Distribution of Ectomycorrhizae in a Mature Douglas-fir/Larch Forest Soil in Western Montana

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Abstract. The top 38 cm (15 inches) of a western Montana forest soil was 60 percent mineral, 23 percent humus, 15 percent decayed wood, and 2 percent charcoal. Most (to 95 percent) of the active ectomycorrhizae were associated with the organic fractions. Five percent of all active ectomycorrhizae occurred in the mineral soil fraction, 66 percent in the humus, 21 percent in the decayed wood, and 8 percent in the charcoal. Thus, the organic reserves in this forest soil were the most important substrates for ectomycorrhiza formation. Therefore, the parent materials (leaves, litter, and woody residues) for soil organic reserves may require management during timber harvesting and prescribed burning to prevent a subsequent loss in the capacity of soils of this type (limestone base) to support ectomycorrhizal associations in mature Douglas-fir/larch forests. ***Forest Sci. 22:393–398.***

Additional key words. Soil microbiology, charcoal, decayed wood, forest fire.

RECENT PROJECTIONS of national wood fiber needs indicate substantial increases in demand for and cost of wood fiber-based products (USDA Forest Service 1973). This demand has resulted in increased efforts to remove the fiber available at harvesting sites (USDA Forest Service 1965, 1973). Increased tree utilization potentially reduces the organic parent materials (litter and woody residues) available for soil-formation processes.

Heretofore, many woody residues left on a site after harvesting were broadcast burned to reduce wildfire hazard. Recent investigations have shown that fire is an integral, functioning component of most forested ecosystems in North America (Wright and Heinselman 1973). The functions of woody residues and their remains after burning (charcoal and ash) need to be determined, particularly their contributions to soil quality.

This study was undertaken as a step toward better understanding functions of the organic reserves of forest soils on the activities of a major group of symbiotic soil microorganisms, the ectomycorrhizal fungi.

Materials and Methods

Site. The study area is in northwestern Montana on the Coram Experimental Forest, approximately 16 km (10 miles) south of Glacier National Park. The study site

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is an approximately 1 hectare (2.4 acre) block of an undisturbed 250-year-old forest typical of the Douglas-fir/larch timber type in western Montana. It is on an east aspect with a slope averaging 55 percent. Soils are derived from argillite and limestone underlying till material of loamy, skeletal soil families. Soil pH averaged 6.9 in the mineral layer and 6.6 in the organic fractions. Organic matter content of the mineral soil averaged 12 percent. Dominant conifers on the study area included Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western larch (*Larix occidentalis* Nutt.), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), and Engelmann spruce (*Picea engelmannii* Parry). Lodgepole pine (*Pinus contorta* Dougl. ex Loud.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and western white pine (*Pinus monticola* Dougl.) occurred occasionally. The vegetation represents an *Abies lasiocarpa/Clintonia* habitat type (Pfister and others 1974).

Sampling Method. Samples consisted of 10- × 38-cm (4- × 15-inch) soil cores taken randomly, 5 from around each of 10 permanent plot centers that were evenly dispersed throughout the study block. A series of matching soil cores were also taken adjacent to and through a decayed log nearest each of the 10 plot centers. Logs selected were in an advanced stage of decay, characterized by a crumbly, friable, cubical texture usually vertically compressed, and dark brown. Such logs were in the process of being incorporated into the forest soil. Samples were taken in October, 2 to 3 weeks after the first fall rain.

Soil Fractionation. Each core was subdivided in the field into the following base fractions: (1) mineral soil, (2) decayed woody material, (3) humus (O_2 horizon), and, in the randomized samples, (4) charcoal. The litter layer (O_1 horizon) contained no mycorrhizae on this study area during the sampling period and therefore was excluded from further study. The decayed wood and charcoal were generally found in the humus or mineral layers. Each fraction was hand separated and placed individually in plastic bags immediately after collection. Approximate volumes occupied by these fractions were determined either by measuring the depth of the layer in the undisturbed core or by placing the material in a graduated cylinder and measuring directly.

Mycorrhizal Root Counts. In the laboratory, each fraction was shaken for 5 minutes in a standard 2-mm soil sieve. The woody fractions, mineral, and humus aggregates were gently crumbled before sieving. Soil and root materials were examined manually. All ectomycorrhizal roots were placed in water in standard 16- × 100-mm petri plates and examined under a dissecting microscope (10 to 50×). All examinations were made within 24 hours after soil cores were collected.

