## Microbial Decomposition of a Forest Leaf Litter as Influenced by Certain Edaphic Factors

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Microbial population, microbial activity and the rate of decomposition of leaf litter of a tropical dry mixed decidous forest of Varanasi, were studied in relation to temperature, moisture and pH. In general, 25% moisture content at 35 C and 6.5 pH were most suitable for microbial growth, microbial respiration and weight loss of the leaf litter. The highest fungal population but the least number of species was recorded at 50 C. Under water-logged condition a few fungi particularly *Aspergillus fumigatus* became dominant while others were suppressed. The number of fungal species was reduced at lower (10%) and higher (40%) moisture levels. A sizable increase in bacterial population was recorded under waterlogged condition and at pH 8.5. The rate of CO2 evolution was high at 50 C during early sampling but declined sharply later on. The minimum weight loss of litter was recorded at 15 C under water-logged condition at pH 8.5.

Key Words Activity Growth Litter Microbe Respiration

Litter decomposition is influenced by a combination of environmental factors including physico-chemical properties of the substrate, the external environment and the organisms active under a particular substrate and environmental conditions (Daubenmire & Prusso, 1963; Williams & Gray, 1974; Meentemeyer, 1978). Witkamp (1966) obtained correlations of temperature with carbon dioxide evolution from forest floor. The influence of environmental factors i.e., temperature and water content on soil respiration has been demonstrated by Wildung et al. (1975), Bunnell et al. (1977a) and Edwards (1975). Meentemeyer (1978) suggested actual evapotranspiration (AET) as an index of the climatic forcing function (energy and moisture) of the specialized decomposers. DEcomposition of litter in relation to environmental factors has been studied by several workers (Witkamp & van der Drift, 1961; Witkamp, 1963, 1966; Wicklow & Moore, 1974; Pal & Broadbent, 1975). Witkamp (1966) demonstrated that microbial populations is influenced in decreasing order by temperature, moisture and age of litter.

A vast quantity of the leaf litter (70%) in the selected forest of Varanasi is decomposed in rainy season (Rai & Srivastava, 1982a). Change in environmental and substratal conditions due to variation in temperature, moisture and pH during this period is expected to exert some definite effect on litter decomposition on the forest floor. Therefore, a short term in vitro study was conducted to determine the microbial population and activity and the rate of decomposition of forest leaf litter as influenced by certain edaphic factors like temperature, moisture and pH.

**MATERIALS & METHODS** Freshly fallen leaf litter of various tree species dominated by *Diospyros melanoxylon*, *Anogeissus latifolia and Ruchnania lanzen* was collected from 50 different spots in a dry deciduous forest floor of Chandraprabha sanctuary, Varanasi and brought to laboratory. Soil, beneath the litter, was also collected from the spots and brought to the laboratory after mixing the samples. Aluminium pots (30 cm diam, 20 cm height) were filled each with 2 kg of forest soil (ground and passed through 2 mm sieve) over which a quantity of 50 g air dried mixed leaf litter per pot was dispersed to study the following aspects.

Effects of Temperature, moisture and pH on microbial population and their activity in the leaf litter To assess the effect of temperature the pots were incubated at 15, 35 and 50 C. The pots were regularly supplied with adequate moisture (25-30%). To see the effect of moisture the pots were

	Table 1	Effec	t of Te	mperat	ture, Moistu (Aver	re and p age Nun	oH on l nber g	Microb <sup>-1</sup> Dry	ial Pop Weigh	ulation of De t )	scompo	sing Le	eaf Litt	er	
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ment	SI	S2	S3	S4	SS	SI	S2	S3	S4	SS	S1	S2	S3	S 4 S5	
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ture (C) 15 35 50	26.89 50.34 230.66	34.75 57.62 256.94	56.38 83.13 327.09	58.50 72.84 281.95	58.17 63.97 258.46	9.05 30.48 16.75	10.74 36.89 20.50	11.80 36.57 22.13	13.54 21.54 19.20	16.09 12.31 18.23	2.05 5.07	11.00 11.50	13.42 17.31	19.87 19.54 24.05 23.17	
Moisture content	•(%)										10-1	71.0	4C.1	4.94 4.34	
10 25 40	14.13 30.46 28.39	22.47 48.81 41.01	49.01 94.59 62.69	45.39 75.78 54.56	43.13 42.37 36.73	0.83 15.51 26.53	1.07 17.82 29.45	4.70 21.72 38 92	3.52 15.99 30.46	2.86 11.17 22.46	3.93 4.57	10.33 13.18	21.36 32.73	19.99 17.19 22.90 19.70	
Water logging	67.06	89.47	111.93	158.62	197.96	2.75	58.55	75.96	133.78	22.40 181.53	0.98	3.06	3.12 0.55	2.58 1.07 0.51 0.40	
ра 4.5 8.5 8.5	25.82 24.16 23.62	45.96 49.75 26.87	86.10 80.70 35.60	74.25 71.91 33.82	61.05 66.92 30.78	4.71 11.26 23.64	4.78 15.82 28.70	6.30 17.54 19.96	7.95 15.02 16.49	9.01 11.21 8.66	0.99 4.43 1.08	1.06 10.39	1.93 23.22 2 75	2.16 2.82 26.38 22.99	
Controlf	37.21	53.75	79.20	65.21	58.95	16.35	19.30	19.26	11.75	431	4 73		03 00	PC-2 17-2 PC-2 PC-2 PC-2 PC-2 PC-2 PC-2 PC-2 PC	1
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Table 2 Relative Abudance (per cent occurrence) of Important Fungal Species Isolated from Decomposing Leaf Litter Incubated at Different Temperature.

