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Fungal diversity in the gut contents of selected earthworms species at two forest stands of Meghalaya differing in altitudes

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A B S T R A C T

Fungal diversity in the gut contents of common earthworms species were studied for a period of two years. The earthworms were collected from the two broad leaved forest stands of Meghalaya. The study sites selected were Upper Shillong at a higher altitude and Mawkyrdep at a lower altitude. The forest stand at a higher altitude is situated at 1861 m above sea level. The other forest stand at a lower altitude is situated at 889 m above sea level. The isolation of fungal species from the earthworm gut contents (foregut, midgut and hindgut) was done following soil plate method Warcup (1950) using Rose Bengal Agar medium, Martin (1950) and incubated at 25±1°C in a B.O.D. incubator for a period of 5-7 days. The colony forming unit (CFU) of fungi was calculated on dry weight basis. The fungal CFU of earthworm gut contents showed the trend foregut > midgut > hindgut. A total of 102 fungal species were isolated from the foregut, midgut and hindgut of the two earthworm species at the two forest stands. Highest number of fungal species were isolated from foregut followed by midgut and least number was isolated from hindgut in case of both the earthworm gut contents. Species of *Absidia, Aspergillus, Cladosporium, Fusarium, Humicola, Minimedusa, Mucor, Penicillium, Pythium* and *Trichoderma* were found to be common in all the three gut contents.

Introduction

Soil is inhabited by vast array of organisms which include both micro and macro flora and fauna. Among soil fauna, earthworms are the most obvious fauna comprising more than 70% of the total biomass (Teng et al., 2012). The lives of soil fauna particularly earthworms and microbes are closely intertwined.

Aristotle called them 'the intestines of the earth' and the eminent nineteenth century biologist, Charles Darwin, spent many years observing their major influence on the formation of humus and transport of soil (Johnson- Maynard et al., 2002).

The spores of many species of microbes which form important diet of earthworms can pass through the earthworm gut without causing any harm. The importance of particular groups of microbes as food of earthworms differs between different earthworm species, particularly those that have remarkably varying feeding habitats.

Earthworms derive nutrients from microbes and promote their activity by shredding and increasing the surface area of the organic matter and making it more available to microbes. Earthworms also pass a mixture of organic and inorganic matter through their guts. As the food materials passes through their gut, the final process in organic matter decomposition or humification is accelerated due to the intestinal micro-flora in the gut.

Although, earthworms have been scientifically studied by man right from the time of Darwin (1881) and though different aspects such as development, physiology and ecology were studied, attention has been paid to the understanding of the relationship between earthworm and microbes only in the last two decades (Parthasarathi, 2007). For better understanding of the effect of earthworm ingestion on microbial processes in soils, knowledge on the microbial biomass in the digestive tract of earthworm may be useful. The present investigation was undertaken to make a comparative study on the fungal diversity of the gut contents of two earthworm species collected from two forest stands differing in altitudes.

Materials and Methods

The earthworms were collected from the two broad leaved forest stands of Meghalaya. The study sites selected were Upper Shillong at a higher altitude and Mawkyrdep at a lower altitude. The forest stand at a higher altitude is situated at 1861 m above sea level. The other forest stand at a lower altitude is situated at 889 m above sea level. Wilke's (1955) hand sorting method was followed for the collection of earthworms from two forest stands of Meghalaya. The earthworms collected were brought to the laboratory, cleansed thoroughly with sterilized distilled water and then killed in 70% alcohol. The body cavity was opened ventrally and the gut was divided into foregut (FG), midgut (MG) and hindgut (HG). They were dissected free and the gut contents were collected in separate sterilized Petri dishes. The samples thus collected were used for the isolation, identification and estimation of fungi.

Isolation, identification and estimation of fungi from gut contents

Soil plate method Warcup (1950) using Rose Bengal Agar medium Martin (1950) was followed for the isolation of fungi. Three replicates were maintained for each sample. The inoculated Petri plates were then incubated upside down at 25 ± 1^0 C for 5-7 days in a sterilized B.O.D. The number of fungal colonies formed was counted and the Colony Forming Unit (CFU) was calculated on dry weight basis.

