle of October after which it gradually decreases. The annual rainfall ranged from 0.1mm to 574 mm.The average humidity ranged from 64.5% to 90% (*figure 1*).

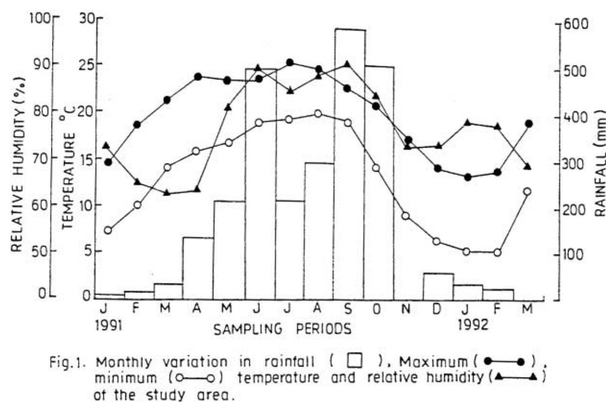

Fig.1. Monthly variation in rainfall (□), Maximum (•—•), minimum (o—o) temperature and relative humidity (▲—▲) of the study area.

Figure 1. Légendes de la figure 1

2.1. Vegetation and soil of the study area

The soil type of the study sites is red loamy with fine silt and gravel constituting the major fractions (sand 54 %, silt 25 % and clay 20 %) and acidic in reaction. The predominant understory vegetation of closed forest stand was dominated by species like *Rubus ellipticus, Osbeckia crinata, O. nepalensis, Cassia mimosoides, Arundinella khasiana, Houttyunia cordata, Hedychium aurentiacum, Trichoranthus* sp and *Eupatorium adenophorum*, while the open forest was dominated by *Elaeagnus latifolia, Eurea japonica, Rhus javonica, Osbeckia crinata, Cassia mimosoides, Ageratum conizoides* and *Eupatorium odoratum.*

2.2. Decomposition and fungal and bacterial activity

The litter comprised only of recently fallen leaves of *Alnus nepalensis* was used for both the sites. Air dried intact litter (10 g) was kept in nylon bags (size 20 × 20 cm, mesh size 1 mm) and spread randomly on the forest floor for decomposition [3]. Six litter bags were collected every month and brought to the laboratory to assess the relative contributions of microorganisms (fungi and bacteria), rates of decomposition and enzyme activities.

2.3. Isolation and counting of platable fungi and bacteria

The dilution plate techniques [22] was employed to count the most important groups of fungi and bacteria in the decomposing litters. The litter was cleaned, air dried, cu into pieces of 1.0 cm and then powdered with a sterilized pestal and mortar. One gram of powdered litter was added into 250 mL conical flask, containing 100 mL of sterilized distilled water and then shaken for 20 min on horizontal shaker. A minimum of 10^{-4}dilution was used to isolate bacteria and 10^{-3} for fungi. 0.5 mL of litter suspension from suitable dilution was spread into each sterilized petriplates containing 20 mL of cooled solidified Rose Bengal Agar [17] and Nutrient Agar [6] media for fungi and bacteria respectively. They were incubated at 25 ± 1°C for fungi and 30 ± 1°C for bacteria. The colony forming unit (CFU) of fungi and bacteria were counted after

7 days and 24 h of incubation respectively. From this data the average number of fungi and bacteria per gram of oven dry weight of litter was computed.

2.4. Determination of moisture content and pH

The moisture content of the decomposing litter was determined by drying 1g of litter to constant weight at 80°C and calculated on a moist weight basis. The pH was determined by grinding a suspension of litter in double distilled water (1:5 w/v) and reading after 1h with an electronic pH meter (Systronics, India).

2.5. Extraction and assay of enzymes

Enzyme activities were estimated by the technique of Spalding [33]. The litter was cleaned to remove adhering soil particles and 5 g were transferred into a Waring blender and ground with 100 mL of chilled acetate buffer (pH 5.5) for 1 min. The homogenate was centrifuged at 9400 *g* at 2°C for 20 min, and the supernatant filtered through a Whatman No. 1 filter paper.