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Pestalotia macrotricha	50	10	44	17	01	08	53	12	01	05	26	67	01	10	32 14 01	11 32 11 01
Phoma hiberrica	15	11	6	10		08	20	8	01	08	07	01	8	8	01 04 -	03-03
Robillarde Arraemiti s		•	•			03	02	01	,	8	03	01	,	8	03 01 -	12 06 05 -
Trichoderma viride		05	•	6	03	07	02	07	13	8	01	8		01		: .
Dark sterile mvčelium	11	03	90	01		01	05		,	01	8		,	!	- 90	01 08
White sterile mycelium		01	10			,	10	'n	'n	01	08	01		01	12	01 10
'other species'	37	42	11	41	01	49	24	46	10	60	34	62	8	2	33 60 03	61 32 60 03
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solated	17	30	17	2	08	00	17	32	11	41	16	5	12	5	CD 07 47	N 07 17 17

I Initial sampling. S1 - S5 , First - Fifth sampling: C Control (room temperature),  $T_1$  15° C,  $T_2$  35° C,  $T_3$  50° C. 20

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Aspergillus flavus	8	03	01	02	03	1	8		<b>4</b> 0	6 1	ŏ	4 0	6	13	01	03	04	6	17	01	03	8	8	13	
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Table 3. Relative Abundance (percent occurrence) of Important Fungal Species Isolated from Decomposing

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regularly supplied with sterile distilled water to maintain the moisture content of the litter at approximately 10, 25 and 40% on oven dry weight basis. Excess of sterile distilled water was added to the pots to maintain water-logging condition throughout the study. For the effect of pH the leaf litter was adjusted initially at pH 4,5, 6.5 and 8.5 using dilute acid or alkali. The pots kept as control, were treated with same amount of sterile distilled water.

The pots were incubated at room temperature (26-33 C) and regularly supplied with sterile water to maintain 25-30% moisture except mentioned otherwise as in the case of experiments set to study the effects of temperature and moisture. The experiments were performed in 5 replicates and the microbial population and activity of the leaf litter were estimated by the method described below:

Isolation of microorganisms The microorganisms were isolated from the decomposing litter at monthly interval. The first isolation was made from the freshly fallen litter which was considered as the 'initial' sample. Subsequent isolations were made from the treated and untreated leaf litter of the pots until 5 months. Dilution plate technique (Parkinson *et al* 1971) was employed for the isolation of fungi, bacteria and actinomycetes from the leaf litter (Rai & Srivastava, 1982b). Czapek-Dox + 0.05% yeast extract agar, Thomton's agar and starch casein agar medium supplemented with mycostatin at 50 µg ml-1, were used for isolation of fungi, bacteria and actinomycetes, respectively. The fungi were assessed qualitatively and quantitatively after 1 week of incubation at  $25\pm2^{\circ}$ C. The bacterial and actinomycete flora were estimated only quantitatively after 4 and 10 days of incubation at  $35\pm2$ C;  $28\pm$ C respectively.

Estimation of microbial activity in terms of  $CO_2$  evolution The rate of  $CO_2$  evolution from the treated and untreated decomposing litter of the pots was determined monthl by inverted box method (Witkamp, 1966). The first estimation of  $CO_2$  evolution was made from the treated and control leaf litter immediately after their dispersion over the soil surface of the pots and was considered as the 'initial' sample. The subsequent estimations were done for 5 months.

