Mycologia, 82(6), 1990, pp. 759–765.

COMMUNITIES OF FILAMENTOUS FUNGI AND YEAST IN DECOMPOSING LOGS OF *PSEUDOTSUGA MENZIESII*

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ABSTRACT

We investigated the communities of filamentous fungi and yeasts in logs in advanced stages of decay. Decay stages are generally defined from class I–V, with class I being the least decayed and class V being the most decayed. Fungi were isolated from cross-sections approximately 15 cm thick of *Pseudotsuga menziesii* logs in decay classes III and IV. Cross sections included portions of logs with and without conifer seedlings. Samples were removed from three vertical positions (top, middle, and bottom) of each cross-section. Comparisons of coefficient of community and detrended correspondence analysis ordinations indicated differences in community structure between class III and class IV logs. No community differences were found between samples with and without conifer seedlings. A total of 18 genera and 36 species of fungi were recovered from samples; more than 50% of recovered genera were dematiaceous hyphomycetes.

Key Words: Ecology, wood decay, fungus communities, *Pseudotsuga*

Most coniferous forests in the Pacific Northwest (PNW) are harvested for timber, and post-harvest treatments often include removal of large coarse woody debris (>2.5 cm diam). Decomposing coarse woody debris (CWD) is important to nutrient cycling and biotic habitat (Harmon *et al.* **1986**; Maser and Trappe, **1984**), and its removal following harvest may negatively affect long-term site productivity.

The above-ground volume of CWD in *Pseudotsuga-Tsuga* forests of the Pacific Northwest is much greater than that found in comparable temperate deciduous forests (Harmon *et al.,* **1986**). The volume of logs in a 250-year-old *Pseudotsuga-Tsuga* forest is 488 m^3/ha, whereas a similarly aged *Quercus* stand has a log volume of 46 m^3/ha (Harmon *et al.,* **1986**). Sollins (**1982**) found that CWD comprises about 50% of the above-ground detrital input of coniferous forests in the Pacific Northwest. Grier and Logan (**1977**) report that on average 1.2 trees died/ha^{-1}/$year^{-1}$ in an undisturbed 470-year-old *Pseudotsuga* stand.

During decomposition, logs undergo structural and biological changes and have been categorized into five decay classes, with class I the least and class V the most decomposed (Fogel *et al.,* **1973**; Maser *et al.,* **1979**; Sollins, **1982**; Triska and Cromack, **1980**). The decay classes and their characteristics are shown in TABLE I. Maser and Trappe (**1984**) determined that residence time of a log is positively correlated with decay class and that the relation is logarithmic. The more decayed a log, the more difficult it is to determine its residence time on the ground. Regardless of residence time, decay classes support unique communities of plants and animals.

The transition of logs from decay class III to decay class IV involves several important biological and structural changes. During this pe-

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TABLE I

CHARACTERISTICS OF FALLEN DOUGLAS-FIR TREES IN FIVE DECAY CLASSES

Characteristics	Decay class				
	I	II	III	IV	V
Bark	intact	intact	trace	absent	absent
Twigs, 0.3 cm	present	absent	absent	absent	absent
Texture	intact	intact to partly soft	hard, large pieces	small, soft, blocky pieces	soft, powdery
Shape	round	round	round	round to oval	oval
Wood color	original	original	original to faded	light brown to reddish brown	red brown to dark brown
Invading roots	none	none	sapwood	heartwood	heartwood
Portion on ground	elevated	elevated slight sag	sagging near ground	on ground	on ground

[a] Adapted from Maser and Trappe (**1984**).

riod, for example, *Pseudotsuga* logs lose about 50% of their volume (Maser and Trappe, **1984**) from the sloughing of partially decomposed bark and sapwood (Graham, **1982**). Tree seedlings, especially *Tsuga heterophylla* (Raf.) Sarg. commonly become established on logs of decay class III-IV (McKee *et al.*, **1982**; Christy and Mack, **1984**). The high water-retention capacities of *Pseudotsuga menziesii* (Mirb.) Franco logs in decay classes III and IV make them an excellent habitat for a wide range of small vertebrates, invertebrates, plants, and microorganisms, especially during summer drought (Maser and Trappe, **1984**). However, not all organisms are favored by these conditions. The high moisture content of logs in these decay classes (150% in class III and >250% in class IV) and advanced exploration by early colonizers may restrict the activity of basidiomycetes and favor ascomycetes (Kaarik, **1974**).

