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## Interactions between Soil Animals and Ectomycorrhizal Fungal Mats

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### ABSTRACT

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Estimates of microbial biomass were made for ectomycorrhizal fungal mats colonizing mineral soil in a 50-75-year-old Douglas-fir stand in western Oregon. The ectomycorrhizal fungal mats are from the basidiomycete, *Hysterangium setchellii*. Numbers and biomass of soil animals including microarthropods and nematodes were estimated for both fungal mat and non-mat areas. The mats generally showed a significantly greater microbial biomass and also greater numbers of soil microarthropods. Protozoans were also sampled and exhibited greater abundance in fungal mats for amoebae and ciliates, but not flagellates. We hypothesize that these mats represent a larger and more active microbial biomass, available as a soil-animal food resource. Fungal mats had greater concentrations of soil C and soil N, and soil respiration and enzyme activity rates were significantly greater in mat than non-mat soil.

### INTRODUCTION

Interactions between soil animals and micro-organisms in decomposition and nutrient-cycling processes are of considerable interest in current attempts to quantify the effects of different faunal and microbial components in their ecosystem functions. Often, such effects are evident on relatively short-term time scales such as seasonal changes in carbon and nutrient pools (Coleman, 1985; Huish et al., 1985). Darwin's classic work on earthworms (Darwin, 1881) was one of the first to demonstrate long-term effects on soil turnover. Recent research by Springett (1985) obtained experimental evidence of earthworm transfer of lime from the soil surface down to depths of 20 cm in the soil within a 9-month time period. As rhizospheres develop, interactions occur between major functional groups such as ectomycorrhizal fungi and bacteria which can

influence development and persistence of these rhizosphere components (Bowen and Theodorou, 1979). The influence of soil and vegetation in a given climatic region helps to determine the qualitative and quantitative assemblage of the below-ground community (Jenny, 1980; Miles, 1985).

The rhizosphere is a current focal point of below-ground ecosystem studies owing to its importance in carbon turnover and nutrient dynamics (Coleman et al., 1983, 1984; Ingham et al., 1985). In recent years, researchers have recognized that the mycorrhizal association with roots can account for a substantial portion of carbon allocation and nutrient dynamics (Fogel and Hunt, 1979, 1983; Vogt et al., 1980). Certain fungi form a sufficiently dense colonization of litter or soil horizons to create characteristic fungal-mat zones which are readily visible (Ramsbottom, 1953; Hintikka and Naykki, 1967; Hintikka, 1970; Fisher, 1972). Some ectomycorrhizal species can form dense fungal mats (Hintikka and Naykki, 1967; Fisher, 1972; Cromack et al., 1979). Wood-rotting fungi also colonize tree boles and roots densely in characteristic decay zones (Johansson and Theander, 1974; Waid, 1974). Fungal mats can impart a distinctive appearance to the soil or litter layers they colonize and can also have different chemical properties from those occurring in adjacent, non-mat soil areas (Hintikka and Naykki, 1967; Hintikka, 1970; Fisher, 1972; Cromack et al., 1979). In the case of ectomycorrhizae, the relatively discrete nature of those species forming fungal mats affords opportunities to study processes associated with micro-organisms and soil animals as components of the mycorrhizosphere. Fungal mats, therefore, can serve as foci for research on biological and biochemical processes.

A previous study by Fogel (1976) found that *H. crassum* = *setchellii* represented an average of 28.4% of the hypogeous fungal sporocarp production during a 3-year-period in a 40-65-year-old Douglas-fir forest ecosystem. Studies undertaken several years later in the same forest by Cromack et al. (1979) found that fungal mats of *H. crassum* = *setchellii* occupied about 15% of the surface area of the mineral soil in the litter-soil interface. Mats contained 31 times more oxalate than non-mat soil, and had substantially greater quantities of Ca as calcium oxalate. In addition, soil pH was lower in the fungal mats, while exchangeable Ca was similar. Calcium oxalate, which is present in both saprophytic and mycorrhizal fungi, may influence soil weathering and soil P availability (Graustein et al., 1977).

The objectives of our research are to obtain estimates of microbial biomass, soil carbon and nitrogen, and soil animal populations and biomass in fungal mats of the ectomycorrhizal basidiomycete *H. setchellii* in contrast to adjacent non-mat areas. In this paper we present the preliminary results of our current research.

#### STUDY SITE

The study site is a 50-75-year-old, young growth Douglas-fir forest, with a sparse understory located on Mary's Peak along the Woods Creek Road about

16 km west of Philomath, Oregon, at an elevation of 460 m (Fogel, 1976). The soils are in the Slickrock series and are derived primarily from weathered sedimentary materials (Knezevich, 1975), but also contain weathered colluvial igneous rock fragments. Rainfall averages 191 cm year<sup>-1</sup>, but little occurs during the summer (Fogel, 1976).