Colony Forming Units (CFU) of fungi was calculated as follows:

$$\text{CFU of fungi } g^{-1} \text{ dry weight} = \frac{\text{Total number of colonies}}{\text{Dry weight of the soil (g)}}$$

The following indices for fungal species diversity and dominance were also calculated:

(a) Index of general diversity (H') or Shannon and Weaver 1949 diversity index

$$H' = \Sigma\ (ni/\ N \log ni\ N)$$

(Where *ni* is the importance value of each species and N is the total importance value)

(b) Index of dominance (C) or Simpson (1949) index of dominance.

$$C = \Sigma (ni/N)^2$$

(Where *ni* is the importance value of each species and N is the total importance value)

Result and Discussion

In both the earthworm species, the fungal CFU of gut contents of the earthworm collected from high altitude forest stand was higher than that at low altitude forest stand. Fungal CFU exhibited monthly variations in the foregut, midgut and hindgut at the two forest stands. Highest fungal CFU was recorded in the foregut followed by the midgut and the least was recorded in the hindgut. The fungal CFU of earthworm gut content showed the trend foregut > midgut > hindgut (Fig. 1).

Table 1 depicts the list of fungal species isolated from the foregut, midgut and hindgut contents of earthworms. Qualitatively, there was not much difference in the fungal species composition. Altogether, 102 fungal species were isolated from the foregut, midgut and hindgut of both the earthworm species at the two forest stands. At the high altitude forest stand, a total of 76 fungal species were isolated of which, 57, 51 and 48 fungal species were isolated from the foregut, midgut and hindgut respectively, whereas, at the low altitude forest stand, a total of 69 fungal species were isolated of which, 55, 45 and 41 fungal species were isolated from the foregut, midgut and hindgut respectively. Maximum fungal genera isolated belonged to Deuteromycotina (16 genera, 65 species) followed by Ascomycotina (8 genera, 14 species), Zygomycotina (5 genera, 19 species) and Mastigomycotina (2 genera, 4 species). Highest number of species of *Penicillium* (23 species) could be isolated followed by *Aspergillus* (11 species), *Mortierella* (8 species), *Trichoderma* (6 species), *Mucor* (5 species), *Fusarium* and *Phoma* (4 species each), *Absidia, Eupenicillium, Paecilomyces, Pythium* and *Talaromyces* (3 species each), *Acremonium, Chaetomium, Cladosporium, Gliocladium, Humicola, Nectria* and *Oideodendron* (2 species each), *Alternaria, Botryotrichum, Cylindrocarpon, Eurotium, Gonytrichum, Minimedusa, Pestalotia, Petriellidium, Phytophthora, Pseudoeurotium, Rhizopus* and *Staphylotricho* (1 species each).

Absidia corymbifera, A. cylindrospora, Acremonium cerealis, Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Fusarium poae, Humicola fuscoatra, H. grisea, Minimedusa polysporum, Mucor circinelloides, M. hiemalis, Penicillium bevicompactum, P. canescens, P. corylophilum, P. simplicissimum, P. verrucossum, Pythium aphanidermatum, Trichoderma koningii and *T. viride* were found to be common in all the three gut contents.

Shannon diversity index of fungal species isolated from all the gut contents were found to be the highest in the foregut. It ranged between 2.65-3.05 in the foregut, 2.60-2.96 in the midgut and 2.40-2.70 in the hindgut at the high altitude forest stand. At the low altitude forest stand, it ranged between 2.50-2.86 in the foregut, 2.45-2.70 in the midgut and 2.35-2.60 in the hindgut (Fig. 2).