Although the role of fungi in early wood decomposition has been well-documented (Kaarik, **1974**; Rayner and Todd, **1979**; Frankland *et al.*, **1982**; Cooke and Rayner, **1984**), their role in the later stages of wood decomposition has received little attention. Fungal communities in CWD may possibly be affected by decay class, presence or absence of *Tsuga* seedlings, and slope orientation. The purpose of this study was to focus on the genera/species of hyphomycetes and yeast present in wood of two advanced decay classes.

We addressed three questions related to community structure of these fungi: 1) do community patterns differ between decay classes III and IV; 2) do community patterns differ in logs with and without *Tsuga* seedlings; and 3) are the fungal communities similar in the top, center, and bottom of decomposing logs?

This study does not encompass white rot fungi, brown rot fungi, mycorrhizal fungi and bacteria. Their presence in wood may be common, but time and fiscal constraints prohibited their identification. Nonetheless, exclusion of these organisms was not based on the assumption that they were unimportant in wood decomposition.

MATERIALS AND METHODS

Study sites.—Two study sites were selected at 400-m elevation near Marys Peak in the Coast Range of Oregon (latitude 44°N, longitude 123°W). Rainfall in the area averages 15.84 cm/month from September through May and 1.24 cm/month from June through August. Average day-time temperatures from September through May range from 20 C to −2.2 C, and from June through August the range is 32 C to 7.7 C. The sites are characterized by 60–80-year-old mixed stands of *Tsuga heterophylla* and *Pseudotsuga menziesii*. A sparse understory of *Acer circinatum* Pursh and *Vaccinium* spp. is present, and bryophytes carpet the forest floor. The canopy affords about 50–70% shade at both sites. Each site was logged 80–100 years ago and left to regenerate naturally.

Sampling of logs.—Three logs of decay classes III and IV were sampled at each site in October, 1984. Logs were selected by using the following criteria: 1) each log was horizontal across sloping aspects; 2) sampled logs were not closer than 50 m; 3) logs were unbroken along a minimum length of 3 m; 4) minimum log diameter was 0.5 m; and 5) *T. heterophylla* seedlings were present on logs.

Two disks including the entire diameter were removed from each log. The average disk diameter was 32 cm with an average thickness of 15 cm. From each log, we selected one cross-sectional disk containing roots of a *T. heterophylla* seedling and one without. The disks were cut at least 3 m from the end of the log with a chainsaw. The average distance between disks was 1.5 m. The vertical orientation of each disk was marked for reference. Each disk was placed in a separate large plastic bag and transported to the laboratory within 1 hr of removal from the log. Disks were stored in the bags up to 18 hr at 4 C before processing.

Processing of samples.—In the laboratory, subsamples from each disk were taken to determine moisture content and for fungal recovery. Three samples, representing top, center, and bottom of each log were taken from the interior of each disk by using ethanol-rinsed, flamed chisels and forceps. Top and bottom samples were taken 5 cm inside the log surface, and the center sample was taken along the midpoint of the vertical diameter. Sample fresh weight was 5–10 g. Surfaces exposed by cutting with the chainsaw were avoided to reduce the possibility of contamination.

A modified dilution-plate method was used to recover fungi from the wood. Samples were placed in a sterile, stainless steel blender with 500 ml of sterile distilled water. All samples were blended at high speed for 45 s or until the samples were broken into very small pieces (*i.e.*, <1 mm^3). The woody material was decanted and subsequently diluted by using wide-mouthed pipettes. Concentrations of about 1/100, 1/1000, and 1/10,000 were prepared. Each dilution was used at the rate of 1 ml per sterile plastic Petri dish; agar at 45 C was then poured into the plates and swirled for 60 s to distribute wood chips throughout the medium. Three media were used: potato-dextrose agar, water agar (15%), and rose-bengal agar. Rose-bengal plates were wrapped in aluminum foil to prevent breakdown of the medium by incidental light. Streptomycin-sulfate was added to all three media at a rate of 100 ppm to retard bacterial growth. Two plates for each dilution and medium were prepared for each sample. Plates were incubated at 15 C in an unlighted incubator and examined daily for fungal growth for 1 month or until they were overgrown. Fungi were subcultured from plates and transferred to PDA slant tubes for subsequent identification and storage.

