



# Truffle abundance and mycophagy by northern flying squirrels in eastern Washington forests

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## Abstract

Although much is known about truffle abundance and rodent mycophagy in mesic Douglas-fir forests in the Pacific Northwest, few data are available for dry interior montane forests dominated by ponderosa pine (*Pinus ponderosa*), Douglas-fir (*Pseudotsuga menziesii*), and grand fir (*Abies grandis*). Our objective was to quantify the relationship between the abundance and diversity of ectomycorrhizal fungal sporocarps in the soil and in the diets of northern flying squirrels (*Glaucomys sabrinus*) in low-elevation forests of the eastern Washington Cascades. We randomly sampled four stands each of three cover types: dry open ponderosa pine, mesic young mixed-conifer forest, and mesic mature mixed-conifer forest. We sampled the soil for hypogeous sporocarps during the spring of 1999 and 2000. We collected fecal pellets from 318 flying squirrels live-trapped during the fall of 1997–2000. We sampled 2400 m<sup>2</sup> of soil surface and found truffles in 40% of 600 plots. Total biomass collected was 609 g. Spring truffle biomass on a kg/ha basis averaged 1.72 in open pine, 3.56 in young, and 4.11 in mature forest. Twenty-two species were collected across all cover types, with all but three species belonging to the Basidiomycotina. Eleven dominant species accounted for 91–94% of truffle biomass in each cover type. Four dominant species accounted for 60–70% of spring truffle biomass: *Gautieria monticola*, *Hysterangium coriaceum*, *Rhizopogon parksii*, and *R. vinicolor*. Truffle assemblages, richness and total biomass differed among cover types: richness and biomass were highest in young and mature mixed-conifer forest, and lowest in open ponderosa pine forest. Fall squirrel diets were composed of 23 genera or groups of fungi, plus about 22% plant material. *Rhizopogon* was the most abundant genus in the diet, followed by plant material, then *Gautieria*, *Leucogaster*, *Alpova*, and *Hysterangium*. Diets in different cover types were similar in the composition, richness, evenness, and the ratio of fungus to plant material. Diet richness varied over the study period. Nineteen truffle genera were detected in fall fecal samples versus 12 in spring soil samples. Management of low-elevation dry forest to maintain or restore stable fire regimes might reduce truffle diversity at stand scales by simplifying stand composition and structure; but, such management might increase long-term beta and landscape truffle diversity and persistence by reducing the occurrence of high-intensity fires and stabilizing inherent fire disturbance regimes.

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## 1. Introduction

Arboreal rodents are important links in the maintenance of ecosystem productivity through their

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consumption of the sporocarps of sequestrate ectomycorrhizal fungi (EMF) and consequent dispersal of fungal spores and nitrogen-fixing bacteria (Fogel and Trappe, 1978; Maser et al., 1978; Li et al., 1986; Carey, 1995). Fungi are the primary food of northern flying squirrels (*Glaucomys sabrinus*) and are important in the diet of Douglas' squirrels (*Tamiasciurus douglasii*), chipmunks (*Tamias* spp.), and other small mammals (Fogel and Trappe, 1978; McIntire, 1984; Maser et al., 1986; Carey et al., 1999)—all important prey for avian and mammalian forest carnivores (Carey, 1993; Carey and Johnson, 1995). EMF form symbiotic relationships with trees and other vegetation in which trees supply carbon from photosynthesis to the fungi, and fungi facilitate uptake of essential minerals and nutrients from the soil by tree roots. This relationship is essential for survival and growth of most coniferous forest trees, and other shrubs and herbaceous vegetation (Fogel and Trappe, 1978). Sequestrate fungi that produce hypogeous, or below-ground, sporocarps (truffles) rely on rodents, among other vertebrates, to disperse spores through consumption and defecation of viable spores (Fogel and Trappe, 1978; Maser and Maser, 1988). Spores dispersed in this fashion germinate to form new fungal mycelia, or anastomose with existing thalli, thus colonizing new areas or potentially increasing the genetic diversity of existing fungi (Fogel and Trappe, 1978).

Much is known about EMF diversity (Fogel and Trappe, 1978; Luoma et al., 1991; North et al., 1997; Colgan et al., 1999; Smith et al., 2002) and rodent mycophagy (Fogel and Trappe, 1978; Maser et al., 1986; Carey, 1995; Carey et al., 1999) in wet Douglas-fir forests in the Pacific Northwest (PNW) west of the Cascade Range crest. Others have studied relatively mesic mixed-conifer fir forests in the interior West (Waters et al., 1997; Rosentreter et al., 1997; Currah et al., 2000; Pyare and Longland, 2001); but, there is scant data for dry low-elevation forests dominated by ponderosa pine (*Pinus ponderosa*), dry Douglas-fir (*Pseudotsuga menziesii*), or dry grand fir (*Abies grandis*) plant associations outside of Arizona (States and Gaud, 1997).

The goal of this study was to quantify truffle diversity, abundance, and consumption by northern flying squirrels as potential correlates to arboreal rodent abundance (Waters and Zabel, 1995; Carey, 1995) in three low-elevation forest cover types in

eastern Washington. Cover types represented a typical complex gradient of composition, structural complexity, temperature, and moisture. The cover types were: (1) dry open ponderosa pine forest with low stand complexity; (2) relatively more mesic, young, mixed ponderosa pine, Douglas-fir, and grand fir (hereafter, mixed-conifer) forests with moderate complexity; and (3) yet more mesic, mature, closed-canopy mixed-conifer forest with high stand complexity. These types had been identified and mapped for a study of resource selection by the northern spotted owl (*Strix occidentalis caurina*) in the same watershed (E. Forsman, US Forest Service, Corvallis, Oregon, unpublished data).

We hypothesized that truffle diversity and abundance in the soil would increase from the open pine to the mature, closed-canopy, mixed-conifer stands. Truffle richness, similarity, and abundance is lower in Arizona ponderosa pine forests compared to mesic Douglas-fir forests in the Pacific Northwest west of the Cascade Range crest (States and Gaud, 1997). We expected the effects of that western regional temperature/moisture gradient on EMF to be similar at the smaller watershed scale in our study. Changing overstory composition and increasing canopy cover, tree density, and woody debris are thought to affect differences in EMF among cover types. As host tree species change in dominance among the types, associated EMF assemblages should also differ (Molina et al., 1992; Carey, 1995; States and Gaud, 1997; Massicotte et al., 1999). Moreover, EMF species richness and abundance increase with canopy cover (Amaranthus et al., 1994; States and Gaud, 1997), stand density in general (North et al., 1997; Colgan et al., 1999), or with increasing amounts of litter and woody debris (Amaranthus et al., 1994; Clarkson and Mills, 1994; North and Greenberg, 1998).

We tested two hypotheses about the fall diets of northern flying squirrels. First, we hypothesized that richness, dominance, and composition of truffles in fall diets among cover types would match general patterns of availability (Carey et al., 2002; Maser et al., 1986), i.e., increase in diversity from open pine to mature mixed-conifer forest. A second hypothesis was that the relative abundance of plant material will show an inverse relationship to the abundance of truffles in diets. As truffle abundance and diversity increase from dry to mesic forest, squirrels will diversify diets and eat less plant material (Carey et al., 1999, 2002).