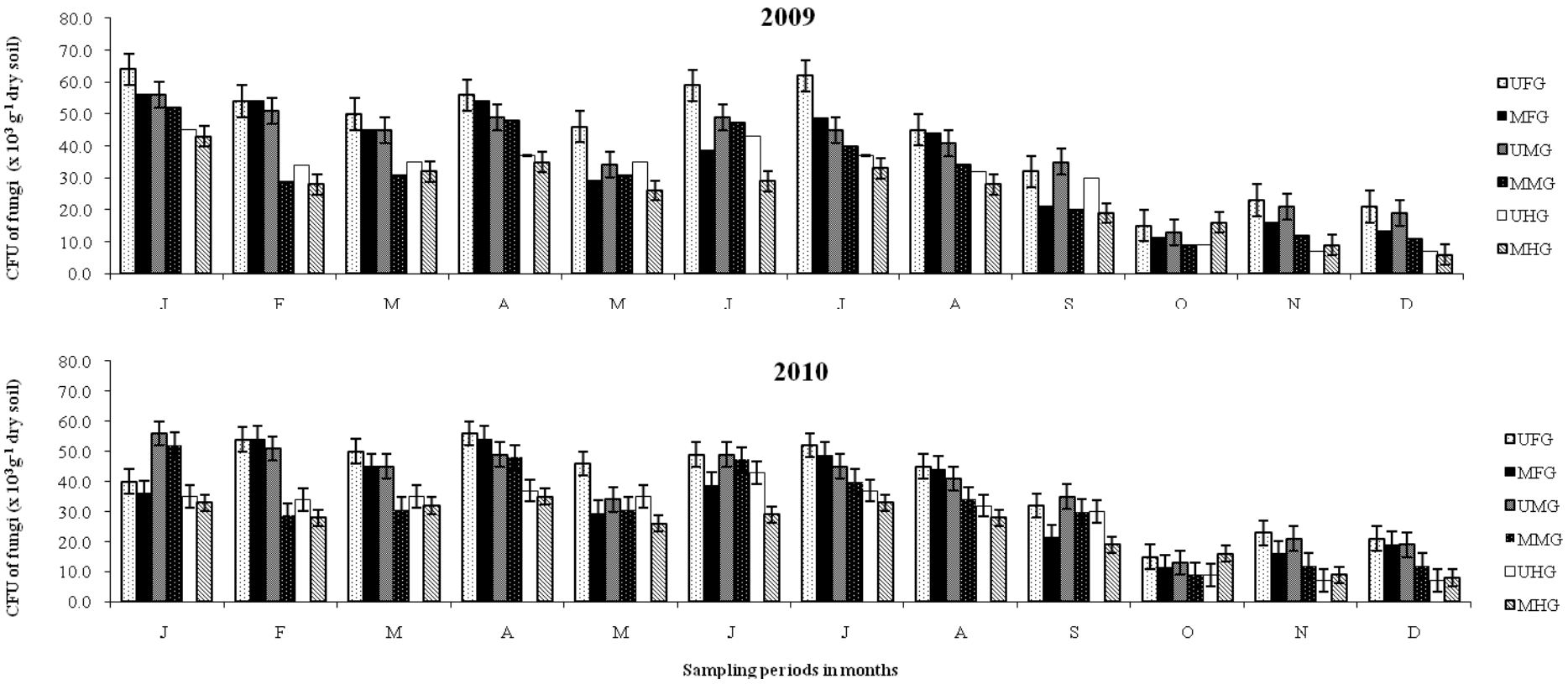
Simpson dominance index of fungal species isolated from all the gut contents were found to be the highest in the hindgut. It ranged between 0.050-0.051 in the foregut, 0.052-0.053 in the midgut and 0.053-0.054 in the hindgut at the high altitude forest stand. At the low altitude forest stand, it ranged between 0.051-0.052 in the foregut, 0.053-0.054 in the midgut and 0.055- 0.056 in the hindgut (Fig. 3).

Table.1 List of fungal species isolated from foregut, midgut and hindgut contents of earthworms collected at the two forest stands during the study periods of 2009 and 2010