Statistical analyses.—Presence or absence of a species was noted for each sample. Species were given a score of 1 if they occurred in any of the 18 plates prepared for a sample. The frequency of occurrence of a species was calculated as the number of samples (n = 36) with the species present divided by the total number of samples cultured.

Differences in species composition among classes, sites, positions, and cross-sections with and without *Tsuga* seedlings were compared by using coefficient of community (Gauch, **1982**). This statistic was calculated from the presence and absence of species and genera in the samples of a category of log (*e.g.*, class III versus class IV). The coefficient of community ranges from 0 (no species or genera in common) to 1.0 (the same species or genera in each class).

Detrended correspondence analysis (DECORANA) was used to ordinate the samples based on presence or absence of species. This method arranges both species and samples along axes so that those that are most similar are closest (Hill, **1979**). Ordinations were performed on the individual samples (the sum of the 18 plates). The importance of species occurring in <10 samples was downweighted by using Hill's (**1979**) method.

RESULTS

A total of 18 genera and 36 species were recovered from the logs (TABLE II). The majority of the fungal genera were dematiaceous hyphomycetes.

Species composition overlapped between decay classes (FIG. 1). The most commonly recovered fungi in both classes of logs were *Penicillium* spp., *Pichia burtonii, Sistotrema brinkmannii, Torulomyces lagena, Humicola* spp., and *Trichoderma* sp. *Penicillium* was recovered in twice as many class III samples as in class IV samples. All 18 species of *Penicillium* were recovered from class III logs, whereas only 5 species were recovered from class IV logs. *Penicillium lividum* was the second most frequently found species (14%) in class III logs and *Penicillium janthinellium*

TABLE II

LIST OF FUNGI ISOLATED FROM CLASS III AND IV LOGS

Species	Abbreviation[a]
Aureobasidium pullulans (de Bary) Arnaud	AP
Basidiomycete unidentified	B
Chalara sp.	CS[b]
Chalara thielavioides (Peyr.) Nag Raj & Kendrick	CS[b]
Chloridium chlamydosporis (van Beyma) Hughes	CC
Chrysosporium pannorum (Link) Hughes	CP
Humicola fuscoatra Traaen	HS[b]
Humicola grisea Traaen	HS[b]
Leptodontidium elatius (Mangenot) Linnemann	LE
Mortierella ramanniana (Moeller) Linnemann	MR
Oidiodendron echinulatum Barron	OE
Penicillium spp.	PS[b]
Penicillium charlesii Smith	PS[b]
Penicillium citrinum Thom	PS[b]
Penicillium corylophilum Dierckx	PS[b]
Penicillium fellutanum Biourge	PS[b]
Penicillium frequentans Westling	PS[b]
Penicillium implicatum Biourge	PS[b]
Penicillium janthinellum Biourge	PS[b]
Penicillium lanosum Westling	PS[b]
Penicillium lividum Westling	PS[b]
Penicillium purpurogenum Stoll	PS[b]
Penicillium raperi G. Smith	PS[b]
Penicillium restrictum Gilman & Abbott	PS[b]
Penicillium rubrum Stoll	PS[b]
Penicillium simplicissium (Oudemans) Thom	PS[b]
Penicillium spinulosum Thom	PS[b]
Penicillium steckii Zaleski	PS[b]
Penicillium stoloniferum Thom	PS[b]
Phialophora sp.	PS[b]
Pichia burtonii (Boidin *et al.*) Kreger van Rij	PB
Sistotrema brinkmannii (Bres.) J. Erikss.	SB
Spiniger sp.	SS
Thermomyces lanuginosus Tsiklinsky	TL[b]
Thysanophora penicillioides (Roum.) Kendrick	TP
Torula herbarum Pers.	TH
Torulomyces lagena Delitsch	TL
Trichoderma sp.	TS

[a] Abbreviations used in FIG. 1.