## 2. Methods

### 2.1. Study area

The study area was in the Swauk Creek drainage of the Cle Elum Ranger District, Wenatchee National Forest, in the east-central Washington Cascade Range. This area is an important ecotone between the dry interior and wet coastal zones, and a center of high biodiversity (Marcot et al., 1998). Forest structure in this area has been extensively altered since European settlement, primarily by fire suppression (Everett et al., 1997) and selective harvest of the largest trees beginning about 1940 (S. Madden, Wenatchee National Forest, Cle Elum, Washington, unpublished data). As a result, most forest stands have an uneven age structure. Average yearly precipitation during the study period was 98 cm, mostly in the form of snow. Maximum snow depth averaged 184 cm. Melt-out of the snowpack occurred on average around 20 April. Summers are hot and dry. Mean maximum temperature during the summer was 34 °C. Study stands ranged in elevation from 900 to 1400 m.

Study stands occurred on montane forest sites characterized by varying dominance of ponderosa pine, Douglas-fir, and grand fir. We randomly selected four sample stands to represent three common cover types: open ponderosa pine, young mixed-conifer, and mature mixed-conifer. Stands were a minimum 15 ha and of largely regular shape to conform to square arboreal rodent trapping grids. Stand overstory and understory vegetation composition and structure were measured from 16, 0.02 ha plots using methods described by Everett et al. (1997).

Open pine stands had canopy closure of 30–45% (by spherical densiometer) and were dominated by scattered medium to large ( $\geq 40$  cm dbh) ponderosa pine (Table 1). A patchy mid-story of smaller ( $< 40$  cm dbh) Douglas-fir and grand fir was often present in mesic microsites, such as draws. Tree density averaged 1200 trees per hectare (tph), of which 10% were medium to large trees. Trees averaged 62-year-old; but, large trees averaged 140-year-old and the oldest trees in a stand ranged from 100 to 348-year-old. Selective logging of mostly large trees occurred during the 1940s, 1960s, and 1980s in most stands. Open pine stands were on warm-dry southerly aspects from 180 to 240°. Plant associations were mostly in

the *Pseudotsuga menziesii* series, predominantly the dry *Pseudotsuga menziesii*/*Calamagrostis rubescens* plant association (Lillybridge et al., 1995).

Young, mixed-conifer stands had moderate canopy closure of 50–70%, with a relatively high density (2595 tph) of trees, most (96%) of which were  $< 40$  cm dbh (Table 1). Dominant tree species were grand fir and Douglas-fir, with a few ponderosa pine. The average tree age was 65 years, and large trees averaged 120 years. The structure of these sites was largely created by three to four entries for selective, and later shelterwood, logging of mostly large trees around 1940, 1960, 1979, and 1983. Plant associations were mostly in the *Abies grandis* series, with *Abies grandis*/*Berberis nervosa*/*Calamagrostis rubescens* the dominant plant association.

Mature, mixed-conifer stands had closed canopies (70–83%) dominated by a mixture of medium to large grand fir and Douglas-fir  $> 40$  cm dbh, with some scattered large ponderosa pine ( $> 64$  cm dbh), and a well-developed mid-story and understory of grand fir and Douglas-fir (Table 1). Tree density (2206 tph) was only slightly less than young-mixed stands, but there were more large trees in mature stands (12%) than in young (4%) or open pine (10%) stands. These sites had no record of being logged since the late 1930s, and were considered to be “old growth” and prime spotted owl habitat (E. Forsman, US Forest Service, Corvallis, Oregon, personal communication). The average tree age was 100 years; but the largest trees averaged 177-year-old with maximum age 180–325 years. The *Abies grandis*/*Achlys triphylla* plant association dominated most sites.

### 2.2. Truffles

Ideally, fall availability of truffles in the soil would be estimated to compare with occurrence in squirrel diets during the same period. However, fall truffle sampling is notoriously difficult in dry interior forests where fall rain that stimulates truffle production (Fogel, 1976; Luoma et al., 1991) is unpredictable and “snow on dust” conditions often preclude fall sampling (D.L. Luoma, Oregon State University, personal communication). That was the situation with this study. Our initial attempt at sampling during fall 1998 failed because of dry conditions through early November when freezing weather was regularly encountered.

Table 1

Environmental attributes of sample stands, and spring diversity or standing biomass of truffles in the soil and in diets of northern flying squirrels for three forest cover types in the eastern Washington Cascades during 1999–2000

	Cover type		
	Open pine	Young mixed-conifer	Mature mixed-conifer
Environmental attributes			
Elevation (m)	1115	1092	1207
Aspect code <sup>a</sup>	3.8	2.5	1.5
Slope (%)	36	30	39
Canopy closure (%)	40	60	75
Basal area (m <sup>2</sup> /ha)	20	24	32
Age largest trees <sup>b</sup>	129	112	156
Coarse woody debris (% cover)	7	13	21
Soft large debris <sup>c</sup> (% cover)	1.2	0.9	3.0
<i>Pinus ponderosa</i> density (trees/ha)	343	164	86
<i>Pseudotsuga menziesii</i> density (trees/ha)	453	319	170
<i>Abies grandis</i> density (trees/ha)	465	1765	1565
Truffles in soil			
Richness ( <i>N</i> species)	7.3 A <sup>d</sup>	9.3 AB	10.8 B
Dominance <sup>e</sup>	0.34 A	0.35 A	0.37 A
Truffle biomass (kg/ha)	1.716 A	3.558 B	4.109 B
Squirrel diets			
Richness ( <i>N</i> items)	7.4 A	8.4 A	8.6 A
Dominance	0.24 A	0.22 A	0.21 A
Fungal/plant material ratio <sup>f</sup>	4.10 A	4.40 A	5.75 A

Sample size  $n = 4$  stands in each cover type.

<sup>a</sup> Aspect code ranges from 1 (cool, moist) to 4 (hot, dry): 1 = north (315–45°); 2 = east (45–135°); 3 = west (225–315°); 4 = south (135–225°).

<sup>b</sup> Largest trees were  $\geq 40$  cm dbh.

<sup>c</sup> Down wood >23 cm diameter in soft decay classes three to five.

<sup>d</sup> Means followed by the same letter are not significantly different ( $P \leq 0.10$ ) by Tukey HSD multiple-comparison test.

<sup>e</sup> Berger–Parker dominance index.

<sup>f</sup> Fungal/plant material ratio: ( $\Sigma$ fungal relative frequencies)/plant material relative frequency.

Spring truffle abundance was reliably assessed during 1999 and 2000 and was used to compare the composition, richness, and biomass of truffles among the three forest cover types, and to compare with flying squirrel fall diet richness among cover types. Comparing spring availability of truffles with fall squirrel diets was not ideal because of seasonal differences in truffle species phenology; but, we focused on differences in diet richness and occurrence of plant material, not species composition, among cover types. We assumed that relative differences in truffle richness and biomass among cover types would not change between seasons (Smith et al., 2002) even though composition might change. Sampling began at the lowest elevation sites each year about 7 weeks after complete snowmelt (6

July 1999 and 15 June 2000) and lasted for 2–3 weeks each spring.

We sampled soils for truffles using the same size and number of plots as the methodology of Luoma et al. (1991), which has been adopted by others for comparative studies (North et al., 1997; Waters et al., 1997; Smith et al., 2002). We systematically located 25 4 m<sup>2</sup> circular plots 40 m apart on an 8 × 8 or 7 × 9 grid used for trapping flying squirrels. We avoided sampling in the same place during consecutive years (1998, 1999, 2000) by shifting plot centers 4 m along the grid line. We raked the soil to 5–10 cm depth to expose truffles in the upper soil layer. We recorded the number of truffles from each “species collection” (a group of truffles of the same species that formed a

cluster with a radius <10 cm), color, and odor. Data from all plots in a stand were pooled as subsamples for a “stand sample” estimate (Luoma et al., 1991), rather than a single subsample plot basis; thus, sample sizes were  $n = 4$  for each of the three cover types. Species biomass was expressed as the mean kg/ha collected during 1999 and 2000. “Dominant” species contributed  $\geq 5\%$  of the total biomass within a cover type (Luoma et al., 1991).