Sl. No.	Fungal species	UFG	UMG	UHG	MFG	MMG	MHG
Mastigomycotina (2 genera, 4 species)							
1	*Phytophthora cinnamomi*	-	+	-	-	-	-
2	*Pythium aphanidermatum*	+	+	+	+	+	+
3	*P. intermedium*	-	-	-	+	+	-
4	*P. irregular*	+	+	+	-	+	+
Zygomycotina (5 genera, 19 species)							
1	*Absidia corymbifera*	+	+	+	-	+	+
2	*A. cylindrospora*	+	+	+	+	+	+
3	*A. glauca*	-	-	+	+	-	-
4	*Acremonium butyric*	+	+	-	+	+	+
5	*A. cerealis*	+	+	+	+	+	+
6	*Minimedusa polysporum*	-	-	+	-	-	-
7	*Mortierella exigua*	-	-	-	+	-	-
8	*M. gamsii*	+	-	-	-	-	-
9	*M. humilis*	+	-	-	-	+	-
10	*M. minutissima*	+	-	-	-	-	-
11	*M. polycephala*	-	+	-	-	-	-
12	*M. polysporum*	-	-	-	+	+	-
13	*M. ramanniana*	-	-	-	+	-	-
14	*Mucor circinelloides*	+	+	+	+	+	+
15	*M. hiemalis*	+	+	+	+	+	+
16	*M. mucedo*	+	-	-	+	-	-
17	*M. piriformis*	-	+	-	-	-	-
18	*M. racemosus*	-	-	+	-	-	-
19	*Rhizopus oryzae*	+	-	+	-	-	-
Ascomycotina (8 genera, 14 species)							
1	*Chaetomium mozdrenkae*	-	-	-	+	-	-
2	*C. tetrasporum*	+	-	-	-	-	-
3	*Cylindrocarpon olidum*	-	-	-	+	-	-
4	*Eupenicillium brefeldianum*	-	-	+	-	-	-
5	*E. javanicum*	-	-	-	+	-	-
6	*E. lapidosum*	-	-	-	+	-	-
7	*Eurotium chevalieri*	-	-	-	+	-	-
8	*Minimedusa polysporum*	+	+	+	+	+	+
9	*Nectria inventa*	+	+	+	+	-	-
10	*N. ventricosa*	-	-	-	-	+	-
11	*Pseudoeurotium zonatum*	+	+	+	-	-	+
12	*Talaromyces emersonii*	-	+	-	-	-	-
13	*T. trachyspermus*	-	-	-	+	-	-
14	*T. wortmanii*	-	-	+	-	-	-
Deuteromycotina (16 genera, 65 species)							
1	*Alternaria alternate*	+	+	+	+	+	+
2	*Aspergillus clavatus*	-	-	+	+	-	-
3	*A. flavus*	+	+	-	+	-	+
4	*A. fumigates*	+	+	+	+	+	+
5	*A. japonicas*	-	-	-	-	-	+
6	*A. melleus*	-	-	-	+	-	-
7	*A. niger*	+	+	-	-	+	+
8	*A. restrictum*	-	-	-	-	+	+
9	*A. sydowii*	+	+	-	+	-	-
10	*A. ustus*	+	+	-	+	-	+
11	*A. versicolor*	+	-	-	-	-	-
12	*A. wentii*	-	-	-	-	+	-
13	*Botryotrichum piluliforum*	+	-	-	-	-	-
14	*Cladosporium cladosporioides*	+	+	-	+	-	-

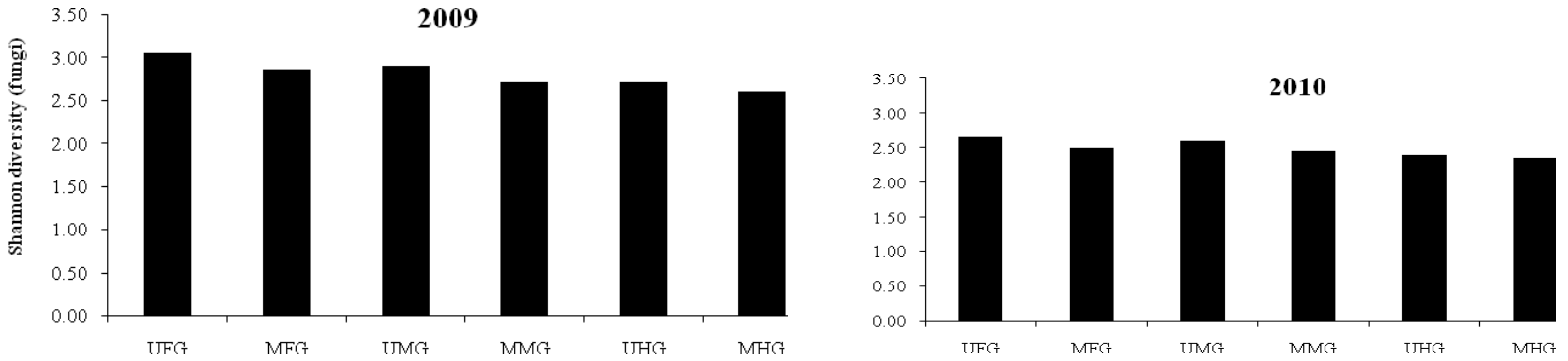
15	*C. herbarum*	+	+	+	+	+	+
16	*Fusarium moniliforme*	-	-	+	-	-	-
17	*F. oxysporum*	+	+	+	-	+	+
18	*F. poae*	+	+	+	+	+	+
19	*F. semitectum*	+	-	-	-	+	+
20	*Gliocladium catenulatum*	-	-	-	+	-	-
21	*G. roseum*	+	+	-	-	+	+
22	*Gonytrichum macrocladum*	-	-	-	+	-	-
23	*Humicola fuscoatra*	+	+	+	+	+	+
24	*H. grisea*	+	+	+	+	+	+
25	*Oideodendron echinulatum*	-	-	-	-	+	-
26	*O. tennuissimum*	-	-	-	+	+	-
27	*Paecilomyces carneus*	+	-	+	-	-	-
28	*P. farinosus*	-	-	-	-	+	-
29	*P. marquandii*	-	-	-	+	+	-
30	*Penicillium atrovenetum*	-	-	+	-	-	-
31	*P. bevicompactum*	+	+	+	+	+	+
32	*P. canescens*	+	+	+	+	+	+
33	*P. chrysogenum*	-	+	+	+	-	-
34	*P. citrinum*	-	-	-	+	-	-
35	*P. corylophilum*	+	+	+	+	+	+
36	*P. expansum*	-	+	-	-	-	-
37	*P. fellutanum*	-	+	+	-	+	-
38	*P. frequentans*	+	+	+	+	-	+
39	*P. implicatum*	+	+	+	-	-	-
40	*P. janthinellum*	-	+	+	+	+	+
41	*P. jensenii*	+	+	-	+	+	+
42	*P. lanosum*	+	+	+	+	+	+
43	*P. oxalicum*	-	-	-	-	+	+
44	*P. purpurogenum*	+	+	+	-	-	-
45	*P. restrictum*	+	-	-	-	-	-
46	*P. rubrum*	+	+	+	+	-	+
47	*P. rugulosum*	-	+	-	-	-	
48	*P. simplicissimum*	+	+	+	+	+	+
49	*P. steckii*	-	-	-	-	+	-
50	*P. stoloniferum*	+	+	-	+	+	-
51	*P. verrucossoum*	+	+	+	+	+	+
52	*P. waksmanii*	+	-	-	-	-	-
53	*Pestalotia* sp.	-	+	+	-	+	-
54	*Petriellidium* sp.	-	-	+	-	-	+
55	*Phoma eupyrena*	+	+	+	+	-	+
56	*P. medicaginis*	-	-	+	-	-	-
57	*P. pomorum*	+	-	-	-	-	-
58	*P. parocandrum*	-	+	-	-	-	-
59	*Staphylotricho coccosporum*	+	-	-	-	-	-
60	*Trichoderma harzianum*	+	-	-	+	-	-
61	*T. hamatum*	-	+	+	-	-	+
62	*T. koningii*	+	+	+	+	+	+
63	*T. polysporum*	+	-	+	+	+	+
64	*T. pseudokoningii*	+	-	-	+	-	+
65	*T. viride*	+	+	+	+	+	+