Effects of temperature, moisture and pH on the rate of litter decomposition. Rate of later decomposition was measured on the basis of loss of dry weight of the litter in small hylon litter bags (Rai & Srivastava, 1982b). Five 15 x 15 cm nylon bags (4 mm sieve), each containing 10 g leaf litter discs, were placed over each pot containing 2 kg forest soil. The litter discs were obtained by cutting different portions of leaves of different tree species with the help of a cork-borer (5 mm diam). To study the effects of temperature, moisture and pH the experiments were set up by the methods described earlier. Five such sets were prepared for each treatment and

control .Five litter bags (one from each replicated pot) from each set were sampled at monthly interval until 5 months. The samples were oven dried at 80 C for 24 h and percentage weight remaining at each sample was calculated.

**RESULTS** Effect of temperature, moisture and pH on the microbial population. The fungal population was least at 15 C and the highest at 50 C. Bacterial count was low at 15 C during early samplings but it increased later on. The litter supported a fairly high bacterial population at 35 C. The population of actinomycetes was lowest at 50 C and the highest at 35 C (Table 1)

The fungal population was low at 10% and high at 25% moisture content of the litter becoming lower at 40%. Exceptionally highest fungal population was recorded under waterlogged condition due to predominance and heavy sporulating nature of Aspergillus fumigatus (Table 3). At 10% moisture level the bacterial population was reduced considerably. There was a regular increase in the bacterial count with increase in moisture. A sizable increase of the bacterial population in the litter was recorded under water-logged condition. The actinomycetes count also declined at 10% and 40% moisture levels. However, the most detrimental effect was noticed under waterlogged condition (Table 1).

The fungal population was slightly low at pH 4.5 and 6.5 at the beginning but enhanced in the successive samples. Fungal population was markedly low at pH 8.5 count was low at pH 4.5 and 6.5 except in the last few samples but pH 8.5 enhanced the bacterial population. Actinomycetes count was reduced due to acid and alkaline treatments whereas it showed values similar to control at pH 6.5 (Table 1).

Effects of temperature, moisture and pH on qualitative nature of the litter mycoflora Less number of fungal species was isolated from the litter incubated at 15 C and 50 C (Table 2). Only a few fungi withstood 50 C. There was a little variation in the number of fungal species between the litter incubated at 35 C and room temperature (26-33 C). Alternaria alternata, A. tenuissima, Curvularia lunata var. aeria, Pestalotia macrotricha, and fungi with dark and white sterile mycelia were favoured while Aspergillus spp. were suppressed at 15 C. A fumigatus and A. nidulans were most dominant species in the litter at 50 C (Table 2).

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A. flavus	5	38	3 3	3 8	3 8	3 3	3 2	1 2	2	8	2	20	10	1	•	1	6	1	1	1	
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'other species'	39	49	48	61	18	44	32	8	2	2	8	5	3						l'	10	
Teral number of species isolated	17	30	27	30	18	36	30	32	18 <sup>`</sup>	41	33	35	5	୍ଗ   ରୁ	~	67			4		

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Initial sampling, S<sub>1</sub>-S<sub>9</sub>, First - Fifth samplings Control, 1 pH 4.5, 2 pH 6.5, 3 pH 8.5 н

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		1 8	100	•	
Treatment	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S4	S <sub>5</sub>
Initial sampling Temperature ('C)			92		
15	95	114	152	• 197	225
35	172	217	335	185	87
50	199	257	297	128	45
Moisture Content (%)					
10	124	136	248	137	88
25	164	182	331	204	80
40	132	128	101	99	99
Water-logging					
рН		·			
4.5	110	142	205	195	86
6.5	118	164	248	171	87
8.5	104	131	196	117	60
Control	125	164	274	160	89

# Table 5. Effect of Temperature, Moisture and pH on the rate of $CO_2$ evolution from decomposing leaf litter (µg g<sup>-1</sup> dry litter hr<sup>-1</sup>)

S<sub>1</sub> - S<sub>1</sub>, First - Fifth sampling.

## Table 6. Effect of Temperature, Moisture and pH on per cent Weight remaining of Decomposing Leaf Litter

Treatment	I	S,	S,	s,	s,	S,
Temperatum (*C)			I	Per cent Weight		
15	· 100	<b>99</b> .	94.	85	77	65
15	100	95.	85	60	40	22
50	100	<b>96</b> .	90	74	62	55
Moisture content (%)		<b>6</b> 4	01	72	60	47
10	100	96	91	13	42	47
25	100	94	8/	01	42	21
40	100	98	94	82	63	28
Water-logging	100	99	95	87	77	68
рН	100	98	90	77	62	49
4.5	100	96	91	70	59	36
6.5	100	00	96	85	72	65
8.5	100	<b>77</b> .			,2	05
Control	100	96	90	67	41	32.