[b] These were combined by genus for FIG. 1.

was the second most frequently found species (13%) in class IV logs. *Sistotrema brinkmannii, Torulomyces, Humicola,* and *Trichoderma* were recovered two to three times more frequently from class IV logs than from class III logs. However, the yeast *P. burtonii* was recovered at a very high rate in class IV logs and to a lesser extent in class III logs regardless of medium used.

Comparing communities by using the coefficient of community indicated class III and IV logs were less similar to each other than they were in community comparisons based on sites, positions, or the presence of seedlings. The coefficient of community for class III and class IV logs was 54%, whereas that for sections with and without tree seedlings was 60%. The same pattern was observed when the presence or absence of genera was used to calculate the coefficient of community.

DECORANA ordination indicated that there was considerable overlap in the species composition of logs in classes III and IV (FIG. 2). Class III logs composed 70% of the samples with first-axis scores >150, and 27% of the samples with first-axis scores of <150. The first DECORANA axis therefore seems to weakly correspond to a time or succession gradient. The ecological interpretation of the second axis was not apparent; it was included in FIG. 2 to display the results more clearly. In contrast to decay class, samples from different positions within the cross-section did not occur in discrete areas of the ordination. The same pattern occurred for cross-sections with or without seedlings. This indicated that species composition for these factors had even more overlap than decay classes. Samples from site 1 tended to have lower scores than those from site 2. This may reflect the greater degree of decomposition in the logs at site 1.

DISCUSSION

Fungal community composition differed most between logs of decay classes III and IV. Differences between categories tested such as the presence or absence of *T. heterophylla* seedlings were not evident in comparisons. Our conclusions about differences in community composition made at the species level remained unchanged when the differences were analyzed at the genus level. Our data suggest that it may be possible to separate fungal communities at the genus level without losing information. We are not, however, advocating an abandonment of species identification in such studies. Each ecological case is different and requires an initial analysis of species/genus community relations before a decision is made to identify fungi only to genus. The frequencies of *Penicillium* and the yeast *Pichia* in these logs is of interest. Our data support the possibility of using relative frequencies of *Pichia* and *Penicillium* to determine the decay class of

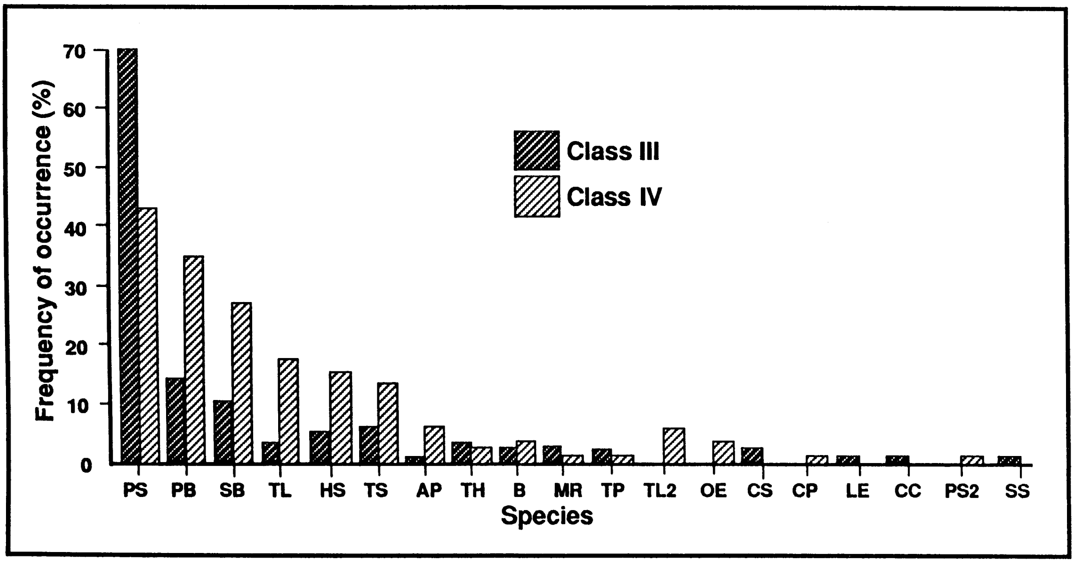

FIG. 1. Frequence of occurrence of fungi isolated from logs in decay classes III and IV. Species abbreviations are given in TABLE II. Although *Penicillium* spp. were combined in this figure, the community analysis was based on individual species.

logs. *Penicillium* was more frequent than *Pichia* in class III logs, whereas in class IV logs the frequencies of these two genera were roughly equal.

