13 Mycorrhizal Mat Communities in Forest Soils

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Ectomycorrhizal Mats as Model Systems

The role of ectomycorrhizal (EM) fungi in forest soil function and tree productivity has become an increasingly important topic for research in light of possible direct large-scale degradation of EM populations in the forests of Europe (Arnolds, 1991). Although there is a rapidly growing body of information being assembled from laboratory studies using pure culture syntheses, there is relatively little known about how these fungi function in the field. EM species of *Gautieria* and *Hysterangium* that form distinctive hyphal or rhizomorph mats have been observed in forests ranging from the subtropical (*Eucalyptus* in Australia) to boreal forests in Alaska (Castellano, 1988; Griffiths *et al.*, 1991b; Griffiths, unpublished observations). The actual quantitative impact of these mat communities on the forest floor remains largely unknown, although Cromack *et al.* (1979) reported up to 27% of temperate coniferous forest mineral soils could be colonized by a single species, *H. setchellii*.

These mats present a novel solution to the problem of how to measure the impact of an EM fungus on its immediate surroundings by greatly magnifying the influence of a single fungal species. The mat communities studied are generally dominated by a single EM species which can have a biomass equivalent to up to half the mass of the soils with which they are associated (Ingham *et al.*, 1991). This level of impact permits one to study the influence of these fungi on associated soil by comparing the biology and chemistry of soils colonized by these fungi with soils that are not so colonized. This approach has now been used to document large differences between mat and non-mat soils over seasonal cycles and in different fungi located in different areas of the Pacific Northwest (Cromack *et al.*, 1988; Griffiths *et al.*, 1990, 1991a).

Acceleratаed Mineral Weathering

An early study of fungal mats produced by presumptive EM fungi in Finland (Hintikka and Naykki, 1967) demonstrated that the mats have the capability of removing plant nutrients from soils and significantly altering soil chemistry. Cromack *et al.* (1979) found elevated oxalate concentrations and evidence for advanced clay weathering in *H. setchellii* mats in the Pacific northwest. The quantitative impact of these mat communities on the forest floor remains largely unknown, although more recently, Entry *et al.* (1987) and Rose *et al.* (1991) have reported elevated DTPA extractable cations associated with mycorrhizal mat communities. Elsewhere in this volume, we report elevated concentrations of a number of cations as well as oxalate and dissolved organic carbon (DOC) in the soil solution of these mats (Griffiths *et al.*, p. 380, this volume). These data all strongly suggest that organic acids are responsible for accelerated weathering of the mineral soils by these mat communities.

Processing of Detrital Nutrient Resources

In distinguishing 'early' and 'late' successional stage EM fungi, Dighton and Mason (1985) proposed that 'late' stage fungi could better utilize organic nutrient pools. Within the EM mat communities, we have found significantly elevated levels of several major soil enzymes which are thought to be responsible for processing complex detrital carbon, nitrogen and phosphorus (Table 13.1).

Table 13.1. Soil and litter enzyme activities in forest ectomycorrhizal mat communities.

Site/community	Cellulase*	Peroxidase†	Phosphatase‡	Proteinase§
Coast Range, Oregon (Douglas fir)				
Non-mat soil	1.34a	2.68a	22.2a	219a
Gautieria monticola	1.54a	87.6b	48.5b	161a
Hysterangium setchellii	3.25b	75.2b	58.3b	414b
Cascade Mountains, Oregon (Douglas fir)				
Non-mat soil	0.66a	0.78a	17.1a	148a
G. monticola	5.82b	98.5b	41.7b	193a
H. coriaceum	4.81b	57.4b	37.0b	376b
H. crassirhachis	4.92¶	58.6¶	35.8¶	289¶
Mendicino CA (Eucalyptus)				
Non-mat litter	2.20a	not detected	46a	391a
H. gardneri	4.94b	not detected	165b	831b

Enzyme activities: * μmol glucose $g^{-1}\,h^{-1}$; † change in $A_{460}\,g^{-1}\,min^{-1}$;
‡ μmol *p*-nitrophenol $g^{-1}\,h^{-1}$, § nmol tyrosine eq. $g^{-1}\,h^{-1}$.
a,b,c: for given site, different letters indicate significantly different ($P \leq 0.05$) enzyme activities.
¶single mat sampled.