For the enzyme assays, 1 mL of substrate solution and 2 mL of enzyme extract were reacted at 37 ± 1°C for 2 h in a test tube. The substrates were 3% carboxymethyl cellulose, sodium salt (Sigma) for cellulase, 6 % soluble starch (Sigma) for amylase and 6 % sucrose (Sigma) for invertase; they were dissolved in the same buffer as used for grinding the litter. The reducing sugars thus formed were determined by the dinitrosalicylic acid method [19], by measuring the absorbance at 575 nm (Hitachi - 220). Enzyme activity was expressed as reducing sugars formed g^{-1} litter h^{-1}.

2.6. Determination of cellulose, total sugars and total nitrogen

Powdered and sieved (< 0.2 mm) litter samples were used to determine cellulose, total sugars and total N. Cellulose and lignin were estimated by Jermyn's [11] method and total sugars were determined by the method of Mahadevan and Sridhar [16]. Total nitrogen was estimated by the micro Kjeldahl method [2]. Three replicates were analysed for each sample and mean values were taken.

2.7. Statistical analysis

Correlations were calculated between fungal and bacterial counts and some properties of litters using Karl Pearson's coefficient [41]. Analysis of variance was calculated between open and closed forest stands concerning number of bacteria and fungal propagules and enzyme activities.

3. RESULTS

3.1. Fungal and bacterial populations

The fungal and bacterial count showed very similar seasonal trends in both the forest stands of alder (*figures 2* and *3*). The closed forest canopy harboured more fungi and bacteria than the open ones. Initially fungi and bacteria were at a minimum, which increased consistently as the decomposition progressed, and then decreased towards the end of the process. Two peaks of fungal counts were recorded in both the forest canopies. The first peak of fungi was in June, after which it decreased in July and again exhibited a second peak in August. Similarly, the bacterial peak was detected in June with a slight decrease in July and August, which increased significantly to maxima in September exhibiting a second peak. Thereafter the population decreased to a low level towards the end of the decomposition.

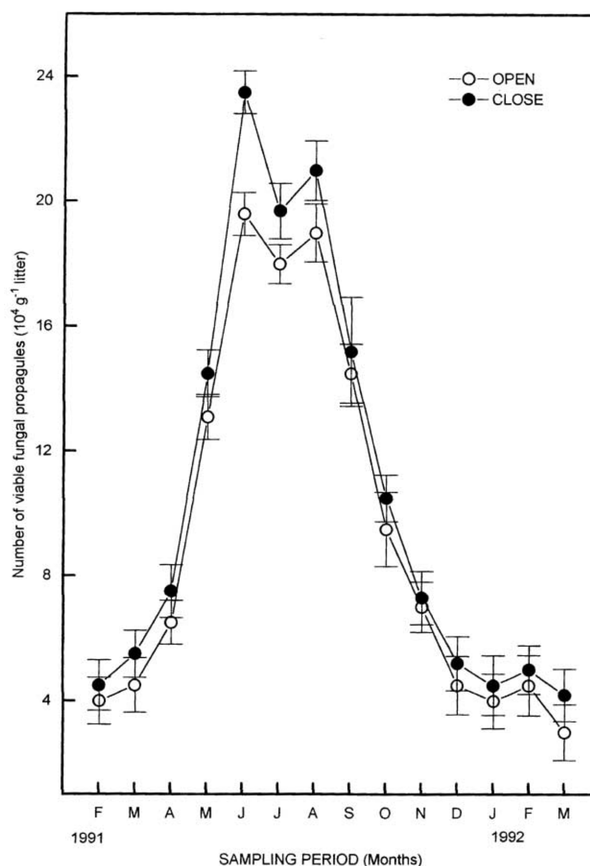

Figure 2. Monthly variation in the number of viable fungal propagules of alder leaf litter in open and closed forest stands. Vertical lines show limits of one SE on either side of mean.