Quantitative Determination of Mycorrhizae. Visual assessments of the number of active ectomycorrhizal root tips were made with the aid of a dissecting microscope (10 to 50×). Because large numbers of root tips were present, only tips in an active metabolic state were counted. No attempt was made to count or differentiate between root tips that were inactive and those that were dead. Each individual tip was counted, even though, in many cases, it was a part of a complex structure.

The following characters were used for designating mycorrhizal tips as "active" or "inactive":

Active—The ectomycorrhiza was turgid, the external surface generally smooth, light brown to grey or white. The distal tips were usually lighter in color than the proximal end. The surface of the structure was associated with cottony or wefty, turgid mycelia, hyphal strands, or rhizomorphs. When sectioned, root tip tissues were light in color.

Inactive—The ectomycorrhiza was locally collapsed, the external surface wrinkled or fissured and dark brown. The distal tips were concolorous with the proximal end. When associated with mycelia, hyphal strands, or rhizomorphs, fungal tissues were plasmolyzed or locally collapsed, contorted,

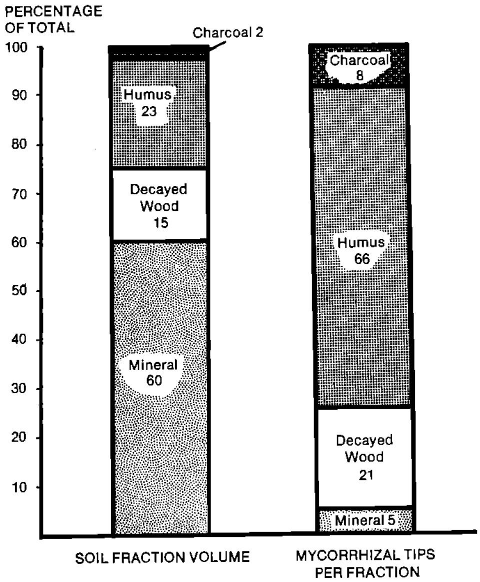

FIGURE 1. *Mean soil fraction volumes (percent) and percent of total active ectomycorrhizae per fraction in fifty 10- by 38-cm (4- by 15-inch) soil cores taken randomly from an approximately 1-hectare (2.4-acre) plot in a 250-year-old Douglas-fir/larch forest.*

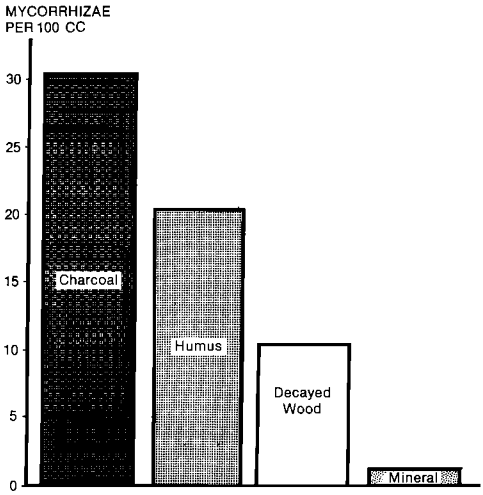

FIGURE 2. *The average number of active ectomycorrhizae per 100 cc of the representative soil fraction. Samples taken from the upper 38 cm (15 inches) of soil from a 250-year-old Douglas-fir/larch forest.*

and dark in color. When sectioned, root tip tissues were dark brown and friable.

Analysis. For the analysis of variance, numbers of soil fractions (n) varied from the sample numbers of 50 and 20, respectively, because only the cores that actually contained the fractions being compared were included in each analysis; not all cores contained decayed wood or charcoal. For these analyses, the interval

$$\left(X_1 - X_2 + t_{(\alpha_1 n-1)} \frac{S_{(X_1-X_2)}}{\sqrt{n}} \right)$$

contains the true difference with the probability 1-α. In all cases, α was 0.1 and $p = 0.9$ was considered significant.

Results

Volume fractionation of the randomized soil cores showed the top 38 cm (15 inches) of soil to contain 60 percent mineral base, 23 percent humus, 15 percent decayed wood, and 2 percent charcoal. Five percent of the active ectomycorrhizae occurred in the mineral fraction, 66 percent in the humus, 21 percent in the decayed wood, and 8 percent in the charcoal (Table 1, Fig. 1).