Most striking of the mat enzyme activities were the elevated levels of peroxidase. The ability of mat-forming EM fungi to decolorize aromatic dyes *in vitro* (Table 13.2) provides presumptive evidence for the ability to degrade lignin (Glenn and Gold, 1983). While this may be important in processing litter, it has also been noted that lignolytic peroxidases can also degrade humic acid (Blondeau, 1989), suggesting a possible mechanism whereby these fungi could gain access to pools of nitrogen and phosphorus contained in recalcitrant soil organic matter.

Of the many papers published on phosphatase production by mycorrhizal fungi, virtually all have used phosphomonoester substrates. However, most organic phosphorus in living tissues, and thus fresh detritus, occurs as phosphodiesters. The abundance of phosphomonoesters, relative to phosphodiesters, in soil organic matter suggests that the phosphodiester pool is more important during litter decomposition. We have found that several species of mat-forming EM fungi are capable of hydrolysing RNA, a major phosphodiester (Table 13.2), providing strong circumstantial evidence supporting Went and Stark's direct nutrient cycling hypothesis (Dighton, 1991).

While the *in vitro* utilization of protein by EM fungi has been demonstrated (e.g. Abuzinadah and Read, 1986; Hutchinson, 1990), detrital proteins may frequently exist as complexes with reactive polyphenols that are resistant to enzyme action (Read, 1991). We have found several mat-forming EM fungi that are capable of growing on an insoluble tannic acid-protein complex. There are also reports of growth of EM fungi on insoluble chestnut tannin–protein complexes.

At this time, we do not know if the elevated mat enzyme activities are due to ectomycorrhiza directly, or enhanced saprophytic populations within the mat community. The ability to culture these fungi for comparison of *in vitro* and *in situ*

Table 13.2. Selected physiological characteristics of pure cultures 'early' successional stage and mat-forming ectomycorrhizal fungi.

EM fungus	Ribonuclease	Reaction on tannin–protein complex[1]	Polyaromatic dye decoloration[2]	
			RBB	R-478
'Early' stage				
Laccaria laccata	–	–	–	–
Hebeloma crustuliniforme	–	–	–	–
Thelephora terrestris	–	–	–	–
Mat-forming				
Chondrogaster sp.	+	+	+	+
Gautieria monticola	+	+	+	+
Hysterangium coriaceum	not tested	+	not tested	
H. gardneri	+	(+)	+	+

Key: 1 reactions:– no visible reaction, (+) Bavendamm-like darkening, + clearing of precipitate.
2 – presumptive assay for lignolytic activity (Glenn and Gold, 1983): RBB, Remazol Brilliant Blue; R-478 (Sigma Chemical Co.).

enzymes make the mat communities novel model systems in which to establish the direct role for these specialized associations in forest nutrient cycling.

Establishment of a Distinct Soil Microhabitat

If these mat-forming mycorrhizal fungi are capable of degrading organic polymers, it is likely that they have some mechanism to transport organic nitrogen and phosphorus released during the degradative process to the host tree. Since the EM fungus has other sources of organic carbon during most of the year, it is likely that only those organic compounds which contain plant nutrients are removed from the soils. If this were the case, we would expect to find higher C:N ratios in mat soils than in non-mat soils: an observation we have made repeatedly in our studies (Griffiths *et al.*, 1990, 1991a). The ratio of chloroform fumigation CO_2 to mineralizable N in *H. setchellii* and *G. monticola* mats over several seasons are consistently higher than in mineral soils not colonized by these fungi (Griffiths *et al.*, 1990, 1991a).