We examined fresh specimens microscopically for diagnostic features of the peridium, gleba, and spores. Microscopic slides were prepared using phloxine to stain cytoplasm, Congo Red to stain cell walls, 3% potassium hydroxide to reinflate collapsed tissues, and Melzer’s solution to test for amyloid (blue–black) and dextrinoid (reddish brown) reactions. We used keys by Hosford (1972), Smith and Smith (1973), Smith and Zeller (1966), and Castellano (1988) to identify taxa. When possible, collections were compared to voucher specimens in the Herbarium at Central Washington University. All identified collections were dried at 105 °C, then weighed to the nearest 0.01 g. Each dried specimen was permanently stored in the Herbarium at Central Washington University, Ellensburg, Washington, USA.

### 2.3. Squirrel diets

We collected fecal pellets from 318 flying squirrels live-trapped during 1997–2000 during 2-week periods from late September to mid-October 1997–2000. From 66 to 96 samples were taken each year, and each cover type was represented by 20–32 samples/year. We collected two to five pellets from each newly captured squirrel, and from squirrels recaptured the first day of the second week of trapping under the rationale that trap bait would have been purged over the weekend when traps were closed (Cork and Kenagy, 1989; Claridge et al., 1999). We collected pellets from the anus of the animal or from a clean uncontaminated surface (e.g., data sheet) where they may have been deposited during handling. We placed pellets in a small plastic or glass vial, labeled, and stored them with the addition of several drops of 50% ethyl alcohol.

We identified fungus spores and plant material using methods described by Cazares et al. (1999), which is an adaptation of method 2 by Colgan et al.

(1997). We pooled samples from individual squirrels in a stand for efficient analysis. Each pooled sample was prepared by randomly selecting four pellets each from a random sample of up to four individual squirrel samples collected in a stand. We prepared two pooled samples for each combination of year (4 years) and stand (12 stands) to yield 96 pooled samples.

We prepared one slide (22 mm  $\times$  40 mm) from each pooled sample according to methods described by Colgan et al. (1997). Each slide was read at 400 $\times$  magnification with a compound microscope in three horizontal lines of 25 fields for a total of 75 fields per slide. Two pooled samples per year  $\times$  stand combination yielded 150 fields per combination. For each field, we recorded the presence of fungal spores to the lowest taxonomic level possible, most often the genus or family, using the key of Castellano et al. (1989). Spores of *Truncocolumella*, an important fall-fruited genus in some Douglas-fir forests (Luoma et al., 1991; Smith et al., 2002), could not be distinguished from *Rhizopogon* in spore samples (Cazares et al., 1999). Plant material (seeds, cell walls, pollen, etc.) also was recorded.

### 2.4. Data analysis

We calculated frequency of diet items as the percentage occurrence in the 75 fields per pooled sample (slide). Percent relative frequency of diet items was calculated as the (frequency/ $\Sigma$ fungal frequencies) per pooled sample (slide). For each stand  $\times$  year combination, we used the mean of the frequencies and relative frequencies from the two pooled samples (slides) in all analyses. We calculated the richness and dominance (Berger–Parker index) of both truffle collections and diets. We calculated the ratio of fungus to plant material in squirrel diets as ( $\Sigma$ fungal species relative frequencies/relative frequency of plant material). Species data for the two years of soil fungal sampling were pooled for each study stand, i.e., no annual effects were analyzed.

We did both univariate analysis of diversity indices and biomass, and multivariate analyses of species composition to evaluate cover types differences and identify indicator species. Species composition, as measured by frequency and abundance, is more often a better measure of community differences than

simple measures of diversity based on counts of species, such as richness and dominance, which are highly variable and difficult to consistently measure within cover types (McCune et al., 1997; McCune, 2000; Peterson and McCune, 2001).

First, we used one-way ANOVA to assess cover type effects on truffle diversity (richness, dominance) and total biomass in the soil from the pooled 2-year dataset. For diets, we used repeated measures ANCOVA to test annual and cover type differences in diet species richness with the number of squirrels in the sample as the covariate. We did repeated measures ANOVA to analyze year and cover type effects on diet species dominance and the fungal/plant material ratio. Exploratory analysis indicated log transformation to meet distributional assumptions for ANOVA only for the fungal/plant material ratio. In addition, the fungal/plant material ratio for one stand (“SV”) during 1997 was a far outlier ( $\sim 12\times$  larger than the overall mean of other values). We minimized the influence of that outlier by changing that value to be one unit larger than the next largest value (Tabachnick and Fidell, 1996). We used Tukey’s HSD test to evaluate multiple comparisons of cover types or annual means. Rank order agreement between truffle genera in the soil and diet was tested with Kendall’s *W*. We used Pearson correlation to examine the associations between characteristics of individual stands and species richness, dominance, and total biomass in soil or diet samples.

Second, we examined compositional similarity among cover types and identified truffle indicator species for both soil and diet samples with two non-parametric multivariate analyses of community structure, as implemented in PC-ORD software (McCune and Mefford, 1999). We analyzed relative frequencies averaged over the entire study period, rather than for each year. We first used multi-response permutation procedures (MRPP) to test the hypothesis of no difference in species composition between cover types based on species frequency and biomass (Zimmerman et al., 1985; Biondini et al., 1988). An *A* statistic measured the grouping “effect size”, or distinctiveness of groups, on a scale of 0–1. Values of *A* > 0.3 are considered fairly high. Monte Carlo permutations calculated probabilities for differences between types. We compared those probabilities with Bonferroni-adjusted *P*-values obtained

by dividing the experiment-wise  $P \leq 0.10$  threshold by three comparisons (see rationale below for type I error level).

We used indicator species analysis (ISA) analysis to identify indicator species, i.e., characteristic species found mostly in a single type and present in the majority of the sites belonging to that type (Dufrene and Legendre, 1997). ISA combined information on both species relative abundance and constancy to estimate indicator values for each species in each group. The maximum indicator value of an item within cover types was tested for statistical significance against the random expectation calculated by Monte Carlo permutation.

We accepted  $P \leq 0.10$  as the observed probability level for significant statistical tests. Although less conservative than  $P \leq 0.05$ , particularly with the relatively small sample size in this study ( $n = 12$  sites), we considered  $\alpha = 0.10$  to be an acceptable chance of type I error for ecological field studies that was well within the bounds of statistical convention and that also allowed for reduced type II error (Zar, 1999). A significant difference is implied where a difference among means is reported; but, we reported exact *P*-values in the text to allow readers to assess the probability of error relative to their own standard of significance (Zar, 1999).

### 3. Results

#### 3.1. Soil diversity and biomass

We sampled 2400 m<sup>2</sup> of soil surface over 2 years and found truffles in 40% of the 600 plots: 27% during 1999 and 53% during 2000. Among cover types, truffles were found in 25, 47, and 48% of the open pine, young, and mature stand subplots, respectively. Most (55%) of the collections were  $\leq 2$  g; one collection of *Gautieria monticola* weighed just over 30 g. Total biomass collected in 240 fungus-bearing plots was 609 g, with 77% collected during the spring of 2000.