Mean totals of active ectomycorrhizae in any organic soil fraction were significantly larger than those in the nonorganic (mineral) fraction (Table 2). The large variation caused by the low number of active ectomycorrhizae in the mineral fractions and the low number of samples of charcoal were limiting statistically to that comparison (Tables 1 and 2). However, charcoal along with other organic fractions ranked among the most suitable of substrates for mycorrhizal activity (Fig. 2).

A comparison of the soil fractions obtained from the matched soil cores, those taken through and adjacent to decayed logs, showed trends similar to those in the randomized soil cores (Fig. 3). Organic fractions were significantly greater in active ectomycorrhizae as compared with the mineral fraction (Table 2). In the nonwoody cores, where the organic matter content was appreciably lower, 89 percent of the active ectomycorrhizal root tips were still found in the organic matter (Fig. 3).

To further examine the relationship be-

TABLE 1. Soil fraction volumes (cc) and numbers of active ectomycorrhizae per fraction in 10- by 38-cm (4- by 15-inch) soil cores taken from a 250-year-old Douglas-fir/larch forest.

Item	Mineral			Humus (O_2 horizon)		
	n	$\bar{x}$	s	n	$\bar{x}$	s
Randomized cores						
Volume	49	1,243.98	499.81	45	532.0	230.77
Mycorrhizae	49	4.24	8.81	45	64.22	94.95
Matched cores taken through decayed logs						
Volume	10	1,035.0	339.29	10	187.5	177.6
Mycorrhizae	10	4.40	8.33	10	54.7	138.44
Adjacent to decayed logs						
Volume	9	981.11	207.45	9	534.44	210.66
Mycorrhizae	9	16.11	21.06	9	128.89	134.06
	Decayed wood			Charcoal		
	n	$\bar{x}$	s	n	$\bar{x}$	s
Randomized cores						
Volume	21	714.52	670.45	12	176.29	179.93
Mycorrhizae	21	44.14	63.12	12	28.58	56.33
Matched cores taken through decayed logs						
Volume	10	1,156.5	619.6	—	—	—
Mycorrhizae	10	75.8	55.96	—	—	—
Adjacent to decayed logs						
Volume	9	0.0	0.0	—	—	—
Mycorrhizae	9	0.0	0.0	—	—	—

TABLE 2. Significance of the numbers of ectomycorrhizae in various soil fractions when compared to other fractions in 10- by 38-cm (4- by 15-inch) soil cores taken from a 250-year-old Douglas-fir/larch forest.

	n	s	$\bar{x}_1 - \bar{x}_2$	Interval
Randomized cores	45	93.19	[1]$\bar{x}_h - \bar{x}_m =$ 59.67	[2](36.32, [3]83.02)
	21	64.10	$\bar{x}_w - \bar{x}_m =$ 41.9	(17.77, [3]66.03)
	17	102.39	$\bar{x}_h - \bar{x}_w =$ 24.53	(−18.83, 67.89)
	12	59.63	$\bar{x}_c - \bar{x}_m =$ 25.25	(−5.67, 56.17)
	10	82.20	$\bar{x}_h - \bar{x}_c =$ 18.1	(−29.55, 65.75)
	6	15.63	$\bar{x}_c - \bar{x}_w =$ 2.67	(−10.19, 15.53)
Matched cores (all)	20	29.13	$\bar{x}_h - \bar{x}_m =$ 77.45	(27.08, [3]127.82)
Through decayed logs (only)	10	20.41	$\bar{x}_w - \bar{x}_m =$ 70.4	(32.99, [3]107.81)
	10	50.59	$\bar{x}_w - \bar{x}_h =$ 27.40	(−65.33, 102.13)
Adjacent to decayed logs (only)	10	39.69	$\bar{x}_h - \bar{x}_m =$ 103.4	(30.65, [3]176.15)

[1] $\bar{x}_h - \bar{x}_m$ is the difference between the mean of the humus (O_2) layer and the mean of the mineral layer, w = decayed wood, and c = charcoal.

[2] $\alpha = 0.1$.

[3] The difference is significant to at least the 0.9 probability level that the interval (see *Materials and Methods*) does not include zero.