There are at least three possible explanations for the large differences in these ratios: (1) there is a proportionately greater concentration of bacteria than fungi

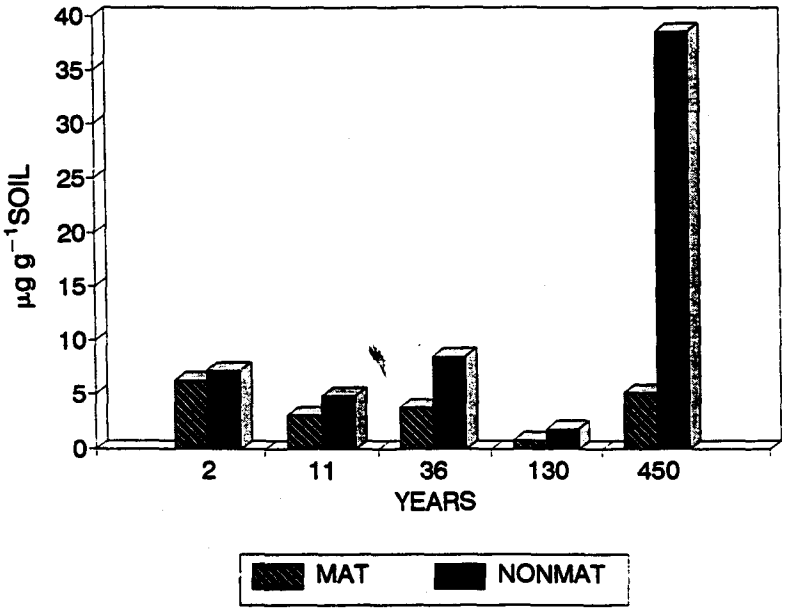

Fig. 13.1. Labile nitrogen in mat and non-mat soils collected in Douglas fir stands that had been disturbed at different times in the past. The years given are the years since the site was burned (130 and 450 years) or harvested and burned (2, 11, and 36 years). The stand that had been harvested and burned 2 years previously still contained approximately 10% of the original old-growth trees. The values given are means of five observations for each treatment. The units are µg nitrogen per g dry weight soil.

in non-mat soils; (2) the fungal mat community is selectively removing organic nitrogen from these forest soils; and/or (3) the fungal community is releasing organic compounds with high C:N ratios into these soils (i.e. organic acids). Direct counts of bacteria and fungi (excluding rhizomorph material) shows no consistent enrichment of bacteria over fungi in mat soils (Ingham *et al.*, 1991); thus alternative (1) appears unlikely. If the organic N is selectively removed, one would expect a reduction in organic N. If the fungal community is releasing compounds of high C:N ratio one would expect to find elevated organic carbon concentrations in mat soils but no difference in nitrogen concentrations. In a recent study of labile carbon and nitrogen in *G. monticola* mats in a chronosequence of Douglas fir forest soils, we found the pattern one would expect if both of these activities were taking place (Figs 13.1 and 13.2).

If mat-forming EM fungi produce exoenzymes which break down organic polymers, they must be in competition with heterotrophic soil bacteria and saprophytic fungi for the break-down products. The question is, have the mat-forming fungi produced conditions in the soil that favour their growth relative to that of competing microorganisms? We have repeatedly observed lower pH values in mat soils and have observed that the mat soils are typically drier and much more hydrophobic than non-mat soils (Griffiths *et al.*, 1990, 1991a). These are conditions that, in general, should favour fungi over bacteria.