#### METHODS

On each of 4 seasonal sampling dates in February, April, September and November 1986, nine pairs of randomly selected *H. setchellii* fungal mats and adjacent non-mat areas were sampled. After removal of the forest floor, three 7.6-cm diameter soil core samples were taken to a depth of 7.6 cm in mineral soil using a bulk density soil sampler from each of the mat and non-mat pairs. One set of cores from each sample pair was placed in a 10-cm diameter plastic pipe which served as a modified Berlese temperature and humidity gradient extractor for microarthropods. A second sample core from each pair was used to obtain subsamples for nematode extraction on a Baerman funnel. Macroinvertebrates, when found, were removed by hand from core samples. In September and November 1986, samples were obtained for estimation of protozoan populations within and adjacent to *H. setchellii* mats, following methods used previously by E. Ingham (Ingham et al., 1986).

A third core from each paired set was used to obtain estimates of microbial biomass by employing the chloroform fumigation technique of Jenkinson and Powlson (1976). Roots were removed prior to incubation of chloroform-treated and control samples. We made the assumption that this method represents labile carbon of varying proportions from both micro-organisms and soil animals. In the case of the present fungal-mat system, which appears dominated by fungal rhizomorphs, the fumigation method is intended to be used for relative biomass comparisons only.

#### RESULTS AND DISCUSSION

Soil characteristics within *H. setchellii* fungal mats and non-mat soil show that the mats are lower in soil pH, have greater quantities of oxalate present in the mat soil, but do not differ significantly in soil exchangeable Ca (Table 1). Substantial quantities of oxalate are present as calcium oxalate within *H. setchellii* tissue (Cromack et al., 1979). Total soil C and organic N are significantly higher in the fungal mats (Table 1). These values may reflect greater amounts of fungal rhizomorph tissue and fungal N within the mats. The higher C and N concentrations in mats may be caused in part by greater turnover of microbial tissue, which could result in higher equilibrium values for these elements.

The presence of a significantly more labile microbial biomass in the fungal

TABLE 1

Soil characteristics of *H. setchellii* fungal mats and adjacent non-mat soil

Characteristic	Fungal mats	Adjacent non-mat soil
pH <sup>1</sup>	4.9*	6.1
Total oxalate <sup>1</sup> (mg g <sup>-1</sup> )	7.30*	0.23
Exchangeable Ca <sup>1</sup> (meq/100 g)	3.26	2.70
Total organic N <sup>2</sup> (%)	0.55*	0.45
Total organic C <sup>2</sup> (%)	12.65*	9.62
Microbial biomass C <sup>3</sup> (μg g <sup>-1</sup> )	385*	150

<sup>1</sup>Soil characteristics within the top 10 cm of mineral soil, exclusive of the forest floor litter layers. Data from Cromack et al. (1979).

<sup>2</sup>Soil characteristics within the top 7.0 cm of mineral soil, exclusive of the forest floor litter layers. Data from Entry et al. (1987).

<sup>3</sup>Microbial biomass within the top 7.6 cm of mineral soil, exclusive of the forest floor litter layers. Data from present study.

\*Denotes means significantly different at  $P < 0.05$ .

mats (Table 1), implies more below-ground allocation to this biotic component. Decomposition of soil animals, such as protozoans, which are effectively eliminated by chloroform (Ingham et al., 1986), could also contribute to the labile biomass C estimated by the Jenkinson and Powelson (1976) chloroform technique. Further, the chloroform method can be less effective in eliminating fungal populations than bacteria or protozoans (Ingham et al., 1986). The Jenkinson and Powelson (1976) technique does not appear to have been tested with fungal rhizomorph tissue in the type of situation represented by the *H. setchellii* fungal mats. The higher labile biomass C observed within the mats, should be considered a relative measure until future comparisons are made using both direct and indirect microbial biomass methods.

The numbers of soil invertebrates, representing microarthropods such as mites and Collembola, were significantly greater within the fungal mats, when data from all 4 sampling dates were averaged (Table 2). Nematodes were also more abundant within fungal mats. Both nematodes and Collembola were significantly more numerous during each of the 4 seasonal samples (B. Fichter, K. Cromack Jr. and A. Moldenke, unpublished data, 1987). Oribatid mites were significantly more numerous in September and November, and total numbers of mites were significantly more numerous for every sampling period except February (B. Fichter, K. Cromack Jr. and A. Moldenke, unpublished data, 1987).