Note: **'+'** indicates present; **'-'** indicates absent**; UFG**= Foregut (Upper Shillong); **MFG**= Foregut (Mawkyrdep); **UMG**= Midgut (Upper Shillong); **MMG**= Midgut (Mawkyrdep); **UHG**= hindgut (Upper Shillong); **MHG**= Hindgut (Mawkyrdep).

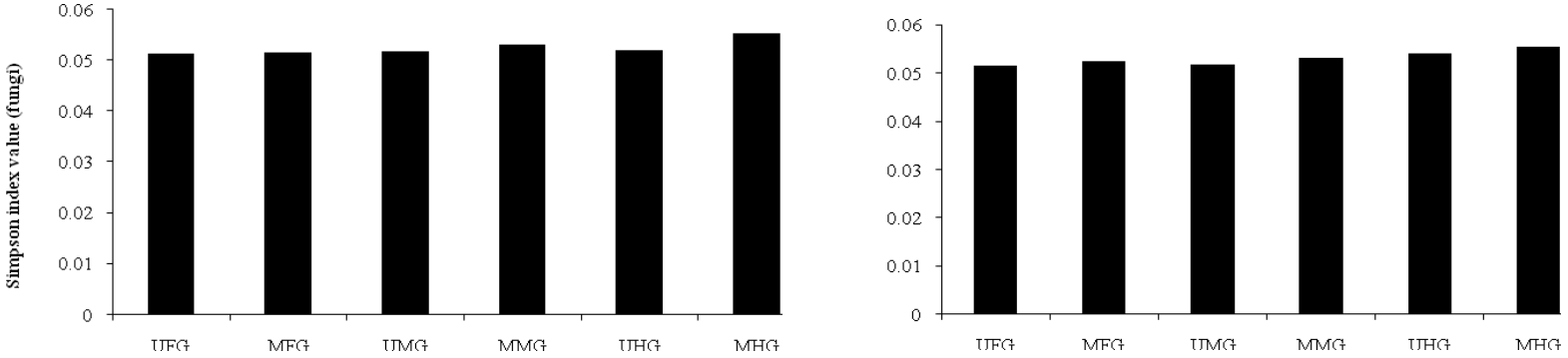


Figs. 1 Monthly variations of fungal CFU of foregut, midgut and hindgut contents of earthworms collected at the two forest stands during the study periods of 2009 and 2010.