We have also observed other chemical differences between mat and non-mat soils which may give EM fungi the competitive edge over other soil microorganisms for organic resources. Quantities of water-soluble phenolics were significantly

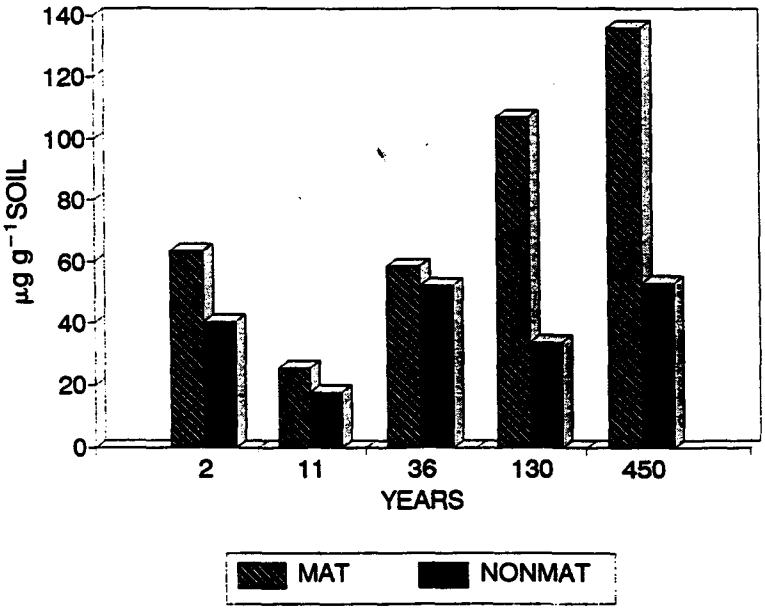

Fig. 13.2. Labile carbon in mat and non-mat soils collected in Douglas fir stands as in Fig. 13.1. The units are µg carbon per g dry weight soil.

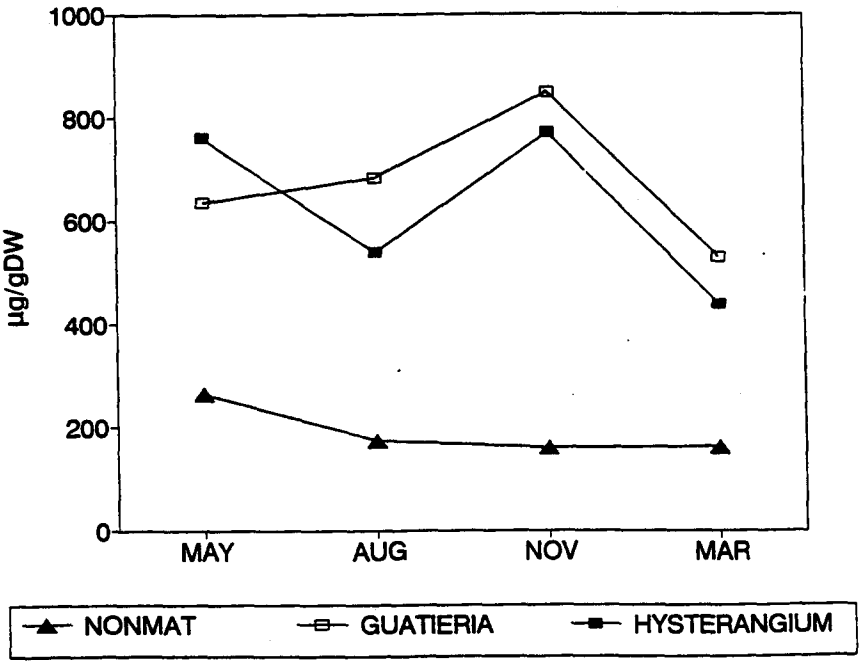

Fig. 13.3. Water-soluble phenolic compounds found in soils collected in *Gautieria monticola* and *Hysterangium setchellii* mats and associated non-mat soils. Soil samples were collected in different mats at each sampling time. The given values are the mean of five soils taken from each soil type. The units are µg gallic acid equivalents per g dry weight soil.

elevated in mat soils during all four seasons (Figure 13.3). In addition, amounts of siderophore-like compounds were also elevated in two of four seasonal samplings in mat soils (Griffiths, unpublished data). Since production of siderophores by one organism can limit Fe^{3+} availability to others this may be a mechanism by which these organisms control competition within mat communities. The high concentrations of oxalate observed in EM mat soil solution may have a similar function although the main function of this compound is most likely to be in mineral weathering (see Griffiths *et al.*, p. 380 this volume). It is known that oxalate can chelate Fe^{3+} in addition to other micronutrients which may have an effect similar to that of siderophores.