Twenty-two species were collected across all cover types, with all but three species belonging to the Basidiomycotina (Table 2). Eleven dominant species accounted for 91–94% of spring truffle biomass in all cover types (Table 3). Four dominant species common

Table 2

Mean spring truffle biomass and indicator value of sequestrate fungal species in three forest cover types in the eastern Washington Cascades during 1999–2000

Name	Biomass (kg/ha)			Indicator value			P-value <sup>a</sup>
	Open pine	Young mixed-conifer	Mature mixed-conifer	Open pine	Young mixed-conifer	Mature mixed-conifer	
Basidiomycotina							
<i>Gautieria monticola</i>	<b>0.259</b>	<b>0.833</b>	<b>1.458</b>	4	34	60 <sup>b</sup>	0.04
<i>Hymenogaster nigrescens</i>	0.075			25	0	0	1.00
<i>Hymenogaster sublilacinus</i>	0.010	0.026	0.088	1	3	41	0.47
<i>Hysterangium coriaceum</i>	<b>0.271</b>	<b>0.801</b>	<b>0.581</b>	13	57	23	0.26
<i>Leucogaster rubescens</i>		<b>0.469</b>	<b>0.280</b>	0	60 <sup>c</sup>	40	0.13
<i>Leucophleps magnata</i>		0.080	0.160	0	21	72 <sup>b</sup>	0.09
<i>Leucophleps spinospora</i>			0.016	0	0	25	1.00
<i>Melanogaster tuberiformis</i>	<b>0.132</b>			50	0	0	0.27
<i>Rhizopogon cokerii</i>		0.010		0	50	0	0.28
<i>Rhizopogon evadens</i>		0.087	<b>0.342</b>	0	5	40	0.48
<i>Rhizopogon occidentalis</i>		0.122		0	25	0	1.00
<i>Rhizopogon pachyspora</i>	<b>0.160</b>			25	0	0	1.00
<i>Rhizopogon parksii</i>	<b>0.373</b>	<b>0.431</b>	<b>0.517</b>	30	32	39	0.87
<i>Rhizopogon</i> sp. (immature)	0.002	0.022	0.011	1	24	23	0.75
<i>Rhizopogon subcaerulescens</i>	<b>0.126</b>	<b>0.263</b>	0.115	24	9	16	0.86
<i>Rhizopogon villosulus</i>	0.076	<b>0.246</b>	0.081	14	11	15	1.00
<i>Rhizopogon vinicolor</i>	<b>0.498</b>	<b>0.355</b>	<b>0.439</b>	13	25	40	0.56
<i>Thaxterogaster pingue</i>			0.008	0	0	25	1.00
Ascomycotina							
<i>Geopora cooperii</i>	<b>0.137</b>	<b>0.209</b>	0.079	33	19	14	0.85
<i>Hydnotrya variiformis</i>	0.013	0.051	0.008	2	39	6	0.48
<i>Tuber</i> sp.		0.039	0.001	0	24	1	1.00
Zygomycotina							
<i>Glomus macrocarpus</i>		0.001		0	25	0	1.00
Total	2.149	4.044	4.273				

Values in bold typeface are dominant species contributing  $\geq 5\%$  of total biomass within a cover type.

<sup>a</sup> P-value calculated as the proportion of 1000 randomized trials with indicator value equal to or exceeding the observed indicator value.  $P = (1 + \text{number of runs} \geq \text{observed}) / (1 + \text{number of randomized runs})$ .

<sup>b</sup> “Strong” indicator value  $P \leq 0.10$ .

<sup>c</sup> “Weak” indicator value  $P \leq 0.25$ .

to each cover type accounted for 60–70% of the fungal biomass: *Gautieria monticola*, *Hysterangium coriaceum*, *Rhizopogon parksii*, and *R. vinicolor*. Another seven species were dominants in one or two cover types, accounting for an additional 21–32% of fungal biomass. The majority (6) of the 11 dominants were *Rhizopogon* species.

Spring truffle assemblages differed overall among the three cover types (MRPPA = 0.146,  $P = 0.012$ ). Mature and young forest had similar truffle assemblages ( $A = 0.004$ ,  $P = 0.531$ ); but, both differed from open pine ( $P \leq 0.028$ ), with differences

increasing from young ( $A = 0.106$ ) to mature conditions ( $A = 0.258$ ). Those compositional differences were supported by similar differences in species richness and total biomass (Table 1). Open pine forest had fewer species ( $\sim 7$ ) than mature forest ( $\sim 11$ ) ( $P = 0.035$ ), but neither differed statistically from the intermediate young forest type ( $\sim 9$  species) ( $P \geq 0.249$ ). Among individual stands, species richness increased with cover of coarse woody debris ( $r = 0.80$ ,  $P = 0.002$ ) and from warm/dry to cool/moist aspects ( $r = 0.74$ ,  $P = 0.006$ ) (Fig. 1).

Table 3

Percentage and rank of spring truffle biomass for dominant ( $\geq 5\%$  total biomass) sequestrate fungal species in three forest cover types in the eastern Washington Cascades during 1999–2000

Species	Open pine		Young mixed-conifer		Mature mixed-conifer	
	Percent biomass	Rank	Percent biomass	Rank	Percent biomass	Rank
<i>Gautieria monticola</i>	12.0	4	20.6	1	34.1	1
<i>Hysterangium coriaceum</i>	12.6	3	19.8	2	13.6	2
<i>Rhizopogon parksii</i>	17.3	2	10.7	4	12.1	3
<i>Rhizopogon vinicolor</i>	23.2	1	8.8	5	10.3	4
<i>Leucogaster rubescens</i>	– <sup>a</sup>	–	11.6	3	6.6	6
<i>Rhizopogon evadens</i>	–	–	2.2	* <sup>b</sup>	8.0	5
<i>Rhizopogon pachyspora</i>	7.4	5	–	–	–	–
<i>Geopora cooperii</i>	6.4	6	5.2	8	1.8	*
<i>Melanogaster tuberiformis</i>	6.1	7	–	–	–	–
<i>Rhizopogon subcaerulescens</i>	5.9	8	6.5	6	2.7	*
<i>Rhizopogon villosulus</i>	3.5	*	6.1	7	1.9	*

Species are listed in order of overall rank importance across all cover types.

<sup>a</sup> Not collected in cover type.

<sup>b</sup> No dominance rank given. Collected in cover type, but not a dominant species.

Truffle biomass in open pine forest (1.72 kg/ha) was 50% of both young (3.56 kg/ha)  $P = 0.100$  and mature forest (4.11 kg/ha) ( $P = 0.037$ ), which were similar ( $P = 0.777$ ) (Table 1). As with richness in individual stands, fungal biomass increased with both cover of coarse woody debris ( $r = 0.85$ ,  $P = 0.001$ ) and with cool/moist aspects ( $r = 0.80$ ,  $P = 0.002$ ) (Fig. 1), but also with increasing canopy cover ( $r = 0.75$ ,  $P = 0.005$ ) (Fig. 2). Species dominance, or evenness, was similar among cover types ( $P = 0.910$ ), so it contributed little to cover type differences in truffle assemblages (Table 1).

Ranking EMF species by total truffle biomass revealed the basis of cover type differences. In open pine forest *R. vinicolor* and *R. parksii* were the most abundant species, relatively more so than in other cover types in terms of percentage of total biomass (Table 3). *H. coriaceum* and *G. monticola* followed in rank as dominant open pine species. Among the four remaining sub-dominant species in open pine forest, *R. pachyspora* and *Melanogaster tuberiformis* were collected only in open pine. No species had significant indicator value, i.e., was uniquely characteristic, in open pine forest (Table 2).

