Diversity and productivity of hypogeous fungal sporocarps in a variably thinned Douglas-fir forest

Wes Colgan III, Andrew B. Carey, James M. Trappe, Randy Molina, and David Thysell

Abstract: Although ecosystem management techniques are designed to enhance species diversity in managed forests, no comprehensive study has been conducted to evaluate effects of such techniques on diversity and productivity of hypogeous fungi (truffles). During this study, truffles were collected in a 55- to 65-year-old Douglas-fir forest from March 1993 through December 1995 at approximately 6-week intervals. Half of the stands served as controls, half were assigned a variable density thinning (VDT) treatment. A VDT stand comprised a mosaic of patches thinned to different densities of standing live trees. To further evaluate the effect of harvesting impacts. this mosaic was divided into two thinning categories, lightly thinned and heavily thinned areas. Truffle standing crop varied greatly but generally was highest in spring with a smaller peak in the fall. At least some sporocarps were found year round. with winter having the lowest biomass and species richness. Overall standing crop biomass (over all seasons) was significantly lower in VDT stands compared with control stands. The abundance of *Gautieria* and *Hvsterangiwn* species was lower in thinned stands, while *Melanogaster* species diversity and productivity were highest in these stands.

Resume : Meme si les techniques d'amenagement des ecosystemes sont concues pour ameliorer la biodiversite des especes dans les forets amenagees, aucune etude detaillee n'a ete realisee pour evaluer les effets de telles techniques sur la diversite et la productivite des champignons hypoges comme les truffes. Dans le cours de cette etude, des truffes ont ete cueillies dans une fordt de sapin de Douglas de 55-65 ans entre mars 1993 et decembre 1995 a environ 6 semaines d'intervalle. La moitie des peuplements ont servi de temoins et l'autre moitie a subi une eclaircie a densite variable. Un peuplement eclairci a densite variable est compose d'une mosaique de surfaces eclaircies en laissant differentes densites d'arbres vivants. Pour evaluer de facon plus complete les effets de la recolte, les eclaircies ont ete divisees en deux categories : zones d'eclaircie faible et zones d'eclaircie forte. La production de truffes variait beaucoup, mais elle etait generalement la plus elevee au printemps avec une pointe plus petite a l'automne. Au moins quelques sporophores ont ete trouves durant toute l'annee. La biomasse et la richesse en especes etaient minimales en hiver. Dans ('ensemble, la biomasse de la production, pour toutes les saisons. etait plus faible dans les peuplements ayant subi une eclaircie a densite variable que dans les peuplements temoins. L'abondance des especes *de Gautieria* et *d'Hysterangium* etait plus faible dans les peuplements eclaircis tandis que la diversite et la productivite des especes de *Melanogaster* etaient plus elevees dans ces peuplements.

[Traduit par la Redaction]

Introduction

Mycorrhizal fungi assist forest trees in the uptake of water and nutrients from the soil and facilitate movement of carbohydrates from host plants into the mycorrhizosphere. This carbon source supports a vast array of microbes, insects, nematodes, bacteria, and other soil organisms (Ingham and Molina 1991; Fogel 1988). Sporocarps of these fungi are a food resource for many forest mammals world-wide (Fogel and Trappe 1978: Maser et al. 1978; Viro and Sulkava 1985: Malajczuk et al. 1987; Blaschke and Baumler 1989; Carey et al. 1992; Launch-baugh and Urness 1992; Claridge and May 1994; Carey 1995). Most of the sporocarps consumed by animals in temperate forests are formed by ectomycorrhizal (EM) fungi that form a symbiotic association with the feeder roots of members of the Pinaceae, Fagaceae, Betulaceae, Myrtaceae. and Salicaceae (Trappe 1962; Molina et al. 1992).

Hypogeous fungi as defined here include the below-ground-fruiting fleshy Ascomycetes (true truffles), Basidiomycetes (false truffles), and some sporocarpic Zygomycetes. For convenience in this paper we refer to them collectively as truffles. They attract animals to their mature sporocarps by producing aromatic compounds (Fogel and Trappe 1978). The animals extract the truffle and consume all or part of it (Trappe and Maser 1977; Fogel and Trappe 1978) including spores, bacteria, all or part of it (Trappe and Maser 1977; Fogel and Trappe 1978) including spores, bacteria and yeasts that live in the sporocarps (Li et al. 1986). The spores, yeasts, and bacteria pass through the digestive tract unharmed and, along with feces, are deposited in new locations (Trappe and Maser 1976). Rain or snowmelt water may then move the fecal contents into the soil where they colonize new roots (Trappe and Maser 1977).

Sexual reproduction (sporocarp formation) of EM fungi has a strong seasonal aspect in the Pacific Northwest (PNW) (Fogel 1976; Hunt and Trappe 1987; Luoma et al. 1991). Studies of truffle abundance in this region have largely examined different forest types and successional stages (Vogt et al. 1981; Luoma et al. 1991; O'Dell et al. 1992; Amaranthus et al. 1994). Luoma (1989) and studies in progress (Pilz and Molina 1996) show differences in the

Received December 3, 1998. Accepted April 1, 1999.

W. Colgan and J.M. Trappe. Department of Forest Science, Oregon State University, Corvallis. OR 97331, U.S.A.
A.B. Carey and D. Thysell. United States Department of Agriculture, Forest Service, Pacific Northwest Research Station. Forestry Sciences Laboratory, 3625 93rd Avenue, Olympia. WA 98512, U.S.A.
R. Molina.[1] United States Department of Agriculture, Forest Service, Pacific Northwest Research Station. Forestry Sciences Laboratory, 3200 Jefferson Way. Corvallis OR 97331. U.S.A.

[1]Corresponding author. e-mail: molinar@fsl.orst.edu

EM fungus community structure by comparing stands at ages of 25 and 50 years and between 80, 160, and 400+ years. Most of these studies have focused on the peak spring and fall fruiting periods of the truffles and other ectomycorrhizal fungi. This approach has left rather large gaps in our understanding of the functional importance of many truffle species at other times of the year. For mycophagous mammals that are active year round (e.g., the northern flying squirrel, *Glaucomys sabrinus*) truffles that fruit during winter and summer are critically important. North et al. (1997) included winter and summer sampling in old-growth, naturally mature, and managed mature forests in the PNW. Hunt and Trappe (1987) and Fogel (1976) documented productivity monthly on sites in western Oregon. These studies show that some species of truffles are available year round in some PNW forests, although lowest diversity and standing biomass occurred in winter. Many of these studies were retrospective (Luoma 1989; North et al. 1997), and others were unreplicated (Hunt and Trappe 1987; Fogel 1976).