In addition to these features, differences in cation chemistry and quantities of DOC have been observed between mat and non-mat soils (Entry *et al.*, 1987; Caldwell *et al.*, 1991) as well as differences in respiration rates (Griffiths *et al.*, 1990, 1991a), and enzyme activities (Griffiths *et al.*, 1987). Microbial biomass levels, as determined by both direct and indirect means, are also higher in mat soils (Ingham *et al.*, 1991). Taken together, these observations suggest that EM mat communities may act as unique soil habitats in forest soils and may play a role in maintaining higher species diversity within forest ecosystems. Significant differences in populations of protozoa, nematodes and microarthropods

(Cromack *et al.*, 1988) between mat and non-mat soils suggest that this may be the case.

In summary, recent work on EM mat communities in the Pacific Northwest of the USA has demonstrated that: (1) these mats are sites of accelerated mineral soil weathering; (2) the fungi involved have the potential to provide access to detrital nitrogen and phosphorus resources; (3) they selectively remove organic nitrogen from the soils, and (4) form specialized habitats in forest soils which could increase overall biological diversity.

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been shown that two species of vesicular-arbuscular (VA) fungi were not able to infect *Brassica* roots, although they could adhere to them and produce swellings resembling appressoria. In this work we studied the non-host genus *Lupinus*. We investigated: (1) the effect of lupin roots on germination and hyphal elongation of *Glomus mosseae*; (2) the effect of excised roots of lupin and luceme (*Medicago sativa*) on appressorium formation by *G. mosseae*.

Lupin roots did not inhibit spore germination and hyphal growth of *G. mosseae*. The hyphae grew on and around excised lupin roots, forming swellings resembling appressoria. Such enlarged structures sometimes produced thin hyphae, growing along and perpendicular to root cell walls. These rapidly aborted, retracting their cytoplasm and forming consecutive septa which isolated hyphal tips. In the presence of excised roots of the compatible host plant lucerne, hyphae extending from appressoria were capable of penetrating adjacent epidermal cells, forming coils, but they soon retracted their cytoplasm and produced septa. Consequently the fungus was not able to spread further into dying roots. When both host and non-host root systems were killed by liquid nitrogen treatment, the fungus did form swellings, but no appressoria and was unable to penetrate even the host roots.

Our results suggest that in lupin roots hyphal adhesion and appressorium formation are inhibited by a factor associated with intact living plants. The failure of fungal infection at a different stage confirms that mycorrhizal infection is a multi-step process, during which signals may initiate consecutive recognition events between host and symbiont, eventually leading to the formation of a functional symbiosis.

Soil Solution Chemistry of Ectomycorrhizal Mat Soils

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Ectomycorrhizal fungal mats are distinct features of Pacific northwest coniferous forests and other forests throughout the world. Organic acids produced by these fungi may play an important role in nutrient availability and mineral weathering within the soil ecosystem. The dissolved chemical elements in soil solutions isolated from two ectomycorrhizal fungal

Table 1. Concentrations (μmol) of dissolved constituents in soil extracts.

	Hysterangium	Soil	*Gautieria*	Soil
H^+	74.1 ***	11.0	31.4 ***	2.1
K	470 ***	120	1 800 ***	210
Ca	710 ***	120	2 000 ***	110
Mg	410 ***	51	1 500 ***	480
Zn	2.67*	1.13	12.6 **	1.23
Mn	10.3 ***	2.00	1 220 ***	9.1
Cu	0.56***	0.20	1.83**	0.18
Fe	39 *	16	320 **	6.3
Al	353 **	56	8 044 ***	55
Oxalate	59 *	5.2	11 638 ***	35
DOC	67 000 **	7 300	188 000 ***	5 200
Total N	550 **	270	1 011 ***	183
CN†	122	32	186	28

*, **, *** = significance of difference between mat and non-mat soil solutions; $P < 0.05$, 0.01 and 0.001 respectively. $n = 5$ (Wilcoxin non-parametric test). † molar ratio for DOC/Total N.