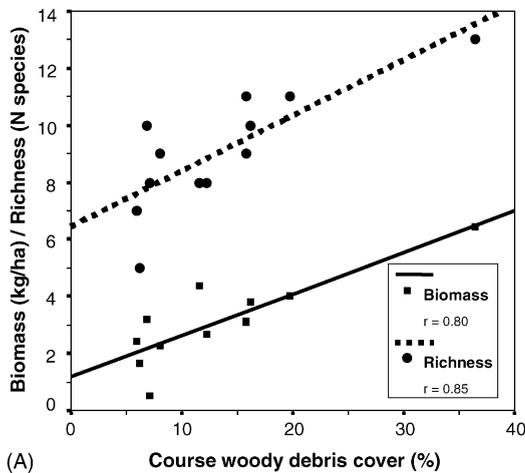
Young and mature forests were similar, but differed from open pine forest in having *G. monticola* and *H. coriaceum* the top ranked species for biomass (Table 3), followed by *R. parksii*, *R. vinicolor*, and

*Leucogaster rubescens*. *G. monticola* was the most abundant species collected overall, and was found in greatest biomass in mature stands where it was a strong indicator species (Table 2). Young forest was unique in having an exceptionally high biomass of *L. rubescens* (Table 3), a weak indicator species for young forest (Table 2) that was not detected in open pine forest. Although not a dominant species, *Leucophleps magnata* was a strong indicator, or uniquely characteristic, of mature forest.

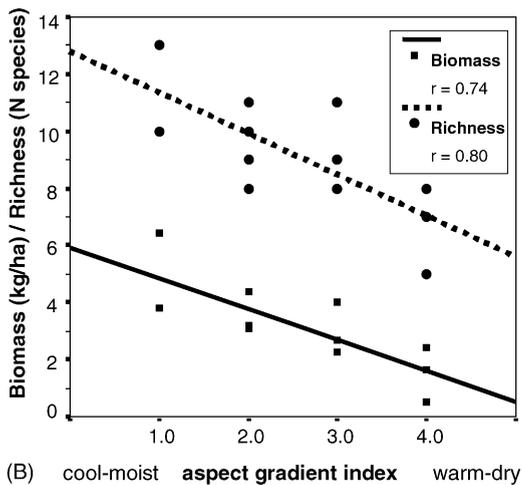
### 3.2. Squirrel diets

Squirrel diets were composed of 23 genera or groups of fungi, plus about 22% plant material (Table 4). *Rhizopogon* was the most abundant genus in the diet, followed by plant material, then *Gautieria*, *Leucogaster*, *Alpova*, and *Hysterangium*. Only two genera were strong indicators, or characteristic, of squirrel diets in particular cover types. *Gymnomyces* was unique and the fifth most abundant genus in diets of open pine forests. *Melanogaster* characterized diets in mature mixed forest stands, and was the eighth most abundant genus there.

Diets in different cover types were similar in the relative abundance and frequency of items (MRPP  $P = 0.262$ ). Likewise, diet diversity was similar among cover types in terms of richness ( $P = 0.568$ )



(A)



(B)

Fig. 1. Relationships between spring truffle richness and biomass in the soil and the cover of course woody debris (A) and along a temperature/moisture gradient indexed by aspect (B) for open ponderosa pine, young mixed-conifer, and mature mixed-conifer montane forest cover types in the eastern Washington Cascades during 1997–2000. Sample size is  $n = 4$  stands per cover type.

and dominance ( $P = 0.333$ ) of food items, and the ratio of fungal/plant material ( $P = 0.261$ ) (Fig. 3, Table 1). Among individual stands, diet richness and the ratio of fungal/plant material were uncorrelated with patterns of spring truffle richness ( $r \leq 0.26$ ,  $P \geq 0.407$ ) or biomass in soil ( $r \leq 0.48$ ,  $P \geq 0.113$ ). The number of diet items (richness) varied over time ( $P = 0.020$ ) (Fig. 3) from about 7–10 items. Diet richness increased from 1997 to 1998 ( $P = 0.054$ ), was similar during 1998 and 1999 ( $P = 0.110$ ), but

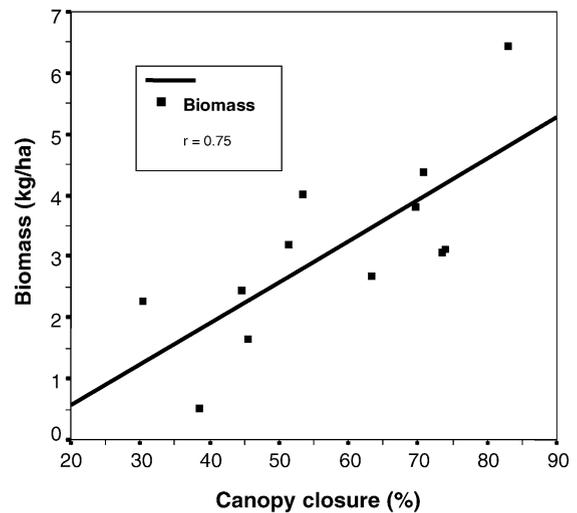


Fig. 2. Relationship between canopy closure and spring truffle biomass in the soil for open ponderosa pine, young mixed-conifer, and mature mixed-conifer montane forest cover types in the eastern Washington Cascades during 1997–2000. Sample size is  $n = 4$  stands per cover type.

then decreased the following year ( $P = 0.008$ ). Although richness varied over time, the evenness (dominance) of items or the ratio of fungal/plant material ( $P > 0.540$ ) did not vary from year to year (Fig. 3). Neither richness, dominance, nor the fungal/plant material ratio varied by cover type among years (cover  $\times$  year interactions  $P > 0.460$ ).

Nineteen truffle genera (not including four family-level taxa) were detected in fecal samples versus 12 in soil samples—a 58% difference (Table 5). Three genera—*Alpova*, *Gymnomyces*, and *Sedecula*—were relatively important in the fall fecal samples (29–50% frequency, 6–11% relative frequency), but absent in the spring soil samples. Four other relatively minor genera ( $\leq 3\%$  frequency,  $< 1\%$  relative frequency) in the fall fecal samples (*Genabea*, *Sclerocystis*, *Elaphomyces*, *Choiromyces*) were absent in the spring soil samples. Rank order of genera common to both soil and diet samples was highly similar (Kendall's  $W = 0.888$ ,  $P = 0.002$ ), but with some minor differences. *Thaxterogaster* ranked much higher in fecal versus soil samples, as did *Melanogaster* to a lesser extent. Relative abundance of *Geopora*, *Leucophleps*, and *Hydnotrya* ranked somewhat lower in fecal versus soil samples.