Little is known of mycorrhizal fungal dynamics during the first 25 years after disturbance. Amaranthus et al. (1994) found significantly lower truffle abundance and diversity in 4- to 27-year-old plantations (regenerated from clearcuts) than in neighboring mature (180-year-old) forest fragments in the Siskiyou National Forest in southwestern Oregon. Waters et al. (1994) is the only published paper that has addressed the effects of thinning on the production and diversity of truffles. That study focused on thinned plots in an *Abies* forest at 17 and 20 years post-thinning. Treatment plots were 0.4 ha arrayed in mosaics of unthinned, thinned, burned structure of truffle species fruiting in these stands. It should be noted that both the Amaranthus et al. (1994) and Waters et al. (1994) studies were small in scale and with limited sample sizes; Waters et al. included only two replicates per treatment and neither study computed species-area relationships to indicate adequacy of sampling. Our study is the first to use a complete randomized block design with a year-round intensive sampling effort and statistical analysis in forest ecosystems dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco).

In addition to performing many key functions, fungi support a number of critical trophic pathways in forest ecosystems. The northern flying squirrel, for example, strongly depends on truffles as the major component of its diet. Carey et al. (1992) and Carey (1995) found that flying squirrel abundance increases with fungal diversity along a north-south cline in the northern Cascades and Olympic Peninsula in northern Washington to the Cascades and Olympic Peninsula in northern Washington to the central western slopes of the Cascades, southern Coast Ranges, and Klamath Mountains of southwestern Oregon. Waters and Zabel (1995) found that flying squirrel densities correlate with sporocarp frequency in fir forests in northeastern California. We poorly understand the role of specific fungal species and the effects of disturbance and forest management (e.g., thinning and tree harvest) on the population dynamics of fungal and small mammal communities.

Intermediate cutting, especially thinning, is frequently proposed as a method of accelerating development of late-seral forest. Long-term effects are thought to be positive. Short-term impacts on fungi may be negative and, thus, could have a depressive effect on mycophagists, including the primary prey of the northern spotted owl (*Strix occidentalis*), the northern flying squirrel. The objective of this study is to document the changes in the productivity and diversity of truffles for the first 3 years following the installation of a variable-density thinning (VDT). This study is one of several interdisciplinary efforts of the Forest Ecosystem Study (FES) (Carey et al. 1999*b*).

Methods

Study area

The FES is located on four blocks of forest. 30 km northeast of Olympia, Wash., on the Fort Lewis Military Reservation. The soils of Star and Stellar blocks are classified as Tenino gravely sand loam. The soils of Hill and Farley blocks are classified as Everett gravely sand loam. All blocks fall into the Southern Puget Trough physiographic province (Franklin and Dyrness 1973). The stands are essentially flat to gently rolling, and slopes rarely exceed 15%. Elevation ranges from 100 to 143 m. annual average precipitation is 91 cm. The forests of the Fort Lewis Military Reservation have been designated as habitat critical to the northern spotted owl because they constitute the only Federal forest between the Cascade Range to the east and the Olympic Peninsula to the west. Fort Lewis is surrounded by urban and agricultural land on the north east and south and Puget Sound on the northwest.

The forests at Fort Lewis are dominated by Douglas-fir. Most of the old growth was cleared in the first half of the 20th century; existing stands regenerated from natural seeding. Farley and Hill blocks were clearcut in 1927 and lightly thinned twice since (1972 and in the late 1980s). They have almost no remaining coarse woody debris (CWD) or large-diameter trees from the original old growth; CWD was intentionally removed from these stands during the early thinning operations. They have developed an extensive understory of *Gaultheria shallon* Pursh, *Berberis nervosa* Pursh, and *Polystichum munitum* (Kaulf.) Presl. Star and Stellar blocks were clearcut in 1937 and had no further silvicultural manipulations before installation of our study. Understories in these blocks were variable and dominated by ground mosses. *Gaultheria shannon* and *P. munitum* occur primarily in scattered canopy openings caused by laminated root rot (*Phellinus weirii* (Murr.) Gilb.). These blocks had considerable residual CWD and numerous large-diameter old-growth trees (about 5/ha).

Experimental design and silvicultural prescriptions

The FES is a complete randomized blocks experiment with four blocks (Star, Stellar, Farley, Hill) and four treatments, with each treatment randomly assigned to one of four 16-ha stands in each block: (*i*) control (no treatment), (*ii*) installation of artificial dens for arboreal rodents (of no importance to hypogeous fungi, and not discussed further here, except as part of the framework for sampling within the experiment), (*iii*) VDT. and (*iv*) a combination of installation of artificial dens and VDT (Carey et al. 1999*a*). The goal of the FES is to determine if development of late-seral forest characteristics can be accelerated through ecologically based commercial thinning. Variable-density thinning is a new approach to commercial thinning that

emphasizes removal of subordinate trees ("thinning from below") and replication of the spatial scale of variation in canopy cover found in old-growth forests (Carey 1995: Carey et al. 1999*a*. 1999*b*). The objectives of VDT are to increase vertical and horizontal heterogeneity, foliage height diversity, and compositional diversity of the plant community in a way that mimics the composition. structure, and spatial pattern of vegetation in naturally old forests and, thus, provide improved habitat for wildlife and increased complexity of trophic pathways (Carey et al. 1992. 1999*a*: Carey 1995; Carey and Johnson 1995). To accomplish the thinning, we surveyed an 8 x 8 grid with 40-m spacing in. each stand: each grid contained forty-nine 0.16-ha cells. Cells within stands were assigned one of three target relative densities (RD: Curtis 1982): two, four, or six. Relative density is a measure of crowding based on quadratic mean diameter at breast height. density of trees, and species growth form. Before thinning, RD averaged 7.6 ± 0.1 (mean ± SE), a density at which crowns become crowded (the case in Farley and Hill) or that occurs as self-thinning ceases (the case in Star and Stellar). The heaviest removal of trees (RD = 2; <40 stems/ha; all low vigor trees removed) was designed as a treatment for root rot (*Phellinus weirii*) pockets and entailed removing all low-vigor trees (regardless of canopy position) out through the margins of the pockets. Pockets were obvious and covered a maximum of 15% of the stands in the Star and Stellar blocks: where <15% of a stand was covered with root-rot pockets. RD-2 removal was applied to randomly selected cells to ensure that 15% of the stand was thinned to RD. In the Farley and Hill blocks (where root-rot pockets were not evident), RD-2 removal was as randomly assigned to seven cells in each treated stand (approximately 16% of stand area). Light (RD = 6) and heavy thinnings (RD = 4) were randomly assigned to the remainder of the cells. Treatment implementation (which incorporated both tree-marking error and logger error) varied somewhat from that planned. Actual percentages of areas treated to RD = 2 were 18% in Star-Stellar and 21% in Farley-Hill. Post-thinning RD averaged 4.1 ± 0.04 (heavy thinning) and 5.7 ± 0.05 (light thinning). Actual percentagesof areas treated in Star-Stellar and Farley-Hill were 35 and 26% for RD = 4 and 47 and 53% for RD ≥6, respectively. These three RD levels are considered the final subtreatments within the FES.