DECORANA analysis indicated a good deal of overlap between the two decay classes in terms of fungal species isolated. This result is reasonable because logs are not uniform but contain pockets of wood in various stages of decay. The first DECORANA axis seemed to correspond to species succession over time. The temporal pattern of species might have been more dramatic if a wider range of decay classes had been examined.

Rayner and Todd (**1979**) discuss the problem of delimiting the fungus individual in wood. When a piece of mycelium is broken, an individual mycelium potentially becomes two mycelia in an instant; additionally, some recovered species are prolific sporulators. With no reliable way to count individuals for statistical analyses, we decided to rely on the presence/absence data for these organisms. Community analysis procedures, such as DECORANA, can reveal community patterns with presence/absence data. Our study design with six samples/log may have limited the number of species recovered from logs. Further work is needed on the number of samples required to make a more complete species list.

One of the more interesting results of this research is that basidiomycetes were not recovered as major fungal components of highly decayed logs, in contrast to logs in early decay states (Rayner and Todd, **1979**; Cooke and Rayner, **1984**; Kaarik, **1974**; Frankland *et al.*, **1982**). These results may have been influenced by media and material processing method. The most prevalent fungi recovered from logs were hyphomycetes. Because we cultured from small pieces of wood, our sampling methods may have excluded some basidiomycetes. Whether a larger piece of wood, and therefore a larger potential volume of hyphae, is required for successful culture of basidiomycetes is not known, but species of basidiomycetes were isolated, leading us to believe that inoculum size was not a totally limiting factor.

Our results suggest that hyphomycetes are highly active in advanced decay stages of *P. menziesii.* The dominance of this group may in part be related to physical and chemical changes associated with decomposition (Maser and Trappe, **1984**). High moisture content throughout much of the year may favor hyphomycetes and ascomycetes over basidiomycetes. The wood chemistry of *P. menziesii* changes markedly during decomposition with an enrichment of lignin and depletion of cellulose and hemicellulose (Means *et al.*, **1985**). Carbon quality therefore decreases