Table 4

Percentage relative and absolute frequencies, and indicator value of plant material and spores of sequestrate fungal genera in feces of northern flying squirrels during fall in montane forest cover types in the eastern Washington Cascades

Genus/group	Percentage relative frequency <sup>a</sup> (absolute frequency)				Indicator value <sup>b</sup>		
	Open pine	Young mixed-conifer	Mature mixed-conifer	Total	Open pine	Young mixed-conifer	Mature mixed-conifer
Plant material	21.65 (92)	20.28 (92)	16.71 (87)	19.54 (89)	37	35	28
Fungi							
<i>Alpova</i>	9.48 (49)	11.47 (50)	12.30 (50)	11.08 (50)	7	9	9
Ascomycete	0.00 (0)	0.27 (2)	2.23 (9)	1.74 (8)	0	1	48
Boletaceae	5.84 (27)	8.21 (36)	9.97 (45)	8.25 (37)	17	31	51
<i>Choirohmyces</i>	0.13 (1)	0.00 (0)	0.00 (0)	0.13 (1)	25	0	0
Cortinariaceae	9.00 (36)	6.51 (28)	5.15 (31)	6.87 (31)	44	31	18
<i>Elaphomyces</i>	0.25 (1)	0.00 (0)	0.00 (0)	0.25 (1)	25	0	0
<i>Gautieria</i>	17.17 (76)	16.37 (78)	15.75 (78)	16.43 (77)	35	33	32
<i>Genabea</i>	0.65 (3)	0.12 (1)	0.72 (3)	0.55 (3)	7	1	33
<i>Geopora</i>	3.58 (17)	3.10 (24)	3.55 (26)	3.41 (22)	21	4	20
<i>Glomus</i>	0.00 (0)	0.17 (1)	0.00 (0)	0.17 (1)	0	25	0
<i>Gymnomyces</i>	10.44 (43)	3.81 (19)	4.81 (27)	6.14 (29)	59 <sup>c</sup>	19	23
<i>Hydnotrya</i>	0.17 (1)	0.44 (3)	0.00 (0)	0.30 (2)	7	18	0
<i>Hymenogaster</i>	4.08 (21)	0.00 (0)	10.71 (65)	6.73 (39)	12	0	38
<i>Hysterangium</i>	11.77 (56)	8.97 (48)	11.68 (59)	10.74 (54)	38	27	35
<i>Leucogaster</i>	13.13 (61)	16.44 (79)	17.77 (85)	15.86 (75)	26	36	39
<i>Leucophleps</i>	0.00 (0)	3.16 (17)	6.72 (40)	4.34 (25)	0	46	27
<i>Melanogaster</i>	6.08 (28)	4.32 (29)	7.05 (40)	6.13 (35)	11	14	59 <sup>c</sup>
<i>Rhizopogon</i>	24.01 (100)	22.07 (100)	21.08 (100)	22.39 (100)	36	33	31
<i>Sclerocystis</i>	0.00 (0)	0.36 (1)	0.00 (0)	0.36 (1)	0	25	0
<i>Sedecula</i>	8.87 (40)	8.93 (51)	0.67 (3)	5.91 (30)	49	16	2
<i>Thaxterogaster</i>	10.21 (50)	6.94 (36)	5.93 (30)	7.44 (38)	18	32	11
<i>Tuber</i>	0.61 (3)	0.88 (5)	1.10 (5)	0.90 (4)	22	14	31
Zygomycotina	0.00	0.13 (1)	0.00 (0)	0.13	0	25	0

<sup>a</sup> Relative frequency: frequency/Σfrequencies.

<sup>b</sup> Indicator value is the combination of relative abundance in cover type vs. all plots, and the relative frequency within the cover type.

<sup>c</sup> Indicator for cover type  $P \leq 0.10$ .

## 4. Discussion

### 4.1. Truffle diversity and biomass in soil

Truffle diversity and abundance in the spring increased along the local cover type gradient as hypothesized, but only partly. Frequency of occurrence, composition, and biomass of EMF truffles in dry open pine forest differed from more mesic young and mature mixed-conifer types, which were found to be relatively similar. Composition of assemblages, diversity, and biomass of truffles in young stands appeared on average to be intermediate between open pine and mature stands; but, variation within young and mature cover types made it difficult to statistically

infer differences. Disassociating stands from cover type categories for regression analyses, however, did support the rationale underlying the hypothesis. We observed increasing truffle richness and biomass with several environmental and habitat factors: a temperature/moisture gradient that influenced microclimate (Amaranthus et al., 1994) and tree composition (Molina et al., 1992; States and Gaud, 1997; Massicotte et al., 1999), and habitat factors of canopy cover (North et al., 1997; States and Gaud, 1997; Colgan et al., 1999) and woody debris cover (Amaranthus et al., 1994; Clarkson and Mills, 1994; North and Greenberg, 1998). We suspect the observed cover type differences and relationships between richness, biomass, and habitat attributes during the spring might

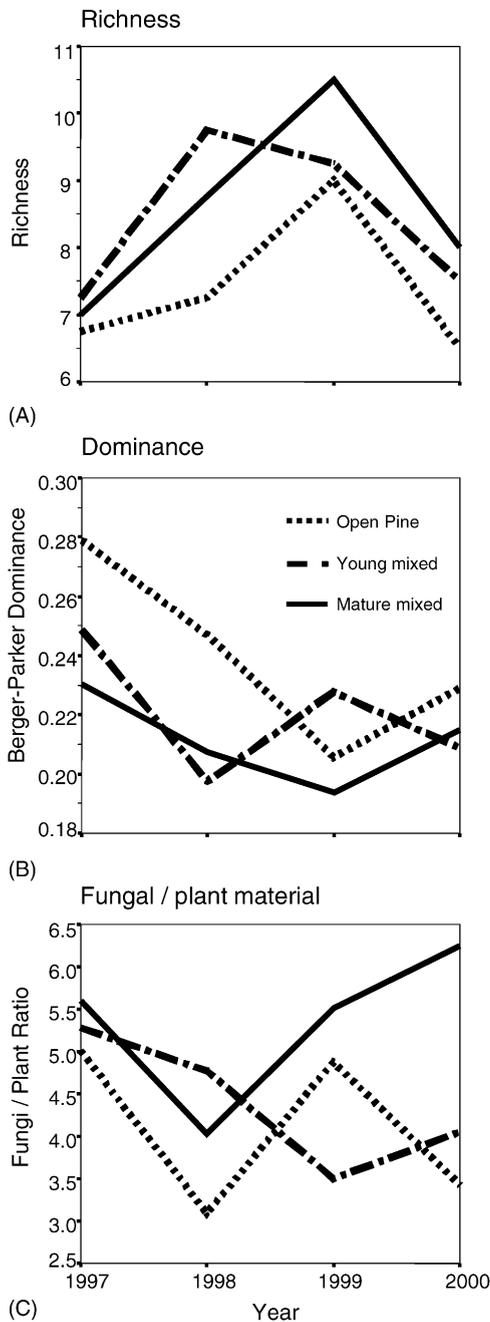


Fig. 3. Mean fall richness (A) and dominance (B) of truffle genera and families; and (C) the ratio of fungal/plant material in feces of northern flying squirrels in three cover types of montane forest in the eastern Washington Cascades during 1997–2000. Richness differed among years ( $P = 0.031$ ,  $1997 < 1998 = 1999 > 2000$ ); but, not dominance or fungal/plant material ( $P > 0.54$ ).

Table 5

Rank order differences in truffle abundance in spring soil samples and fall fecal samples of northern flying squirrels in montane dry forest of the eastern Washington Cascade Range

Genera	Feces rank <sup>a</sup>	Soil rank <sup>b</sup>
<i>Rhizopogon</i>	1	1
<i>Gautieria</i>	2	2
<i>Leucogaster</i>	3	4
<i>Alpova</i>	nr <sup>c</sup>	Absent
<i>Hysterangium</i>	4	3
<i>Thaxterogaster</i>	5	11
<i>Gymnomyces</i>	nr	Absent
<i>Sedecula</i>	nr	Absent
<i>Melanogaster</i>	6	8
<i>Hymenogaster</i>	7	7
<i>Geopora</i>	8	5
<i>Leucophleps</i>	9	6
<i>Tuber</i>	10	10
<i>Genabea</i>	nr	Absent
<i>Hydnotrya</i>	11	9
<i>Sclerocystis</i>	nr	Absent
<i>Elaphomyces</i>	nr	Absent
<i>Glomus</i>	12	12
<i>Choironomyces</i>	nr	Absent

Rank order was highly similar between sets (Kendall's  $W = 0.888$ ,  $P = 0.002$ ).