Fungal sampling

Field sampling took place about every 6 weeks from 14 April 1993 through 5 December 1995. Fungal sporocarps were collected from each of 10 circular, 4-m^2 plots, located at about 10-m intervals along randomly placed transects (modified fromLuoma et al. 1991), in each of the control stands of each block at each sampling period. Transects were curved or separated into segments when plot boundaries were reached before all 10 plots in a transect were completed. Thinned stands of each block were sampled more intensively to determine if thinning of subtreatments affected sporocarp production differently. Originally, the three subtreatments noted above were to be sampled with 10 randomly placed 4-m^2 plots. totaling 30 plots per thinned stand per sampling period. However, because treatment implementation varied somewhat from that planned (see above paragraph), we found that some of our sampled transects did not correspond to the original sub-treatment design. We thus found it necessary to collapse the three subtreatments into two thinning categories based on basal area (BA) removed. Hereafter we refer to these thinning categories as lightly thinned (<48% of the BA in the grid cell removed, RD <4) and heavily thinned (≥48% of the BA in the grid cell removed. RD≥4). Lightly thinned areas averaged 18 ± 0.8% BA removed (82 ± 0.8% retained). Heavily thinned areas averaged 73 ± 1.6% BA removed (27 ± 1.6% retained). Twelve transects that spanned grid cells of different thinning categories were excluded from the analysis.

Each collection (one to several sporocarps of the same species in close proximity to one another on a single 4-m^2 sample plot) was placed in a wax paper bag with a tag recording plot number. stand number, and other pertinent information. Each plot was then raked with hand tools to a depth of at least 5 cm into mineral soil to expose hypogeous sporocarps. Field characteristics of sporocarps were noted (bruising reactions. odor. etc.) for each collection. All plots were marked with a plastic pin flag and the duff was replaced. No plots were sampled twice. All fungal samples were dried the day of collection with a forced air dehydrator set at 49°C and then returned to the Forestry Sciences Laboratory in Corvallis, Oreg. for identification and weighing to the nearest 0.01 g. Voucher specimens were placed in the Mycological Herbarium at Oregon State University (OSC).

Much of the labor involved in field sampling was volunteer: about 80 individual volunteers participated. 'Volunteers included Professors of Mycology at Universities, postdoctoral fellows and graduate students in mycology, undergraduate student interns in mycology, and amateur mycologists from the local community, Native Plant Societies of Washington and Oregon. and a number of natural history organizations. Volunteers worked in teams of two to four lead by professional mycologists (W.C., J.M.T., and D.T.).

Analysis

Frequency (presence or absence in 4-m^2 truffle plots) was calculated for each species encountered in each thinning category and in the control stands. Biomass values were standardized for each transect (ten 4.0-m^2 truffle plots per thinning category) to kilograms per hectare dry mass. Stand-level biomass estimates were calculated by weighted mean values (based on percentage of the area in stand occupied by each thinning category). Biomass data for VDT stands and control stands were compared by using analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD) with the significance level at 5%. Overall frequency of occurrence of truffles from each thinning category and control were compared using chi-square goodness of fit tests with significance level at 5%.

Species diversity was evaluated by two indices: the Berger-Parker index for species dominance based on sporocarp biomass in each thinning category, and the Margalef's index for species richness based on numbers of sporocarps in each of the thinning categories (formulae from Magurran 1988). Indices from each thinning category were compared by chi-square goodness of fit tests with significance level at 5%. Because of an uneven number of sample transects, Margalef's index was calculated by randomly selecting an equal number of transects in each of the thinning categories. These two

Table 1. Percent frequency of each truffle species found on plots at the Fort Lewis Military Reservation April 1993 through December 1995. in control, lightly thinned, and heavily thinned stands.

Taxon	Control	Lightly thinned	Heavily thinned
Alpova diplophloeus (Zeller & Dodge) Trappe & Smith			0.11
Elaphomyces granulatus Fr.	0.11	0.18	0.22
Elaphomyces muricatus Fr.			0.22
Endogone lactiflua Berk. & Broome	0.98	3.11	2.00
Endogone pisiformis Link:Fr.	0.11	0.24	0.56
Gautieria monticola Harkn.	1.52	0.18	0.11
Gen.nov. and sp.nov. No. 1	0.11		
Gen.nov. and sp.nov. No. 2	0.11		
Genabea cerebriformis (Harkn.) Trappe	0.22	0.06	
Genea harknessii Gilkey			0.11
Genea intermedia Gilkey	0.11		
Glomus macrocarpum Tul. & C. Tul.	0.22	0.12	0.22
Glomus microcarpum Tul. & C. Tul.		0.12	
Glomus mosseae (T.H. Nicolson & Gerd.) Gerd. & Trappe		0.06	
Glomus sp.nov.		0.06	0.11
Hydnotrya variiformis Gilkey		0.36	
Hymenogaster sp.	0.11	0.06	0.11
Hymenogaster sublilacinus A.H. Smith	0.11	0.24	
Hysterangium coriaceum R. Hesse	1.85	0.42	
Hysterangium crassirhachis Zeller & Dodge	1.41	0.12	
Hysterangium setchellii E. Fisch.	1.52	0.30	
Leucangium carthusianum (Tul. & C. Tul.) Paol.		0.18	
Leucogaster candidus (Harkn.) Fogel comb. ined.	0.11	0.18	
Leucogaster citrinus (Harkn.) Zeller & Dodge	0.22		0.11
Leucogaster gelatinosus Fogel nom. ined.	0.22	0.12	0.11
Leucogaster rubescens Zeller & Dodge	0.22	0.06	
Leucogaster sp.nov.		0.06	
Leucophleps magnata Harkn.	0.33	0.36	
Leucophleps spinispora Fogel	0.11		
Melanogaster ambiguus (Vitt.) Tul. & C. Tul.		0.18	
Melanogaster euryspermus (Zeller & Dodge) Zeller		0.30	0.33
Melanogaster natsii Wang, Trappe & Castellano. nom. ined.		0.30	0.11
Melanogaster thiersii Wang, Trappe & Castellano. nom. ined.			0.11
Melanogaster trappei Wang nom. ined.		0.66	0.22
Melanogaster tuberiformis Corda	0.65	0.48	1.67
Melanogaster variegatus (Vittad.) Tul. & C. Tul.		0.06	
Pachyphloeus thysellii Colgan, nom. ined.		0.06	
Radiigera fuscogleba Zeller		0.06	
Rhizopogon hawkerae A.H. Smith	1.20	0.90	0.78
Rhizopogon rogersii A.H. Smith		0.06	
Rhizopogon subareolatus A.H. Smith		0.06	
Rhizopogon villosulus Zeller	0.43	0.24	0.11
Rhizopogon vinicolor A.H. Smith	6.44	4.37	1.78
Rhizopogon vulgaris (Vittad.) M. Lange	0.11		
Truncocolumella citrina Zeller	0.65	0.18	0.33
Tuber anniae Colgan & Trappe	0.22	0.12	
Tuber gibbosum Harkn.		0.06	
Tuber monticola Harkn.	2.17	1.50	1.56

measures were used because indices that attempt to represent both richness and evenness (such as the Shannon index) often provide no information beyond that offered by richness alone (Magurran 1988).