Note: UFG= Foregut (Upper Shillong); **MFG**= Foregut (Mawkyrdep); **UMG**= Midgut (Upper Shillong); **MMG**= Midgut (Mawkyrdep); **UHG**= hindgut (Upper Shillong); **MHG**= Hindgut (Mawkyrdep)



Figs.2 Shannon diversity index of fungi isolated from foregut, midgut and hindgut contents of earthworms collected at the two forest stands during the study periods of 2009 and 2010



Figs. 3 Simpson dominance index of fungi isolated from foregut, midgut and hindgut contents of earthworms collected at the two forest stands during the study periods of 2009 and 2010.

Higher fungal CFU of earthworm gut contents were recorded at the high altitude forest stand. The higher fungal CFU at the high altitude forest stand may be due to moisture content which renders moist soil condition at the high altitude forest stand. Fungal CFU was highest in the foregut followed by midgut and the least was recorded in the hindgut. This trend may be attributed to the stimulation of fungal species during passage through the gut. This is in agreement with the earlier findings of Dkhar and Mishra (1991) who reported maximum microbial populations in the foregut and minimum in the hindgut.

Almost similar fungal species were isolated throughout the study period in the gut contents at the two forest stands; however, few were restricted to each stand. Qualitatively and quantitatively, there was not much difference in the fungal species of the gut contents. A decrease in the number of fungal CFU throughout the gut length from the foregut, midgut to hindgut may be due to the fact that probably some of them were killed during passage or fungal mycelia and spores got digested as they passed through the gut (Dash et al., 2009). It may also be due to large amount of mucus that the earthworms secrete in their gut which may be assimilated by the microbial community in the gut (Scheu, 1993). Alauzet et al (2001) also reported that the slight decrease in the number of fungi in the guts show that earthworms regulate the number of fungi mostly by digesting them. Schonholzer (2002) also observed that large, metabolically active microbes are preferentially digested in the digestive tract of the worm and by the time they reach the hindgut only few of them survive. Moody et al (1996) found that the effect of passage through the earthworm gut on the viability of spores of saprophytic fungi was found to vary depending upon the fungal and earthworm species. Some of the fungal species were present throughout the gut canal as well as in the soil, this may be due to the fact that various fungal spores have thick-walled or wrinkled coats Dash et al (2009) or these are resistant to breakdown by intestinal enzymes of the earthworms (Striganova et al., 1989) thus leading to their survival during passage through the alimentary canal (Harinikumar and Bagyaraj, 1994). The survival of the fungal spores and mycelia such as species of *Aspergillus* and *Penicillium* may be attributed to their antibiotic producing capacity (Reddy and Grisham, 1998). The variation in the mycoflora in the different regions of the gut suggests that some of the fungi which are in dormancy get broken down while passing through the gut (Reddy and Grisham, 1998; dash et al., 2009). Tiwari et al (1990) suggested that there exists a gradient with regard to the digestive capability of different regions of the gut of earthworms for utilisation of microfungi as food. As a whole, various reasons have been attributed to the survival of microbes in the earthworms guts such as: (a) production of antibiotic or inhibitory substance by *Aspergillus* sp. and *Penicillium* sp. Dash et al (2009) (b) presence of strong outer coat apparently protecting them from digestion and (c) production of phytotoxic metabolite by *Fusarium* sp. Ghosh et al. (1989) or may be due to the production of antibiotic and/or inhibitory substance and/or presence of strong outer coat and/or production of phytotoxic metabolites, as reported in earlier studies (Ghosh et al., 1989; Pizl and Novokova, 2003).

Conclusion

It can be concluded that altitudinal and climatic differences of forest stands influenced fungal diversity in the earthworm gut contents. *E. foetida* (exotic) exhibited

slightly higher gut fungal diversity than that of *P. excavatus* (indigenous). Also, considering the potential contribution of earthworms to soil fertility management, there is the need to consider them in agroecosystem management decisions. The earthworms can specifically affect soil fertility that may be of great importance to increase sustainable land use in naturally degraded ecosystems as well as agroecosystems.

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