<sup>a</sup> Rank based on relative frequency in fecal samples (this study).

<sup>b</sup> Soil rank based on biomass sampled by Gould (2002).

<sup>c</sup> Not ranked for comparison with soil sample—found only in fecal samples.

be even stronger during the relatively dryer late summer and fall when moisture stress may limit production.

Spring truffle assemblages, dominant species, and biomass in sampled eastern Cascades forests, especially in young and mature stands, were similar to those in more mesic Douglas-fir and western hemlock forest west of the Cascades. *Gautieria monticola*, *Hysterangium coriaceum*, *Rhizopogon parksii*, *R. vinicolor*, *Leucogaster rubescens*, and *Rhizopogon* species in general are dominant in Douglas-fir and grand fir forests on both sides of the Cascade Range (Luoma et al., 1991; North et al., 1997; Colgan et al., 1999; Smith et al., 2002; this study). The relative importance of *Melanogaster tuberiformis* in relatively dry open canopy sites (Carey et al., 2002; North et al., 1997; Colgan et al., 1999) was confirmed in this study. The apparent insignificance of *Elaphomyces granulatus* in the eastern Cascades was an important differ-

ence from mesic western Cascades forests where some studies have shown it a dominant species (Luoma et al., 1991; North et al., 1997; Smith et al., 2002). *Rhizopogon parksii* was a dominant species in the spring soil samples, whereas it typically has been considered a strong fall-fruiting species west of the Cascades (Luoma et al., 1991; Smith et al., 2002). Either the eastside phenology differs, or fall truffles persist during cold winters (D.L. Luoma, Oregon State University, Corvallis, Oregon, personal communication).

Our spring-only sampling provided a minimal list of east Cascades truffles: more spring samples likely would increase the species list (Colgan et al., 1999) as would summer and fall sampling. However, if spring is the primary season for truffle production because of dry conditions during summer and fall, then our results probably represent the dominant pattern of truffle species occurrence and abundance in these forest types. In lieu of fall soil sampling, addition of seven EMF genera found in fall squirrel fecal samples, but not in spring soil samples, augmented the list of known genera in eastern Cascades forests. The fecal sample also might provide a general picture of relative fall abundance of genera, if flying squirrels generally ate what was available during the fall in proportion to its abundance (i.e., little selection) (Carey et al., 2002), or they did not eat spring truffles that were cached or residual in the soil.

There is some cautionary evidence of truffle selection by squirrels or truffle carryover into summer and fall, both of which would limit use of diet information to infer truffle availability. Zabel and Waters (1997) found weak selection among three truffle genera (only one of three possible comparisons) in limited cafeteria trials with flying squirrels. North et al. (1997) noted apparent truffle selection in a field study, but concluded that the evidence was inconclusive. We did not observe flying squirrels caching truffles, and there is little evidence of that behavior from field studies (Wells-Gosling and Heaney, 1984; A.B. Carey, US Forest Service, Olympia, Washington, personal communication). However, flying squirrels are well known for raiding truffles from tree caches and ground middens of pine squirrels (*Tamiasciurus hudsonicus*, *T. douglasii*) (Mowrey and Zasada, 1984; Hall, 1991; Rosentreter et al., 1997), which occur (*T. douglasii*) in the study area. Truffle persistence in the soil from spring through summer into fall has been considered

implausible in mesic forest west of the Cascades (Luoma et al., 1991, but see North et al., 1997); but, it has been observed in dry forests of the eastern Cascades (D. Luoma, Oregon State University, Corvallis, Oregon, personal communication). The dominance in fall diet samples of several species (*G. monticola*, *H. coriaceum*, *M. tuberiformis*) that are predominantly spring producers in forests west of the Cascades (Luoma et al., 1991; Smith et al., 2002), as well as in this study, indicates either persistence of those truffles into fall, active searching and selection by squirrels of small fall crops, or a different phenology than found in mesic forest. Given the uncertainties, fall abundance from squirrel diets should be inferred with some caution.

Standing biomass of spring truffles in eastern Cascades forests was at the high end of ranges (0.23–5.4 kg/ha) reported for spring in mesic Douglas-fir and western hemlock (*Tsuga heterophylla*) forests west of the Cascade Range, and was comparable to drier true-fir forests in interior northern California (Table 6). Spring biomass values for the open ponderosa pine type (1.7 kg/ha) were similar to those found in fall post-monsoon ponderosa pine forest in northern Arizona (Table 6).

#### 4.2. Squirrel diets

Flying squirrel diets in our eastern Cascades forests were similar in diversity and species composition to squirrel diets in other parts of the Pacific Northwest. The 19 genera we detected in fecal samples, not including 4 taxa identified to family level, was at the high end of the range reported by Carey's (Carey et al., 2002) review of his extensive work: 11 taxa in western hemlock forests of the Olympic Peninsula, Washington; 16 taxa in Douglas-fir forests of the Puget Trough, Washington; and 27 taxa in Douglas-fir forests of southwestern Oregon. As in westside Cascades forests (Carey et al., 2002; Carey, 2000) *Rhizopogon*, *Gautieria*, *Leucogaster*, *Hysterangium*, *Melanogaster*, and *Hymenogaster* species dominated the diet. *Alpova* and *Thaxterogaster* were two other important genera in eastside Cascades diets, as they were in other dry forests in California (Hall, 1991; Waters et al., 1994; Pyare and Longland, 2001) and Washington (Rhoades, 1986). The 21–24% of plant material in east Cascades diets was at the high end of the 10–30% range reported

Table 6

Mean spring biomass of truffles in mesic Douglas-fir and western hemlock forests of western Washington and Oregon, and in dry interior ponderosa pine, Douglas-fir, or true fir forests of the interior Pacific Northwest and Arizona

Moisture regime	Region, state <sup>a</sup>	Forest type <sup>b</sup>	Biomass (kg/ha)	Source
Mesic, coastal	Olympic Peninsula and North Cascades, WA	TSHE	4.0–4.5	North et al. (1997)
	Puget Trough, WA	PSME	1.2–1.6 <sup>c</sup>	Colgan et al. (1999)
	Puget Trough, WA	PSME	1.8	Carey et al. (2002)
	Central Coast Range, OR	PSME	2.3–5.4	Fogel (1976)
	Central Coast Range, OR	PSME	2.0–3.2	Hunt and Trappe (1987)
	Central Cascades, OR	PSME	0.7–2.2	Luoma et al. (1991)
	Central Cascades, OR	PSME	0.82–4.9	Smith et al. (2002)
	Southern Cascades, OR	PSME	0.23–0.48	Cazares et al. (1999)
	Dry, interior	Eastern Cascades, WA	PIPO, PSME, ABGR	1.7–4.1
Southern Cascades, CA		ABCO, ABMA	1.3, 5.2 <sup>d</sup>	Waters et al. (1994)
Southern Cascades, CA		ABCO, ABMA	2.4	Waters et al. (1997)
Coconino Plateau, AZ		PIPO	0.64, 2.0 <sup>e</sup>	States and Gaud (1997)

Data are for spring season unless noted.

<sup>a</sup> WA: Washington; OR: Oregon; CA: California; AZ: Arizona.

<sup>b</sup> Dominant tree species. TSHE: *Tsuga heterophylla*; PSME: *Pseudotsuga menziesii*; PIPO: *Pinus ponderosa*; ABGR: *Abies grandis*; ABCO: *Abies concolor*; ABMA: *Abies magnifica*.

<sup>c</sup> Year-round mean.

<sup>d</sup> Summer (July) and fall (September) collections only.