Results

During the course of this study, 1786 truffles were collected from 3680 plots (14720 m^2). Forty-eight species were identified: six species were new to science, and two were from undescribed genera. Species frequency over the entire study ranged from 0 to 6.4% of plots in any thinning category or control. Four species were encountered only in heavily thinned plots, 12 only in lightly thinned plots, and five species only in control plots (Table 1).

Overall, 15% of the plots sampled contained ≥1 sporocarp (85% had no sporocarps); 18% in control stands contained ≥1 sporocarps. Frequency declined to 13% in VDT stands, with 14% in lightly thinned areas and 10% in the heavily thinned areas. Thinning significantly reduced frequency of

Fig. 1. Truffle standing crop biomass for the Fort Lewis Military Reservation for control and variable-density thinned (VDT) stands at each sampling period. The December 1994 sample was canceled owing to snow cover. Error bars are 95% confidence interval of the mean.

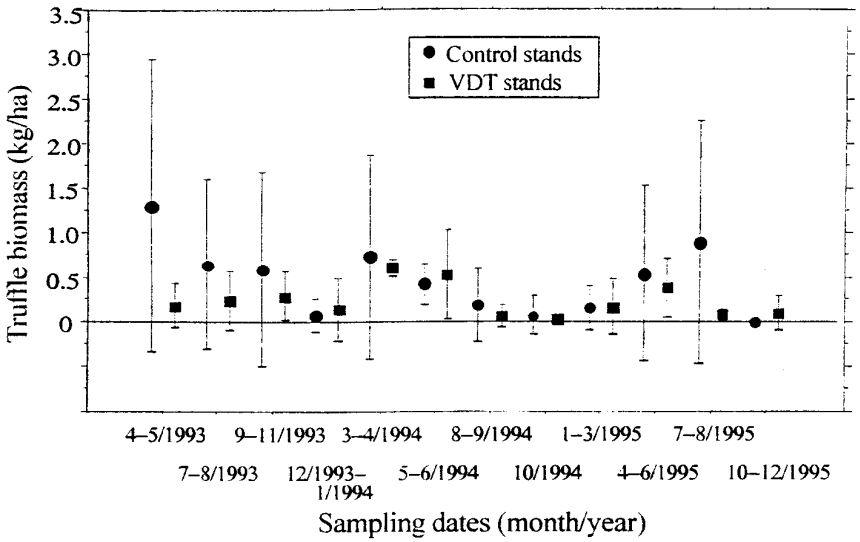
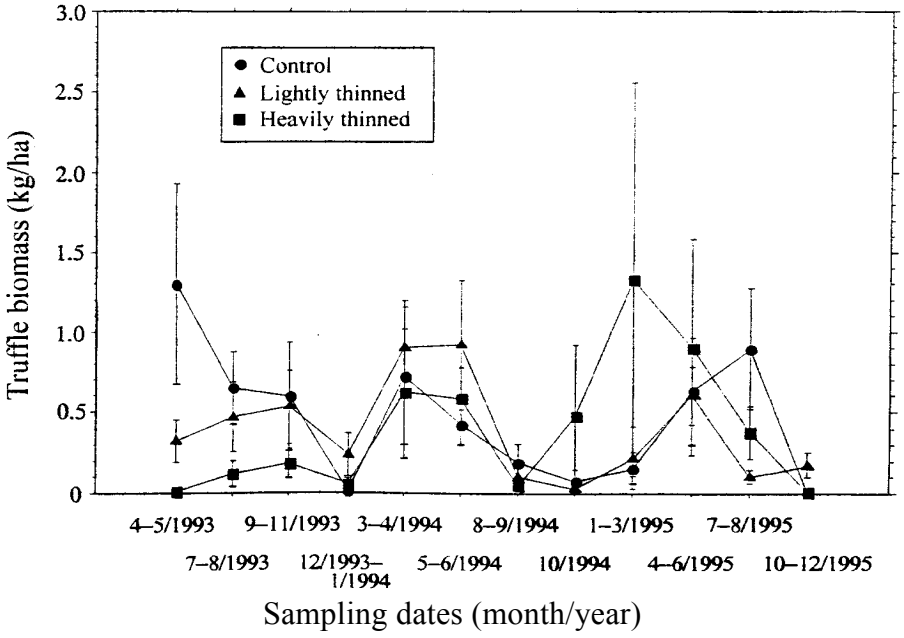


Fig. 2. Mean truffle standing crop (kg/ha) for control, lightly thinned, and heavily thinned stands at each sampling period. The December 1994 sample was canceled owing to snow cover. Error bars are SE.



sporocarps (χ^2 = 6.22, df = 1, P = 0.0134), and heavy thinning markedly reduced frequency of sporocarps (χ^2=22.714, df = 1. P < 0.0001). The total biomass collected was 0.610 kg. The largest transect estimate (11.1 kg/ha) was from March 1995, Star Block, heavily thinned area. The next largest transect estimate was 5.6 kg/ha from a lightly thinned area in the Stellar Block. Several transects (from all three treatments) contained no detectable sporocarps. The VDT-treated stands had significantly lower biomass than the control stands on average (ANOVA Fisher's PLSD, df = 1, P = 0.0333; Fig. 1). Study blocks did not differ significantly in biomass, and no significant interaction occurred between treatment and block. Average standing crop biomass ranged from a low of 0.008 ± 0.008 kg/ha in control stands (October-December 1995) to 1.331 ± 1.226 kg/ha in the heavily thinned stands (January-March 1995) (Fig. 2).

Control stands averaged 0.486 ± 0.089 kg/ha, and stands averaged 0.233 ± 0.034 kg/ha (Table 2). Annual variation in standing crop biomass was high, with year-1 control stands averaging almost twice the biomass of any other. Year 2 VDT stands approached the average found in control stands but then fell off sharply in year 3 (Table 2).

Six species each accounted for ≥5% of the biomass collected and collectively 80% of the biomass in the control stands (Table 3); 22 species accounted for the remaining 20%. Five species accounted for 64% of the biomass in the lightly thinned stands, with 33 species accounting for the remaining 36%. In the heavily thinned

Table 2. Mean truffle standing crop (kg/ha) by year and stand treatment.

Year	Stand treatment	Standing crop Mean	SE
1	Control	0.654	0.192
1	VDT[a]	0.251	0.463
2	Control	0.350	0.109
2	VDT	0.308	0.077
3	Control	0.403	0.149
3	VDT	0.178	0.047
Overall	Control	0.486	0.089
Overall	VDT	0.233	0.034

[a]Represents data from all heavily thinned and lightly thinned plots.

Table 3. Percentage of total biomass collected for dominant truffle species (accounting for >5% of the biomass collected) for each treatment.