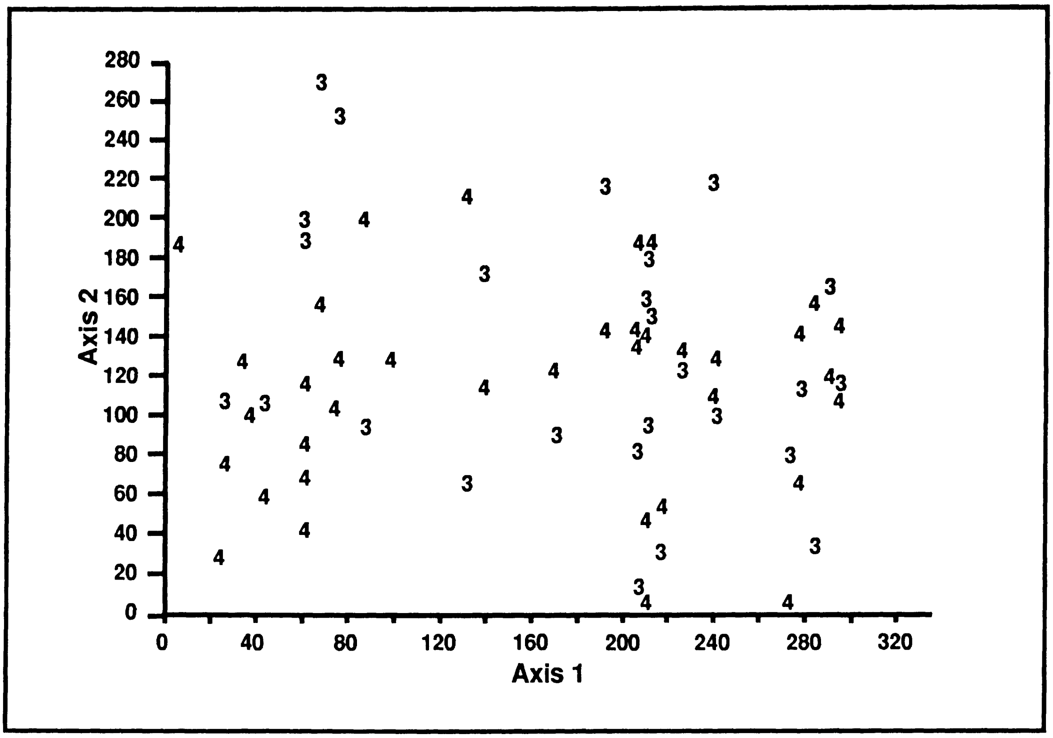

FIG. 2. DECORANA ordination of the 72 samples from which fungi were isolated. A 3 indicates class III log, and a 4 indicates a class IV log sample.

during decomposition and the activity of wood-degrading basidiomycetes is likely to decrease. In contrast, ascomycetes and hyphomycetes may decompose metabolites produced from plant, animal, fungal, or procaryote activity in the log. Some ascomycetes, however, have been shown to have cellulotic activity *in vivo* (Merrill and French, **1966**). Further study is needed on the chemistry of logs in the later stages of decomposition and the effect on microbial activity.

We originally expected to find some differences between fungal communities around roots of *Tsuga* seedlings and in wood without seedlings, but our results indicated no significant differences in community structure. We think that biochemical effects of the rhizosphere on saprophytic fungi in rotten wood may: 1) extend far beyond the immediate vicinity of the roots themselves, thus equally affecting the fungal community in portions of the logs uncolonized by roots; 2) not extend far enough beyond the root surface to affect the surrounding microbial community; or 3) not affect the fungal community. Closer investigation is needed of rhizosphere relations in the environment of the rotten log.

The importance of the various mechanisms responsible for dispersing fungi between and within logs is not clearly understood. Since fungi lacking wind-dispersal mechanisms are widespread in highly decayed conifer wood, we speculate that they move from log to log by: 1) active growth; 2) dispersal of propagules by water percolation; and 3) dispersal by animals. The spores of *Pichia burtonii,* for example, are not adapted for wind dispersal, and their spread is likely due to insect dispersal. Propagules of many ascomycetes can be carried in litter and soil by microarthropods (Visser *et al.,* **1987**). Additionally, propagules of many ascomycetes may be introduced to the surface of logs by canopy throughfall. *Pichia* grows very slowly in culture, producing minute hyphae with multitudes of spores along the hyphae: its movement in logs is more likely due to the spread of its conidia than to rapid penetration by hyphae. Although spores of some genera, such as *Penicillium,* are easily dispersed to new logs by wind, this mechanism is not active deep in wood; again, active growth, dissemination of propagules by water percolation, and the action of animals are the likely mechanisms for within-log movement of such fungi.

The fungal community present in logs determines, in part, the rate and type of decomposition and nutrient cycling processes. Although our results indicate fungal communities in logs of decay classes III and IV are similar, they seem to be different from those found in prior studies of early stages of decay (Kaarik, **1974**; Rayner and Todd, **1979**; Frankland *et al.,* **1982**; Cooke and Rayner, **1984**). Our study suggests that basidiomycetes may be less active than ascomycetes and hyphomycetes, but more work is required before this question is completely addressed.

ACKNOWLEDGMENTS

This research was supported by NSF Ecosystems grants BSR-8516590 and BSR-8514325; NSF Systematics grant BSR-8500434; a grant from the cooperative project "The Fallen Tree—An Extension of the Live Tree" that involves the U.S. Department of the Interior, Bureau of Land Management; U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station; and Agricultural Research Service; Oregon State University, Department of Forest Science, and the Oregon Department of Fish and Wildlife. Special thanks to Harlan Fay and Dan Wilcox for technical assistance.