<sup>e</sup> Spring (April–June) and post-monsoon fall (September–November) collections.

for the Pacific Northwest (Carey, 1995; Rosentreter et al., 1997; Carey et al., 1999).

We found no support for our hypotheses that diet diversity follows patterns of availability among cover types, nor that the consumption of plant material increases in sites with low truffle diversity (i.e., open pine forest). Compared to the spring truffle sample, we expected diets in open pine forest to be less rich and less even, and have more plant material than in either young or mature mixed-conifer forest. Mean diet attributes for cover types (Table 1) seemed to support those expectations: diet richness and the fungi/plant ratio were nominally lower and dominance higher in open stands than in young and mature stands. However, variation within cover types and among years was high such that average differences were not significant (Figs. 1 and 3). Power of those tests to detect a significant difference was low (0.14–0.20); and power analysis indicated that about 100 stand samples for each cover type would be needed to have power  $\geq 0.90$ . The multi-species MRPP analysis, however, did support the conclusion that diet composition was similar among cover types.

Several alternative explanations can be tendered as to why diets in open pine were similar to young and mature forest when they were expected to differ based on spring soil samples: (1) spring samples were not comparable, hence good indicators, of fall truffle diversity, and fall diversity was similar in all cover types, as reflected by diet samples; or (2) spring samples were unbiased estimators of fall truffle diversity among cover types, but fecal samples were biased by squirrel foraging behavior and sampling error; or (3) there is bias in both spring soil and fall diet samples; i.e., cover types have similar fall diversity, squirrel diets differ among types, and little can be said about truffle diversity in soil or diets with this study. The most likely explanation is the second one: spring samples are unbiased estimates of fall diversity and biomass among cover types, but fall diet samples are biased for cover types.

Spring truffle samples likely were unbiased estimators of relative fall diversity among cover types, even though species occurrence or abundances could vary between seasons. Smith et al. (2002) found similar patterns of relative richness and biomass among most forest age classes in western Oregon during spring and

fall, despite changes in truffle species abundances. If truffle diversity between open pine and both young and mature forests does vary between spring and fall, we would expect the observed pattern to hold and differences between cover types to be accentuated by the effects of prolonged summer drought, i.e., greater in dry open pine sites versus mesic mixed-conifer sites.

If patterns of spring truffle diversity and biomass among cover types were similar in the fall, then diet sampling bias associated with sample stand area and the range of squirrel foraging movements likely explains the lack of difference in squirrel diets among cover types. The minimum convex polygon summer home range of flying squirrels in open pine forest was 4.6 ha, compared to smaller home ranges of 2.5 ha in young and mature forest (J. Lehmkuhl, unpublished data). Hence, flying squirrels compensated for relatively low truffle richness and biomass in open pine forest by foraging over much larger areas that likely encompassed patches of young and mature forest with richer and more abundant truffle resources. Evidence for this can be seen in the primary association of preferred *Gautieria* and *Leucogaster* species (Carey et al., 2002; Zabel and Waters, 1997) with mature and young forest, respectively, in this study; whereas, both were well represented in the diets of squirrels in open pine, young, and mature forest. Carey et al. (2002) found similar anomalies between truffle availability and feeding as a result of wide-ranging foraging behavior. Also, squirrels perhaps were better samplers of open pine sites, being better able to find truffles than humans (Carey et al., 2002; Carey, 1995) and to better sample the within-stand patchiness of closed and open canopy sites than our sampling design, especially for important taxa like *Gautieria*, which typically have a very clumpy distribution (Carey et al., 2002; Luoma et al., 1991). Squirrel sampling intensity also has a temporal aspect with relatively long retention times of spores in squirrel guts. These foraging strategies undoubtedly contribute to their success in harvesting truffles during the dry summer and fall seasons when truffle production likely is relatively low and patchy, a condition not well sampled by current field techniques (J.E. Smith, US Forest Service, Corvallis, Oregon, personal communication; W. Colgan, Louisiana Tech University, Ruston, Louisiana, personal communication). Cache raiding also may be another foraging

strategy to overcome potentially low truffle abundance during the dry season and winter (Mowrey and Zasada, 1984; Hall, 1991; Rosentreter et al., 1997).

#### 4.3. Management implications

Currently, forest management in the dry forest zone represented by the study area is focused on conservation of habitat for the northern spotted owl, restoration of stable fire regimes, and on the maintenance of healthy dry forest ecosystems that have developed anomalously under nearly a century of fire exclusion (Okanogan and Wenatchee National Forests, 2000). Management in spotted owl habitat, represented by the mature mixed-conifer type in this study, is primarily conservatory with little active manipulation of forest structure. However, active management of dry forest types represented by the open pine and much of the young mixed-conifer types is being designed and implemented for restoration of healthy ponderosa pine ecosystems. Management objectives for dry forest are: (1) reduce stand density; (2) alter species composition for more fire-tolerant species; (3) reduce fuel loads to levels consistent with pre-settlement fire regimes; and (4) reduce susceptibility to insects and disease outside of endemic levels (Okanogan and Wenatchee National Forests, 2000). The primary management options for achieving those objectives are: (a) pre-commercial or commercial thinning; (b) pruning; (c) favoring insect/disease resistant tree species; or (d) prescribed fire.

The result is that managed open pine and young mixed-conifer stands might have fewer trees, have a less complex and more open canopy structure with some likely warming and drying effects on stand microclimate, and have greater dominance of ponderosa pine than Douglas-fir and grand fir. Our data suggest that shifting stand microclimate to relatively more xeric conditions will result in lower richness and biomass of truffles, but would favor species associated with dryer conditions. Our data and others (Amaranthus et al., 1994; Clarkson and Mills, 1994; North and Greenberg, 1998) also indicate that retention of the largest woody debris may ameliorate that effect by creating or maintaining relatively mesic microsites through shading or moisture retention without increasing hazardous fuels. Carey et al. (2002) recommends that  $\geq 15\%$  cover of coarse woody debris be retained in mesic Douglas-fir forests to maintain critical truffle

habitat. That recommendation seems well suited to our eastside forests where woody debris averaged 13–21% cover in young and mature stands, which had very high truffle biomass. Changing tree species composition to pine dominated stands likely would reduce the occurrence and biomass of species associated with Douglas-fir and grand fir (Molina et al., 1992; Carey, 1995; States and Gaud, 1997; Massicotte et al., 1999). Retaining some patchiness in stand density and species composition, as opposed to homogenous thinning, also may reduce the impact on truffle richness and abundance and consequent impacts on mycophagous rodents (Amaranthus et al., 1994; Carey et al., 2002). Such patchiness, however, may be difficult to sustain with a regular program of post-thinning prescribed fire, unless fires are frequently prescribed with consequent reduced fire intensities and increased patchiness of fire effects.

The potential negative stand-scale impacts of dry forest thinning for fire and fuel management on truffle diversity are traded off for improved long-term stability and persistence of low-elevation dry forest landscapes. Moreover, the potential loss of truffle diversity at stand-scales would be balanced by increased beta diversity across the landscape. Potential losses from silviculture or prescribed fire are trivial compared to the large-scale losses of forest that following 5000–60,000 ha stand replacement fires have occurred in the area during the last decade (Gaines et al., 1997). Moreover, dry forest silvicultural practices to restore or maintain stable fire regimes and fuel management also aim to restore single-story large ponderosa pine forests (Hann et al., 1997; Lehmkuhl et al., 1997; Hessburg et al., 1999) and associated wildlife (Lehmkuhl et al., 1994; Wisdom et al., 2000) that have declined during the last century.

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