Species	Control	Lightly thinned	Heavily thinned
Endogone lactiflua	—	14.78	—
Gautieria monticola	14.74	—	—
Hysterangium coriaceum	12.53	—	—
Hysterangium setchellii	14.58	—	—
Melanogaster euryspermus	—	9.47	6.60
Melanogaster natsii	—	9.43	—
Melanogaster thiersii	—		7.04
Melanogaster trappei	—	11.70	—
Melanogaster tuberiformis	13.08	—	58.88
Rhizopogon hawkerae	7.76	—	6.06
Rhizopogon vinicolor	18.13	18.38	6.22
Total	79.74	63.76	84.80

stands, five species accounted for 85% with one, *Melanogaster tuberiformis*. accounting for 59% of the biomass. Seventeen other species accounted for the remaining 15%.

The heavily thinned stands showed the greatest dominance by a single species (Berger-Parker index, $x^2 = 3.125$; P = 0.022; df = 11; Table 4). Margalef's index of species richness was highest in the lightly thinned stands (Table 5), but the thinning treatments did not differ significantly.

Collections of sporocarps (one to several truffles of the same species in close proximity to one another on a single plot) differed among thinning categories; 233 collections were found in control stands, 345 collections were found in lightly thinned stands, and 115 collections were found in heavily thinned stands. The largest average number of sporocarps per collection occurred in the heavily thinned stands, with 2.8 sporocarps/collection; control stands averaged 2.6 sporocarps/collection, and lightly thinned stands averaged 2.4 sporocarps/collection. *Rhizopogon vinicolor* and *R. hawkerae* both had significantly more sporocarps/collection in the heavily thinned stands compared to control stands (Table 6).

Species diversity increased rapidly with number of samples then leveled off. The lightly thinned stands

Table 4. Species dominance for each treatment and sampling period.

Sample period (month and year)	Control	VDT[a]	Lightly thinned	Heavily thinned
4–5/1993	1.95	2.25	2.23	2.40
7–8/1993	3.09	2.88	2.20	2.10
9–11/1993	1.64	6.86	2.63	2.30
12/1993–1/1994	1.24	1.29	1.19	1.91
3–4/1994	1.87	5.09	3.57	1.48
5–6/1994	2.33	2.67	2.85	2.25
8–9/1994	1.54	1.18	1.22	2.19
10/1994[b]	1.05	1.03	1.00	1.04
1–3/1995	2.46	1.39	1.05	1.09
4–6/1995	2.17	2.38	2.36	1.19
7–8/1995	2.64	3.26	3.38	2.26
10–12/1995	1.00	3.23	3.12	1.26
Overall	5.61	4.40	11.93	1.70*

Note: Values are the Berger–Parker index using sporocarp mass. Values close to 1 indicate dominance. higher values indicate more evenness.
[a]Represents data from all heavily thinned and lightly thinned plots.
[b]The 12/1994 sample was canceled owing to weather.
*Significantly different (χ^2, $P = 0.022$, df = 11).

Table 5. Margalef's index of diversity using sporocarp numbers and number of species (in parentheses) by treatment and sampling period: calculated by randomly selecting equal numbers of transects in each treatment category (higher values indicate Greater species richness).

Sample period (month and year)	Control	VDT[a]	Lightly thinned	Heavily thinned
4–5/1993	2.00 (12)	2.36 (12)	1.90 (9)	1.44 (2)
7–8/1993	2.70 (11)	2.84 (13)	1.57 (6)	1.30 (6)
9–11/1993	1.53 (7)	3.17 (15)	2.11 (9)	2.12 (7)
12/1993–1/1994	0.00 (1)	2.12 (11)	0.25 (2)	0.56 (3)
3–4/1994	1.63 (6)	3.49 (21)	2.39 (11)	1.20 (7)
5–6/1994	2.03 (10)	2.84 (15)	1.70 (8)	1.66 (7)
8–9/1994	1.52 (5)	2.34 (7)	2.16 (4)	1.12 (3)
10/1994[b]	1.44 (2)	0.26 (2)	0.00 (1)	0.28 (2)
1–319/95	0.56 (3)	2.34 (12)	0.98 (5)	0.64 (3)
4–6/1995	2.66 (10)	2.45 (13)	1.31 (6)	1.46 (5)
7–8/1995	2.73 (9)	2.38 (9)	1.24 (3)	2.09 (6)
10–12/1995	nc (1)[c]	2.63 (11)	1.82 (7)	0.62 (2)
Overall	4.29 (28)	5.90 (43)	5.38 (32)	3.69 (22)

[a]Represents data from all heavily thinned and lightly thinned plots.
[b]The 12/94 sample was canceled owing to weather.
[c]Cannot be calculated owing to small number of sporocarps.

began to level out at 3600 m^2 sampled, with 38 species total. Control stands were asymptotic at 2400 m^2 sampled and contained 28 species total. Heavily thinned stand species composition continued to rise throughout this study with 22 species total. Figure 3, which shows the number of species per area curves, shows a classical exponential form, approaching an asymptote quickly after reaching a shoulder. As collections continued, a few additional species were found as would be expected. The total species-area curve became asymptotic after 7600 m^2 had been sampled. The VDT stands did not level off until 5760 m^2 and exhibited a slight increase at 9100 m^2 (third

Fig 3. Curves for the number of truffle species per area for the Fort Lewis Military Reservation.

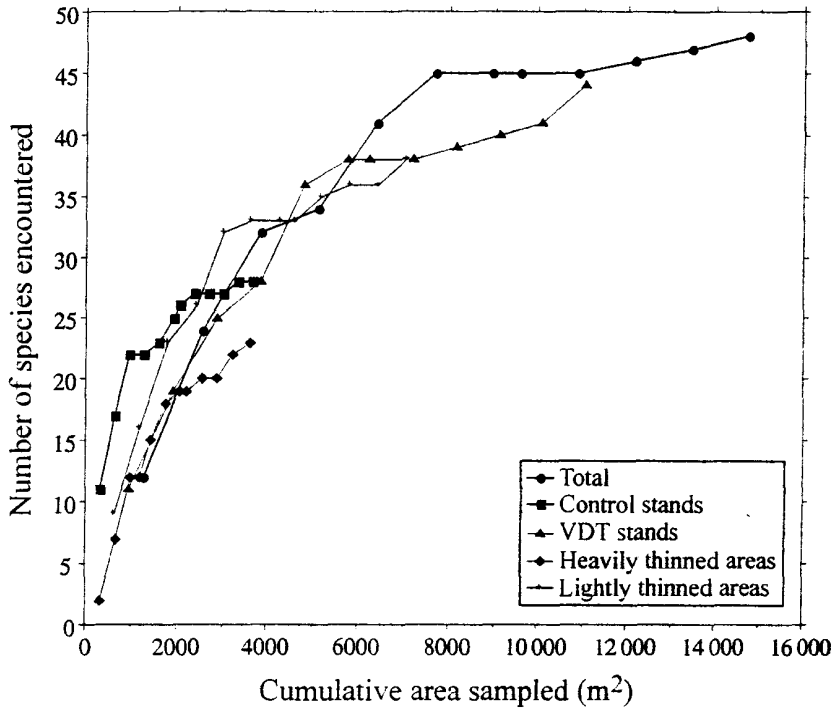


Table 6. Mean number of sporocarps per collection of two *Rhizopogon* species found in control, lightly thinned, and heavily thinned stands.