Soil animal biomass did not differ significantly for data averaged over all 4 sampling dates (Table 2). For individual sampling dates, there was significantly greater mite biomass in September and November 1986, and in November for Collembola and nematodes. Soil fauna such as myriapods and

TABLE 2

Soil animal numbers and biomass within *H. setchellii* fungal mats and adjacent non-mat soil

Group	Number g <sup>-1</sup> dry wt		Biomass (μg g <sup>-1</sup> dry wt)	
	Mat	Non-mat	Mat	Non-mat
Collembola	2.07*	0.78	2.72	1.34
Oribatid mites	2.82*	0.86	3.90	2.52
Total mites	4.28*	1.42	5.36	3.38
Nematodes	38.00*	20.42	1.86	1.59

\*Denotes means significantly different at  $P < 0.05$ .

TABLE 3

Active protozoan populations within *H. setchellii* mat and non-mat soil

Group	September 1986		November 1986	
	Mat <sup>1</sup>	Non-mat <sup>1</sup>	Mat <sup>1</sup>	Non-mat <sup>1</sup>
Flagellates	4953	5285	26532	27903
Amoebae	2077*	470	3018*	0
Ciliates	32*	6	74*	0

<sup>1</sup>Means given are no. g<sup>-1</sup> wet weight of soil. In September 1986, soil moisture averaged 23.9% and 22.4% for mat and non-mat soil, respectively. In November 1986, soil moisture averaged 46.3% and 48.0% for mat and non-mat soil, respectively. Soil moisture data are from B. Fichter, K. Cromack Jr. and A. Moldenke (unpublished data, 1987)

\*Denotes means significantly different at  $P < 0.05$ .

enchytraeids, for example, contributed little to total numbers but were a substantial fraction of faunal biomass (B. Fichter, K. Cromack, Jr. and A. Moldenke, unpublished data, 1987).

Soil protozoa were significantly more numerous as active amoebae and ciliates in fungal mats during both September and November 1986 (Table 3). Although flagellates were not significantly higher in *H. setchellii* mats, they did increase 5-fold between September and November. The fall represents a peak of fungal sporocarp production (Fogel, 1976). Increased soil moisture and increased above-ground litter inputs in the fall, which would stimulate decomposer activity, would also contribute to more food resources for soil fauna, such as protozoans.

Our preliminary observations on soil macrofauna do not suggest an association with fungal mats. When active, these fauna could migrate freely across mat and non-mat boundaries. Invertebrates, such as millipedes and earthworms, could utilize fungal-mat resources on an opportunistic basis. Spiers et al. (1986) found that earthworms of the genus *Arctiostrotus* can colonize both

soil and rotting wood in coastal forest ecosystems on Vancouver Island. *Arctiostrotus* ingests fungal hyphae and can digest calcium oxalate present on fungi in these acid forest soils.

In our forest ecosystem, small mammals would have the opportunity to utilize the hypogeous (truffle) sporocarps of *H. setchellii* as a food resource, and in turn, to inoculate this mycorrhizal species at other rhizosphere sites. Owing to the potential importance of small mammal mycophagy in our forest ecosystems (Maser et al., 1978), these animals should be considered as an integral component of the soil biota. As is the case for soil invertebrates, small mammals contribute to soil turnover and other soil processes through tunnelling and digging activities in soil and litter.

In addition to serving as sites of increased resource availability for soil animals, the *H. setchellii* fungal mats function as sites of enhanced microbial activity (Caldwell et al., 1988). Fungal mats had significantly enhanced respiration, phosphatase, peroxidase, protease, cellulase, xylanase and laminarinase in comparison with non-mat soil. Peroxidase activity was greater than 30-fold that of non-mat soil, while other enzymes were 2-3 times higher in fungal mats. Respiration was 4-fold greater. Thus, in addition to having greater microbial biomass, increased soil C and N, and more abundant invertebrate fauna, the *H. setchellii* fungal mat microbial community represents sites of increased respiration and enzyme activity relevant to P and N mobilization and breakdown of complex C substrates (Caldwell et al., 1988).

#### CONCLUSIONS

Ectomycorrhizal fungal mats of the type represented by *H. setchellii* are sites of greater labile microbial biomass and increased concentrations of soil C and N. Soil fauna such as mites, Collembola and nematodes are more abundant within mat-colonized soil. Protozoan groups such as amoebae and ciliates were also more abundant in the fungal mats. With the exception of some sampling dates for individual groups, the soil fauna did not have an increased biomass within these mats. Fungal mats also exhibited increased respiration, phosphatase, protease and complex C-decomposing enzyme activity.

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