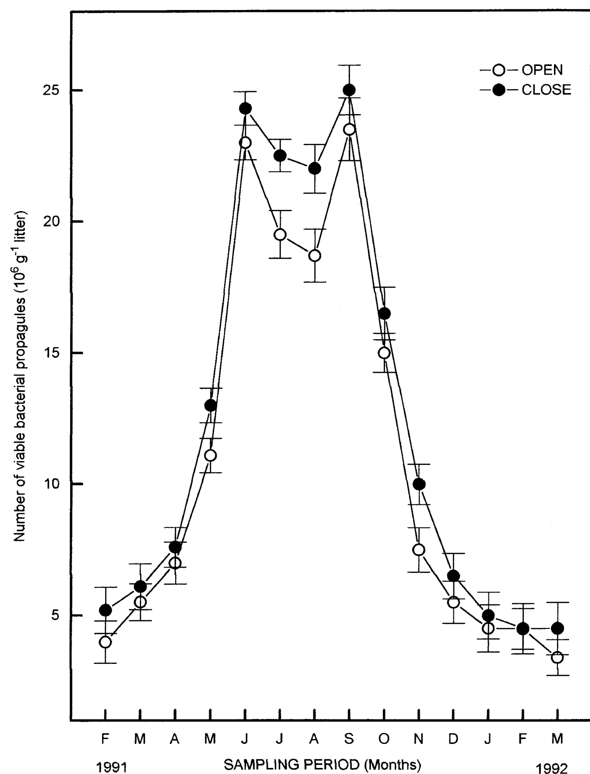

Figure 3. Monthly variation in the number of viable bacterial propagules of alder leaf litter in open and closed forest stands. Vertical lines show limits of one SE on either side of mean.

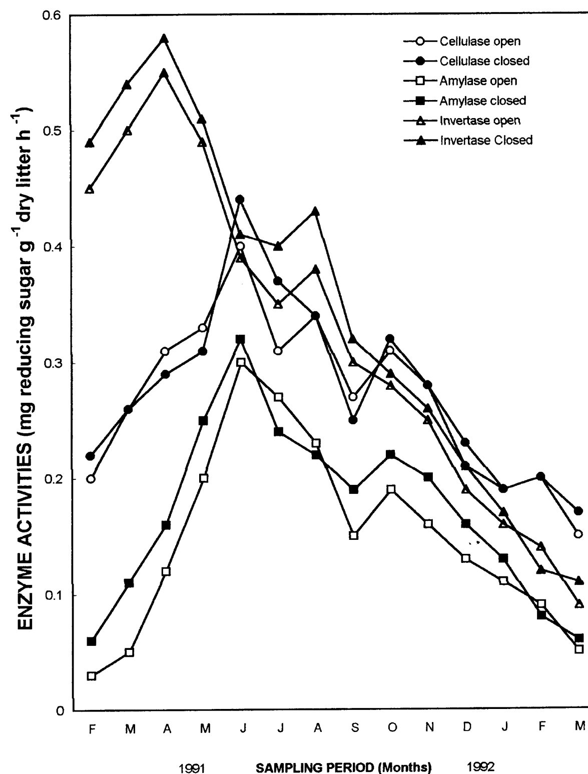

Figure 4. Monthly variation in enzyme activities of alder leaf litters in open and closed forest stands. Cellulase: mean SE ± 0. 031 (closed); 0. 026 (open); amylase: mean SE ± 0. 023 (closed); 0.021 (open) and invertase: mean SE ± 0. 019 (closed); 0.017 (open).

3.2. Cellulase, amylase and invertase activities

Extractable cellulase activity was higher in alder litter of closed forest than in the open forest. In both the forest canopies the cellulase activities were less at the beginning and increased consistently from May onwards and attained a peak in June which ultimately decreased from July to September but again increased slightly in October exhibiting a second peak (*figure 4*). A positive correlation between cellulase activity and the fungal and bacterial numbers and moisture content was found in both the litters, and negatively with the cellulose content of the litters (*table I*).