LITERATURE CITED

Christy, E. J., and R. N. Mack. 1984. Variation in demography of juvenile *Tsuga heterophylla* across the substratum mosaic. *J. Ecol.* **72**: 75–91

Cooke, R. C., and A. D. M. Rayner. 1984. *Ecology of Saprotrophic Fungi.* Longman, London. 415 p.

Fogel, R., M. Ogawa, and J. M. Trappe. 1973. Terrestrial decomposition: a synopsis. U.S. I.B.P. Coniferous Forest Biome Internal Report 135. University of Washington, Seattle, Washington. 12 p.

Frankland, J. C., J. N. Hedger, and M. J. Swift. 1982. *Decomposer Basidiomycetes: Their Biology and Ecology.* Cambridge University Press, London. 355 p.

Gauch, H. G., Jr. 1982. *Multivariate Analysis in Community Ecology.* Cambridge University Press, Cambridge. 298 p.

Graham, R. L. 1982. Biomass dynamics of dead Douglas-fir and western hemlock boles in mid-elevation forests of the Cascade Range. Ph.D. Thesis, Oregon State University, Corvallis, Oregon. 152 p.

Grier, C. C., and R. S. Logan. 1977. Old-growth *Pseudotsuga menziesii* communities of a western Oregon watershed: biomass distribution and production budgets. *Ecol. Monogr.* **47**: 373–400.

Harmon, M. E., J. F. Franklin, F. J. Swanson, P. Sollins, S. V. Gregory, J. D. Lattin, N. H. Anderson, S. P. Cline, N. G. Aumen, J. R. Sedell, G. W. Lienkaemper, K. Cromack Jr., and K. W. Cummins. 1986. Ecology of coarse woody debris in temperate ecosystems. *Adv. Ecol. Res.* **15**: 133–302.

Hill, M. O. 1979. DECORANA: a FORTRAN program for detrended correspondence analysis and reciprocal averaging. Ecology and Systematics, Cornell University, Ithaca, New York. 30 p.

Kaarik, A. A. 1974. Decomposition of wood. Pp. 129–174. *In: Biology of Plant Litter Decomposition.* Vol. 1. Eds., C. H. Dickson and G. J. F. Pugh.

Maser, C., R. G. Anderson, K. Cromack Jr., J. T. Williams, and R. E. Martin. 1979. Dead and down woody material. Pp. 78–95. *In: Wildlife habitats in managed forests, the Blue Mountains of Oregon and Washington.* Ed., J. W. Thomas. USDA Agricultural Handbook 553.

———**, and J. M. Trappe.** 1984. The seen and unseen world of the fallen tree. USDA Forest Service Gen. Tech. Rep. PNW-164. 56 p.

McKee, W. A., G. LaRoi, and J. F. Franklin. 1982. Structure, composition, and reproductive behavior of terrace forests, South Fork Hoh River, Olympic National Park. Pp. 22–29. *In: Ecological Research in National Parks of the Pacific Northwest.* Eds., E. E. Starkey, J. F. Franklin, and J. W. Matthews. National Park Service Cooperative Studies Unit, Corvallis, Oregon.

Means, J. E., K. Cromack Jr., and P. C. MacMillan. 1985. Comparison of decomposition models using wood density of Douglas-fir logs. *Canad. J. For. Res.* **15**: 1092–1098.

Merrill, W., and D. W. French. 1966. Decay of wood by *Alternaria* and *Penicillium. Phytopathology* **56**: 301–303.

Rayner, S. D. M., and N. K. Todd. 1979. Population and community structure and dynamics of fungi in wood. *Adv. Bot. Res.* **7**: 333–420.

Sollins, P. 1982. Input and decay of coarse woody debris in coniferous stands in western Oregon and Washington. *Canad. J. For. Res.* **12**: 18–28.

Triska, F. J., and K. Cromack, Jr. 1980. The role of wood debris in forests and streams. Pp. 171–190. *In: Forests: Fresh Perspectives from Ecosystem Analysis.* Proc. 40th Annual Biology Colloquium, Oregon State University Press, Corvallis, Oregon.

Visser, S., D. Parkinson, and M. Hassall. 1987. Fungi associated with *Onychiurus subtenuis* (Collembola) in an aspen woodland. *Canad. J. Bot.* **65**: 635–642.

Accepted for publication July 26, 1990