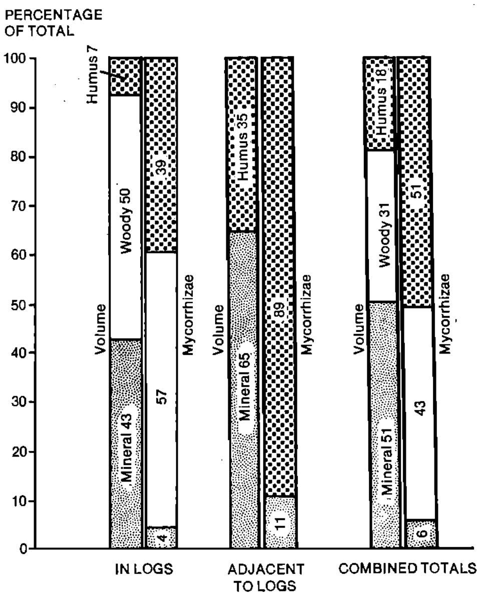

FIGURE 3. *Comparison of the mean fraction volumes (percent) and the percent of the total active ectomycorrhizae in ten 10- by 38-cm (4- by 15-inch) soil cores taken from 10 paired locations (20 cores) in and adjacent to decaying logs in the process of being incorporated into the soil system of a 250-year-old Douglas-fir/larch forest.*

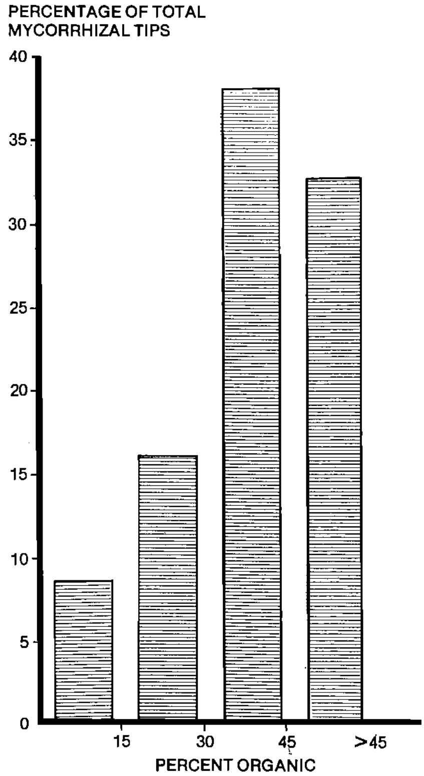

FIGURE 4. *Comparison of the percent of the active ectomycorrhizae at four organic matter levels (humus, decayed wood, and charcoal) with the combined total in all seventy 10- by 38-cm (4- by 15-inch) soil cores. All were taken from a 250-year-old Douglas-fir/larch forest.*

tween active ectomycorrhizal root tips and soil organic matter, the results from all 70 cores were combined and the percent organic matter volume (decayed woody, humus, and charcoal fractions inclusive) of each was calculated. The cores were then segregated into four approximately equal groups according to their total organic matter volume. The mean percentage ectomycorrhizae in the organic fractions of each were compared as a percentage of the overall mean. Figure 4 illustrates the relationship between soil organic matter levels and ectomycorrhizal roots. Since all factors other than organic matter content remained constant, these data show that under these conditions soil organic matter was a primary associate of site quality.

Notable throughout the sampling period was the consistent predominance (visual basis only) of a single morphological type of active ectomycorrhiza. In most cases, this occurred as a pinnate or compound pinnate form; external features are described in *Materials and Methods.* Rarely, perhaps as an immature form, such roots appeared as monopodial branches that were otherwise identical to those of the pinnate form.

Infrequently, the distinctive mycorrhizal structures of *Cenococcum graniforme* (Sow.) Ferd. et Winge and *Rhizopogon* sp. were observed. No specific attempts were made to determine the fungi responsible for predominate mycorrhizae on host species of this experimental area. However, during the sampling period, fruiting structures of *Russula brevipes* Pk. and *Suillus cavipes* (Opat.) Smith et Thiers were frequently observed in close association with the roots of Douglas-fir and larch, respectively.