Amylase activity was also generally higher in litter at closed forest than at the open forest stand. It, too increased with litter decomposition, and showed a marked seasonal variation, with values generally highest in June which ultimately decreased from July to September but again increased slightly in October exhibiting a second peak (*figure 4*). A positive correlation between amylase activity and the fungal and bacterial numbers, moisture content and pH of litters was found in both the forests (*table I*).

Invertase activity was also generally higher in litter at closed forest than at the open forest stand. Unlike cellulase and amylase activity, invertase activity was highest at the beginning of litter decomposition exhibited a peak in April after which it gradually decreased towards the successive months of litter decay in both the forests (*figure 4*). Invertase activity was correlated positively and generally significantly with litter total nitrogen, but negatively with weight loss in both the forests. Neither cellulase nor amylase activity was correlated significantly with total nitrogen or weight loss in any of the litters (*table I*).

4. DISCUSSION

Cellulase activity was negatively related to cellulose content suggests that the amount of cellulose acts as a limiting factor for the activity of cellulase [12]. The high activity in litter at closed forest may be attributed to its high cellulose and nitrogen contents (*table II*). The improved cellulase activity in May-June was assigned to its significant positive correlation with the fungal and bacterial numbers. The positive correlation of cellulase activity with the moisture content of the litter, suggesting the favourable role of moisture in the synthesis of cellulase [29, 35]. The activity of this enzyme is markedly influenced by moisture content [9].

Table I. Correlation coefficient (*r*) of enzyme activities with the fungi, bacteria, moisture content, pH, total nitrogen, weight loss and total sugars contents of *A. nepalensis* litter.

Source of variation	DF	Open forest			Closed forest		
		Cellulase	Amylase	Invertase	Cellulase	Amylase	Invertase
Fungal population		0.84***	0.91***	0.31	0.87***	0.86***	0.30
Bacterial population	12	0.81***	0.89***	0.27	0.74***	0.78***	0.17
Moisture content	12	0.74***	0.82***	0.14	0.73***	0.82***	0.16
pH of litter	12	0.44	0.56**	0.03	0.45	0.69***	0.46
Total nitrogen	12	0.37	0.41	0.87***	0.39	0.42	0.80***
Weight loss	12	– 0.41	0.20	– 0.95***	– 0.44	– 0.19	– 0.97***
Cellulose	12	– 0.46*	–	–	– 0.47*	–	–
Total sugars	12	–	–	0.93***	–	–	0.95***

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

Table II. Cellulose, lignin, total sugar, nitrogen and weight remaining of decomposing alder litters at two sites (open and closed) during initial and final stage of the study.

Leaf litter	Site	Stage of study	Cellulose (%)	Lignin (%)	Total Sugar (μg 100 mg^{-1})	Total N (%)	Weight remaining (%)
Alder	Open	Initial	39	15	221	1.4	100
		Final	5	27	24	0.5	4
	Closed	Initial	41	16	228	1.5	100
		Final	8	21	28	0.7	8

All data mean of three replicates.

The high invertase activity at the initial stages of decomposition was associated with content of soluble sugars in the litter and it appears to originate in part from plant materials [27]. The low activity of invertase towards the end of the decomposition may have been caused by exhaustion of specific substrates and lower numbers of microbes [36]. Ross [25] has also observed more invertase activity in fresh leaves and less activity in an organic horizon.

The changes in amylase activity during litter decomposition signified to the changes in the numbers of microorganisms, confirming the probable microbial origin of this enzyme [12, 27]. The low concentrations of these enzymes in litter of open forest canopy may be the result of low microbial numbers. The significant differences (significance level, $p < 0.05$) between open and closed forest concerning number of fungal and bacterial propagules and enzyme activities may be due to the concentrations of different labile and recalcitrant compounds [13]. Soluble polysaccharides, cellulose and lignin all seemed to be important in determining numbers and types of microorganisms and resultant enzyme activities [23, 24, 32].

The results of the present investigations suggested that the reduced microbial population numbers and their activities in the open forest may lead to decreased mineralization and thus slowing the successional process. The different nature of the understorey vegetation and changes in forest canopy may also act as an additional factor for regulating the activities of microbes.

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