Truffle species	Control	Lightly thinned	Heavily thinned
R. hawkerae	2.53 (0.47)*	1.81 (0.25)*	6.57 (2.50)
R. vinicolor	1.38 (0.12)**	1.75 (0.16)	2.66 (0.69)

Note: Values are means with *SE* given in parentheses.

*Significantly fewer sporocarps per collection than heavily thinned area. Fisher's PLSD (P = 0.0064 and 0.0015 for control and lightly thinned stands, respectively).

**Significantly fewer sporocarps per collection than heavily thinned area. Fisher's PLSD (P = 0.043).

Table 7. Percentage of sporocarps and biomass of truffle genera collected accounting for >5% winter standing crop.

Group	Sporocarps	Biomass
Endogone	54	33
*Rhizopogon**	35	39
Tuber	6	7
Total	95	79

*Includes closely related *Truncocolumella citrina*.

spring after thinning). Three genera accounted for 79% of the total biomass collected during winter sampling at Fort Lewis. These same three genera accounted for 95% of all sporocarps collected during winter samplings (Table 7). *Endogone lactiflua* and *E. pisiformis* were the most frequently encountered, and *Rhizopogon* spp. (primarily *R. hawkerae*) contributed most to standing crop biomass.

Discussion

The most striking results of this study were the shift in species dominance within the lightly thinned and heavily thinned treatments and the presence of 16 species found only in the thinned stands. This suggests that some species were induced to fruit by the thinning operations. Species immigration is not likely as only 3 years elapsed during this study. The forests at Fort Lewis were essentially homogenous within each block before study installation. Some of the measured increase may be artificial because of the increased sampling effort in the VDT stands. Diversity increased, (albeit by only three species compared with control) however, even when the excess replication was removed in the lightly thinned treatments (Table 5). Frequency and diversity of sporocarpic *Glomus* species was highest in the thinned stands. This corresponds well with the increase of herbaceous plants and broadleaf shrubs with which *Glomus* species form arbuscular mycorrhizae.

Species dominance shifted most in heavily thinned areas. The genus *Melanogaster* preferentially fruited in VDT stands. Six of seven *Melanogaster* species encountered in this study were found only in the VDT grids. One species (*M. thiersii*) was found only in heavily thinned areas. *Melanogaster tuberiformis* accounted for almost 60% of all truffle biomass collected in heavily thinned areas. The overwhelming dominance of this and four other closely related species in VDT stands strongly suggests that the thinning promotes sporocarp formation by *Melanogaster* species but not necessarily others. This is particularly interesting as *Melanogaster* is second only to *Rhizopogon* in the diets of the mammals at Fort Lewis (Colgan 1997). *Melanogaster* is also one of the most

nutritious (caloric value per gram of truffle) for small mammals (A.W. Claridge, unpublished data). Waters et al. (1994) found a similar increase in *Gymnomyces* spp. in shelterwood stands of *Abies* spp. in northeastern California. The increase in productivity of *Gymnomyces* spp. offset the decrease of productivity in *Hysterangium* and *Gautieria* in their study stands.

Contrary to the increase in *Melanogaster*. *Hysterangium* and *Gautieria* declined in frequency and productivity in the VDT stands. *Hvsterangium setchelii, H. coriaceum,* and *Gautieria monticola* accounted for 42% of the biomass collected in control stands. These species declined markedly in VDT stands and did not account for a substantial portion of the biomass collected. None of the three species of *Hysterangium* found during this study occurred in the heavily thinned stands; *G. monticola* occurred only once. All these species form dense hyphal mats in Douglas-fir forests (Griffiths et al. 1991). These mats are known to host a greater concentration of soil organisms and soil microbial biomass and have substantially different soil chemistry than surrounding "nonmat" soils (Aguileria et al. 1993; Cromack et al. 1988; Griffiths et al. 1990). Such mats may occupy up to 28% of the forest floor in Douglas-fir forests (Cromack et al. 1979). Many factors may relate to the decline of sporocarp production by these fungi in thinned stands. Their hyphal mats may be susceptible to mechanical damage by logging operations. The loss of photosynthate due to removal of the host trees may compromise their ability to support large hyphal networks, thereby decreasing the carbohydrate allocated to sporocarp production. Likewise, the thinning may have changed the microclimate to the detriment of these species. This closely resembles the trend found by Waters et al. (1994), who found that relative frequency of *Hysterangium* and *Gautieria* were significantly less in the shelterwood stands 17 and 20 years after timber harvest. Evidently considerable time is needed for these fungi to produce sporocarps after disturbance. McIntire (1984) found a significant decrease in *Hysterangium* spores in fecal pellets of Siskiyou chipmunks *(Eutamias siskiyou)* in shelterwood stands in southwestern Oregon. A study at Fort Lewis showed that the amount of forest floor covered by the hyphal mats was significantly lower in the VDT stands than in nonthinned stands (Y. Valochovic, unpublished data). This corresponds well with our observation of sporocarp frequency and productivity. These observations are supported by Aguileria et al. (1993) who found *Gautieria* mats only in the rooting zone of the retained old-growth trees in a 2-year-old shelterwood stand in the Oregon Cascades. They report that mats were scarce in a nearby 11-year-old clearcut site. Further studies are necessary, but these fungi may well be indicators of soil disturbance in PNW forests.

In a thinning project installed by S.L. Miller (University of Wyoming). ectomycorrhizal fungi were collected and fruiting locations mapped in a lodgepole pine (*Pinus contorra* Dougl. ex Loud.) forest in Wyoming (S.L. Miller, unpublished data). His data suggest that removal of host trees and creation of small canopy gaps increased ectomycorrhizal sporocarp production on a very local scale. Although species were found across all treatments, some fruited more prolifically in the openings and others occurred only in controls and very small openings. His data parallel our study in many ways. *Rhizopogon vinicolor* was the most frequently encountered truffle in our control stands and remained high in the lightly thinned areas as well but was only one third as frequent in heavily thinned areas as in controls (6.4% of plots compared with 1.8%). However, *Endogone lactiflua* productivity was greatest in lightly thinned and heavily thinned areas. All species that were restricted to one treatment can be considered rare (<5% of the biomass in the subtreatment). *Melanogaster thiersii* was collected only once during this study, as a single cluster in a heavily thinned area.