I Initial sampling : S - S , First - Fifth samplings .

The r. er of fungal species was less in the litter at low moisture content (10%). The diversity in fungal composition increased by increasing moisture content of the litter to 25%. Further increase in moisture content i.e., 40% caused a decline in fungal species (Table 3). Waterlogging caused a marked reduction in the number of fungal species and the dominant species isolated was *A.fumigatus*. In addition, *Absidia butleri*, *A. repens*, *A. scabra*, *Aspergillus sydowi* and *Cunninghamella echinulate* were also well represented (Table 3).

Alteration in pH of the litter caused reduction in the number of fungal species. The most significant effect was recorded at pH 8.5 and the least at pH 6.5 (Table 4). Alternaria alternate, Pestalotia spp. and Trichoderma harzianum were abundant at pH 4.5. At pH 8.5, Aspergillus spp., particularly A. luchuensis, A. nidulanes A. nigar and A variecolor, were most abudant in the litter.

Effects of temperature, moisture and pH on  $CO_2$ evolution from the litter Carbon dioxide evolution from the decomposing leaf litter under different temperature, moisture and pH is presented in Table 5. The rate of CO2 evolution was low at 15 C in the first three samples but increased subsequently. Incubation of litter at 35 C enhanced the rate of  $CO_2$  evolution. The rate of  $CO_2$ evolution at 50 C was high in the first two samples but declined sharply later on showing values even less than the litter at room temperature (26-33 C).

 $CO_2$  evolution at 10% moisture content was low. Moisture content at 25% favoured  $CO_2$  evolution except in the last sample. Further increase in moisture content (40%) reduced the rate of  $CO_2$  evolution except in the first and last samples. Water logging of the litter resulted in the minimum rate of  $CO_2$  evolution.

Alteration in pH of the litter lowered the rate of  $CO_2$ evolution and the least rate was recorded at pH 8.5. However, the rate was high for the fourth samples at pH 4.5 and 6.5.

Effects of temperature, moisture and pH on weight loss of the litter. The litter lost maximum weight (78%) at 35 C and the minimum (35%) at 15 C (Table 6). The maximum weight loss of litter was recorded at 25% (72%) followed by 10% moisture content (53%). The minimum loss of litter weight occurred under water-logged condition (31.97%). The litter adjusted at pH 4.5, 6.5, and 8.5, lost

less weight compared with the control. The minimum weight loss of the litter was recorded at pH 6.5.

DISCUSSION A change in temperature, moisture and pH will alter the species composition of the active flora and at the same time will have a direct influence on each organism within the community in terrestrial ecosystem (Alexander, 1977). Our results indicate that the optima of temperature, moisture and pH lie somewhere near 35C. 25% and 6.5, respectively, for microbial growth in the litter. However, the reason for an exceptional highest fungal population at high temperature and under water. logged condition might be attributed to predominance and rich sporulation of Aspergillus fumigatus and A.nidulans in the case of former and only A. fumigatus in case of the latter. The dominance of these two species under adverse situation and at high temperature has been reported by other workers (Evans, 1971; Tiwari, 1975 and Upadhyay 1987). The bacterial population was recorded higher under water-logged condition as obligate and facultative anaerobic bacteria might have grown under such condition. The relaxation in number of species at these extremes might be considered a factor for some of the microbes to grow.

The optima of the three factors for growth of microorganisms corresponded with the optima of microbial metabolic activity. Edwards (1975) found a strong relationship betweenCO2 evolution and mean daily litter temperature. The microbial activity was slow at 15C but rapid at 35 C. The CO<sub>2</sub> evolution declined at temperature above 35 C except in case of thermophilic microbial activity. Adequate moisture (i.e. 25% moisture content of the litter) might have induced greater microbial activity in the litter. The pH also influences the type of microorganisms associated with the carbon cycle or any habitat (Alexander, 1977). Therefore, reduction in microbial activity in the litter at altered pH was apparent. The rapid decomposition of the litter occured at pH 6.5 which is in accordance with the pH optimum for microbial count and  $CO_2$  evolution. The lowest rate of decomposition was recorded at pH 8.5 which might be attributed to the adverse effect of alkaline reaction on fungi.

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