(*Hysterangium setchellii* and *Gautieria monticola*) mat soils were compared with those from adjacent soils with no visible mat development. The concentrations of dissolved constituents were greater, in all cases, for the mat soils (Table 1).

Concentrations of cations, oxalate, dissolved organic carbon (DOC) and total N were always greatest in mat soils with concentrations in *G. monticola* mat solutions usually being higher than in *H. setchellii* mat solutions. The chemical constituents showing the largest differences between mat and non-mat soils for both mat types included: Al, Fe, Mg, Mn, oxalate and DOC. The reduced pH and elevated oxalate and DOC concentrations in the mat soils suggest that organic acids produced by the fungal mat community accelerated weathering of the soil mineral phase causing an increase in pore-water cation concentrations. The elevated C:N ratios in mat soil solutions may be caused by the input of organic acids by the ectomycorrhizal fungus and/or by the selective removal of dissolved organic nitrogen by the fungus resulting in an enrichment of DOC. This process may be responsible for releasing plant nutrients to be transported by the fungi to the host tree.

Fungicide Interactions with VA Fungi in *Ananas comosus* Grown in a Tropical Environment

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The aim of the study was to determine the effects of several fungicides (fosetyl-Al, etridiazole, captan and maneb) on VA infection development and plant growth in pineapple (*Ananas comosus*).

Micropropagated plantlets of Queen and Smooth Cayenne (clone CY0) were inoculated with *Glomus* sp. (LPA 21) in an acid soil (pH 5.0) and growth in a growth chamber with artificial tropical conditions (300 $\mu E\,s^{-1}\,m^{-2}$, 12 h day, 29/25°C). Previous studies showed that under these conditions, endomycorrhizal pineapple plants have better growth than non-mycorrhizal plants. Fungicides were applied at field rates recommended for root disease control: fosetyl-Al (10 $g\,m^{-2}$), etridiazole (3 $g\,m^{-2}$), captan (1.2 $g\,m^{-2}$) and maneb (1.5 $g\,m^{-2}$).

Fungicide application did not negatively influence endomycorrhizal growth effects on shoot and root fresh mass, except for etridiazole, which caused a decrease in shoot growth. Captan positively affected non-mycorrhizal and endomycorrhizal plants and in certain cases it reinforced the endomycorrhizal effect (+34% shoot and +68% root fresh mass of Queen, +25% root fresh mass of Smooth Cayenne). This was also true for fosetyl-Al and maneb treated endomycorrhizal plants of the Queen variety (+46% root fresh mass) and Smooth Cayenne variety (+48% root fresh mass), respectively.

The root:shoot ratio was not modified by fungicide application, except for Queen variety plants treated with fosetyl-Al (+30%) and etridiazole (+41%); plants treated with the two fungicides showed increases in root production.

The tested fungicides were not harmful to the endomycorrhizal infection by *Glomus* sp., except in relation to arbuscule frequency of plants treated with captan and maneb, which were reduced respectively by 24% and 32% for the Queen variety and of 37% and 34% for Smooth Cayenne. However, this decrease in infection did not affect the growth of plants.

These results show that certain fungicide treatments are compatible with the positive effect of controlled endomycorrhization of micro-propagated plantlets of pineapple during the post-*vitro* weaning period, and they open interesting perspectives for combining the positive effect of endomycorrhizas in micropropagated plant production with the chemical control of fungal pathogens.

1463

Mycorrhizas in Ecosystems

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