The decrease in number of collections combined with the significant increase in number of sporocarps per collection of *R. vinicolor* and *R. hawkerae* suggests that localized flushes may be a stress reaction of these fungi (S.L. Miller, personal communication). Many flowering plants will allocate a greater proportion of their available resources to reproductive structures when under stress. Conceivably the mycorrhizal fungi could similarly respond to the loss of a host tree due to logging operations. Alternately, these fungi may be responding to reduced competition from *Hvsterangium* and *Gautieria* spp. in the heavily thinned areas. Furthermore, *R. vinicolor* ectomycorrhizae are frequently found in buried wood (Zak 1971). Because the heavy thinnings were focused on *Phellinus* root rot pockets, these areas may have a significant legacy of coarse woody debris which could provide favorable habitat for *R. vinicolor.* It should be noted, however, that *Phellinus* root-rot pockets were also present in control and light thinned plots, and these areas were included in sporocarp sampling. More research is necessary to test these hypotheses.

At 0.5 kg/ha. the control stands had considerably less standing crop biomass of hypogeous fungi than reported by other researchers. Old-growth and natural mature Douglas-fir forests in the Oregon Cascades averaged 2.3-5.4 kg/ha of hypogeous fungi (Luoma 1991). Hunt and Trappe (1987) found 2.0-3.0 kg/ha hypogeous fungi in a 35- to 50-year-old Douglas-fir forest in western Oregon. These are substantially more than our study, even if winter samples are excluded. Our estimates are similar to those observed by North et al. (1997) in natural mature stands (averaged 0.78 kg/ha) in their nonexclosure plots. The carrying capacity of managed forests for mycophagous small mammals requires further study. This question is particularly interesting, as North et al. (1997) estimated that 60% of all truffle biomass produced in their young managed stands was consumed by mycophagists.

Species-area curves (Fig. 3) are often useful to visualize the species composition of the community and evaluate adequacy of the sample size. All our curves began to plateau rather quickly, except in the heavily thinned treatment, thereby suggesting that species heterogeneity was greatest in the VDT stands.

Food resources available during times of lowest abundance may be considered limiting to the animals that are active year round. Truffle abundance during the coldest parts of the winter in the PNW may limit northern flying squirrel densities. North et al. (1997) found that consumption of truffles in managed young stands is closest to the estimated standing crop during winter; that is, truffles were not limiting during times of peak production. In their study, evidence of

of failed attempts by mammals to enter exclosure plots were found only in those in place over winter.

Three genera accounted for the majority of available fungal food resource during winter at Fort Lewis. *Endogone lactiflua* was one of the more frequently encountered species during this study, but it did not account for a substantial portion of the biomass in the control or heavily thinned areas overall. This species and *E. pisiformis* were highest in frequency and standing crop biomass in the VDT stands. Luoma (1991) found *E. lactiflua* to be one of the more frequently encountered species but also concluded it did not meaningfully contribute to overall biomass. The majority of his collections were from mesic study sites. Many of the collections of *E. lactiflua* at Fort Lewis occurred during winter months, and many were found in or near heavily decayed coarse woody debris (W. Colgan. unpublished data).

Rhizopogon (along with *Trutcocolumella*) contributed most to standing crop biomass during the winter months. *Truncocolumella* is included with *Rhizopogon* because the two genera are closely related and of similar nutritive value for the small mammals (A.W. Claridge. unpublished data). Frequency of winter-fruiting species in this group did not change between the thinned stands and controls. North et al. (1997) suggested that these are some of the more "palatable" (most consumed) species in his study sites. Carey (1995) found *Rhizopogon* to be the most frequently encountered fungal spore type in feces from flying squirrels in the same stands in 1987. This group of truffles is the primary yearround dietary item for mycophagous small mammals at Fort Lewis (Colgan 1997). *Tuber* was the third genus to contribute to winter standing crop. Frequency of *Tuber monticola* did not change substantially in the thinned stands. *Tuber gibbosum* was found only once during this study, in a lightly thinned area.

Although standing crop of hypogeous fungi in VDT stands declined significantly compared with controls, diversity appeared to increase at least in the lightly thinned areas. Our data suggest that greater amounts of truffle may have been available in the VDT stands during times of lowest abundance, and some of the more nutritious truffles were most abundant in the VDT stands. Future studies are required to determine the full impact of VDT on truffle availability and usage by mycophagists during times of low abundance. Studies on small mammal densities and use of the truffle resources in thinned stands are underway. This multidisciplinary approach to ecosystem research will allow for a comprehensive evaluation of this ecosystem management technique.

Acknowledgments

We thank the more than 80 volunteers who contributed over 2000 h to sample truffle plots at Fort Lewis Military Reservation, often under adverse conditions. We give special thanks to volunteers from the Pacific Northwest Research Station, USDA Forest Service, The Evergreen State College, Oregon State University, the Native Plant Societies of Washington and Oregon. and the South Sound Mushroom Club. Funding was provided by the U.S. Army, Fort Lewis Military Reservation; the USDA National Research Initiative Competitive Grants Program (Grant No. 9401095*):* and the USDA Forest Service, Pacific Northwest Research Station. Monika Myrdail, Peter Molina, and Jim Eblin provided crucial data entry functions. Many thanks to Andrew Claridge, Doni McKay, Jane Smith, Mitch Kiel, Caitlin Cray, and Daniel Luoma, who contributed to this project in numerous ways. Special thanks to Dr. Steve Miller who share his thoughts on the effects of thinning on sporocarp production.

References

Aguileria, L.M., Griffiths, R.P. and Caldwell, B. 1993. Nitrogen in ectomycorrhizal mat and nonmat soil of different aged Douglas-fir forest. Soil Biol. Biochem. **25:** 1015-1019.

Amaranthus, M.P. Trappe, J.-M.. Bednar. L.. and Arthur, D. 1994. Hypogeous fungal production in mature Douglas-fir forest fragments and surrounding plantations and its relation to coarse woody debris and animal mycophagy. Can. J. For. Res. **24:** 2157-2165.

Blaschke, H.. and Baumler. W. 1989. Mycophagy and spore dispersal by small mammals in Bavarian forests. For. Ecol. Manage. **26:** 237-245.

Carey, A.B. 1995. Sciurids in managed and old growth forests in the Pacific Northwest. Ecol. Appl. 5: 648-661.

Carey, A.B., and Johnson, M.L. 1995. Small mammals in managed, naturally young, and old-growth forests. Ecol. Appl. **5:** 336-352.

Carey, A.B., Horton. S.P., and Biswell, B.L. 1992. Northern spotted owls: influence of prey base and landscape character. Ecol. Monogr. **62:** 223-250.

Carey, A.B., Kershner, J., Biswell, B., and De Toledo, L.D. 1999*a*. Ecological scale and forest development: squirrels, dietary fungi, and vascular plants in managed and unmanaged forests. Wildl. Monogr. **142:** 1-71.

Carey, A.B., Thyseil, D.R, and Brodie, A.W. 1999*b*. The forest ecosystem study: background, rationale implementation, baseline conditions, and silvicultural assessment. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-457.