Discussion

These data show that soil organic matter, in the form of soil humus and decayed wood, provides an important and stimulatory substrate for the formation and activity of ectomycorrhizae within the Douglas-fir/larch timber type of western Montana. Mycorrhizae will form in soils with a low organic matter content (Björkman 1956), as was noted in the mineral fractions of this study; however, previous reports have noted strikingly beneficial effects of organic composts and humus, as soil amendments, on formation of mycorrhizae and growth of conifers (Mikola 1973; Rayner 1936, 1938). Similarly, decaying roots in soil have been credited with promoting formation of mycorrhizae (McMinn 1963).

The relationship of soil organic matter to this symbiotic association is interesting because ectomycorrhizal fungi are said to be unable to utilize residues in these forms as a direct source of carbon or nitrogen (Hacskaylo 1973). The water-holding capacity of organic materials, particularly decayed wood, may be important in this regard.

Although the number of soil samples that contained charcoal in this study were limited, the large proportion of active ectomycorrhizae associated with charcoal appears to be important and needs more research. Past research has indicated variable effects of fire on the mycorrhizal association. Relatively hot fires can suppress mycorrhizae in the top 5 to 10 cm (2 to 4 inches) of soil for 1 to 2 years (Mikola and others 1964, Tarrant 1956, Wright 1971, Wright and Tarrant 1957). However, modest fertilization with wood ash reportedly has benefited mycorrhiza formation and the subsequent growth of coniferous seedlings (Björkman 1941). The present report shows that charcoal contained in forest soils of this study area had a strong stimulatory effect on the formation and activity of ectomycorrhizae.

The data contained in this report are pertinent only to a mature forest and should not be interpreted as a strong indication of the status of a young or regenerating forest.

Literature Cited

BJÖRKMAN, E. 1941. Die Ausbildung und Frequenz der Mykorrhiza in mit Asche Gedüngten und ungedüngten Teilen von entuässeetem Moor. Medd. Skogsförsöksanst. Stockholm 32:255.

———. 1956. Uber die natur der Mykorrhizabildung unter besonderer Berucksichtigung der Waldbaume und die Anwendung in der forstlichen Praxis. Forslwiss Zentralbl 75:265–286

HACSKAYLO, E. 1973. Carbohydrate physiology of ectomycorrhizae. p. 207–230 *in* Ectomycorrhizae. Their Ecology and Physiology, G. C. Marks and T. T. Kozlowski, eds. Acad. Press, New York and London. 444 p.

MCMINN, R. G. 1963. Characteristics of Douglas-fir root systems. Can J Bot 41:105–122.

MIKOLA, P. 1973. Mycorrhizal symbiosis in forestry practice. p. 383–411 *in* Ectomycorrhizae. Their Ecology and Physiology, G. C. Marks and T. T. Kozlowski, eds. Acad. Press, New York and London. 444 p.

———, O. LAIHO, J. ERIKAINEN, and K. KUVAJA. 1964. The effect of slash burning on the commencement of mycorrhizal associations. Acta For Fenn 77:1–11.

PFISTER, R. D., B. L. KOVALCHIK, S. F. ARNO, and R. C. PRESBY. 1974. Forest habitat types of Montana. USDA Forest Serv, Intermt Forest and Range Exp Stn and North Reg, Mimeo Rep, 213 p.

RAYNER, M. C. 1936. The mycorrhizal habit in relation to forestry. II. Organic composts and growth of young trees. Forestry 10:1–22

———. 1938. The use of soil humus inocula in nurseries and plantations. Emp For J 17: 236–243.

TARRANT, R. F. 1956. Effects of slash burning on some soils of the Douglas-fir region. Proc Soil Sci Soc Am 20:408–411.

USDA, FOREST SERVICE. 1965. Timber trends in the United States. Forest Resour Rep 17, 235 p.

———. 1973. The outlook for timber in the United States. Forest Resour Rep 20, 367 p.

WRIGHT, E. 1971. Mycorrhizae on ponderosa pine seedlings. Oregon State Univ Bull 13, 36 p.

———, and R. F. TARRANT. 1957. Microbiological soil properties after logging and slash burning. USDA Forest Serv, Pac Northwest Forest and Range Exp Stn Pap 157, 5 p.

WRIGHT, H. E., JR., and M. L. HEINSELMAN 1973. The ecological role of fire in natural conifer forests of western and northern America: a symposium. Quaternary Res 3:317–513