Claridge, A.W., and May, T.W. 1994. Mycophagy among Australian mammals. Aust. J. Ecol. **19:** 251-275.

Colgan. W., III. 1997. Diversity, productivity and mycophagy of hypogeous fungal sporocarps in a variably thinned Douglas-fir forest. Ph.D. thesis, Oregon State University, Corvallis.

Cromack, K., Jr., Sollins, P., Graustin. W., Spidel, K., Todd. A., Spycher, G. Li, C.Y., and Todd, R.L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hvsterangium crassum.* Soil Biol. Biochem. **11:** 463-468.

Cromack, K., Jr., Fichter, B.L., Moldenke, A.M., and Ingham, E.R. 1988. Interactions between soil animals and ectomycorrhizal fungal mats. Agric. Ecosyst. Environ. **24:** 161-168.

Curtis, R.O. 1982. A simple index of stand density for Douglas-fir. For. Sci. **28:** 92-94.

Fogel. R. 1976. Ecological studies of hypogeous fungi. II: Sporocarp phenology in a western Oregon Douglas-fir stand. Can. J. Bot. **54:** 1152-1162.

Fogel, R. 1988. Interactions among soil biota in coniferous ecosystems. Agric. Ecosyst. Environ. **24:** 69-85.

Fogel, R., and Trappe, J.M. 1978. Fungus consumption (mycophagy) by small animals. Northwest. Sci. **52:** 1-30.

Franklin, J.F., and Dyrness, C.T. 1973. Natural vegetation of Oregon and Washington. USDA For. Serv. Gen. Tech. Rep. PNW-8.

Griffiths, R.P., Caldwell, B., Cromack, K.. Jr., and Morita, R.Y. 1990. Douglas-fir forest soils colonized by ectomycorrhizal mats. I. Seasonal variation in nitrogen chemistry and nitrogen cycle transformation weights. Can. J. For. Res. **20:** 211-218.

Griffiths, R.P., Castellano, M.A., and Caldwell. B. 1991 Hyphal mats formed by two ectomycorrhizal fungi and their association with Douglas-fir seedlings: a case study. Plant Soil. **134:** 255-259.

Hunt, G.A., and Trappe, J.M. 1987. Seasonal hypogeous sporocarp production in a western Oregon Douglas-fir forest. Can. J. Bot. **65:** 438-445.

Ingham, E.R., and Molina, R. 1991. Interactions among mycorrhizal fungi. rhizosphere organisms. and plants. In Microbial mediation of plant-herbivore interactions. *Edited by* P. Barbosa., V.A. Kirsk, and C.G. Jones. John Wiley & Sons. Inc.. New York. pp. 169-197.

Launchbaugh, K.L., and Urness, P.J. 1992. Mushroom consumption (mycophagy) by North American cervids. Great Basin Nat. **52:** 321-327.

Li, C.Y. Maser, C., Maser, Z., and Caldwell, B. 1986. Role of three rodents in forest nitrogen fixation in western Oregon: another example of mammal - mycorrhizal fungus - tree mutualism. Great Basin Nat. **46:** 411-414.

Luoma, D.L. 1989. Biomass and community structure of sporocarps formed by hypogeous ectomycorrhizal fungi within selected forest habitats of the H.J. Andrews Experimental Forest. Oregon. Ph.D. thesis. Oregon State University. Corvallis.

Luoma, D.L. 1991. Annual changes in seasonal production of hypogeous sporocarps. *In* Wildlife and vegetation of unmanaged Douglas-fir forests. *Edited by* L.F. Ruggiero. K.B. Aubry, A.B. Carey. and M.H. Huff. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-285. pp. 83-89.

Luoma, D.L., Frenkel, R.E., and Trappe, J.M. 1991. Fruiting of hypogeous fungi in Oregon Douglas-fir forests: seasonal and habitat variation. Mycologia, **83:** 335-353.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.

Malajczuk, N., Trappe, J.M., and Molina, R. 1987. Interrelationships among some ectomycorrhizal trees, hypogeous fungi and small mammals: western Australian and northwestern American parallels. Aust. J. Ecol. **12:** 53-55.

Maser. C., Trappe. J.M., and Nussbaum. R.A. 1978. Fungal - small mammal interrelationships with emphasis on Oregon coniferous forests. Ecology. **59:** 799-809.

McIntire, P.W. 1984. Fungus consumption by the siskiyou chipmunk within a variously thinned forest. Ecology. **65:** 137-146

Molina, R., Massicotte, H.B., and Trappe, J.M. 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. *In* Mycorrhizal func- tioning: an integrative plant-fungal process. *Edited by* M. Allen. Chapman & Hall Inc., New York. pp. 357-423.

North, M.J., Trappe, J.M., and Franklin, J. 1997. Standing crop and animal consumption of fungal sporocarps in Pacific Northwest forests. Ecology. **78:** 1543-1554.

O'Dell, T.E., Luoma, D.L., and Molina, R.J. 1992. Ectomycorrhizal fungal communities in young, managed, and old-growth Douglas-fir stands. Northwest. Environ. J. **8:** 166-168.

Pilz. D., and Molina. R. 1996. Managing forest ecosystems to con- serve fungus diversity and sustain wild mushroom harvests. USDA For. Serv. Gen. Tech. Rep. PNW-371.

Trappe. J. 1962. Fungus associates of ectotrophic mycorrhizae. Bot. Rev. **28:** 538-606.

Trappe. J.M., and Maser. C. 1976. Germination of spores of *Glomus macrocarpus* (Endogonaceae) after passage through a rodent digestive tract. Mycologia. **67:** 433-436.

Trappe, J.M., and Maser, C. 1977. Ectomycorrhizal fungi: interactions of mushrooms and truffles with beasts and trees. *In* Mushrooms and man. an interdisciplinary approach to mycology. *Edited by* T. Walters. Linn-Benton Community College. Albany, Oreg. pp. 165-179.

Viro, P., and Sulkava, S. 1985. Food of the bank vole in northern Finnish spruce forests. Acta Theriol. **30:** 259-266.

Vogt. K.A., Edmonds, R.L.. and Grier, C.C. 1981. Biomass and nu- trient concentrations of sporocarps produced by mycorrhizal and decomposer fungi in *Abies amabilis* stands. Oecologia, **50:** 170-175.

Waters, J.R., and Zabel, C.J. 1995. Northern flying squirrel densities in fir forests of northeastern California. J. Wildl. Manage. **59:** 858-866.

Waters. J.R., McKelvey, K.S., Zabel, C.J., and Oliver, W.W. 1994. The effect of thinning and broadcast burning on sporocarp production of hypogeous fungi. Can. J. For. Res. **24:** 1516-1522.

Zak, B. 1971. Characterization and classification of mycorrhizae of Douglas-fir. II. *Pseudotsuga menziesii* + *Rhizopogon vinicolor*. Can. J. Bot. **49:** 1079-1084.