Spring 2015

Truffle abundance and mycophagy of small mammals in northern New England forests

Tyler J. Remick
tji72@wildcats.unh.edu

Truffle abundance and mycophagy of small mammals in northern New England forests

Keywords
truffles, small mammals, mycophagy, ectomycorrhizal, New England

Truffle abundance and mycophagy of small mammals in northern New England forests

Honors Thesis
2015

By Tyler Remick
Wildlife Conservation Biology

Advisor Dr. Rebecca Rowe
Natural Resources and the Environment

ABSTRACT

Truffles are the fruiting bodies of ectomycorrhizal fungi which are important for the uptake of water and nitrogen in tree roots. Since these fruiting bodies form within the soil they rely upon small mammals to uncover, ingest, and disperse their spores, and in turn can comprise a large portion of a small mammals diet. The relationships between forest structure, truffle abundance, and small mammal species has been heavily studied in the Northwestern United States, but have received little attention in the Northeast. To provide insight 768 4m² truffle plots were excavated in the Bartlett Experimental Forest, White Mountain National Forest, New Hampshire. A total of 6,326 truffles from five genera were collected. Truffle richness and biomass of individual trufle species were compared to nine habitat variables. Within the same area small mammals were live captured and the fecal pellets of three rodent species, *Myodes gapperi*, *Napaeozapus insignis*, and *Tamias striatus,* were examined for fungal spores. Basal area of hemlock was the strongest habitat variable significantly impacting the biomass of three truffle species and truffle richness, although overall models explained little of the variance seen. These findings suggest the local presence of hemlock may be important for truffle abundance and richness, but truffles are more likely structured by large scale environmental factors and soil characteristics. Fungal richness in the diets *T. striatus* and *N. insignis* significantly differed across the trapping season, but not in *M. gapperi*. Changes in the abundances and richness of truffle species could have cascading effects on the diets of small mammals and overall health within northern New England forests.

INTRODUCTION

Ectomycorrhizal fungi aid plant roots in the uptake of water and nitrogen in exchange for carbohydrates (Simard et al. 2012). The presence of these fungi is important in structuring the plant community since plants may not establish if the fungi is not already present within the soil

(Dighton et al. 1986). These symbiotic fungi often form fruiting bodies called truffles which fruit underneath the leaf litter and into the soil. The presence and abundance of these fruiting bodies is affected by environmental factors such as temperature and precipitation which directly relate to season (Mehus 1986). These fruiting bodies are enclosed packets of spores and are essential for the dispersal of the fungi, but since they form underground, spores cannot be readily dispersed by the wind. Therefore ectomycorrhizal fungi must rely on other mechanisms, in particular the consumption by animals, for dispersal (Cázares and Trappe 1994).

Small mammals (mice, voles, and squirrels) are known to be drawn to the scent of fruiting truffles and will consume the fungus, a process called mycophagy, and defecate the truffle's spores where they travel (Frank et al. 2006). In turn these fungi can provide a substantial portion of small mammal's diet. However, the amount of fungi consumed can vary across species. Northern flying squirrels (*Glaucomys sabrinus*) can have up to 90% of their diet comprised of fungi (Maser et al 1985) whereas the diet of the white footed mouse (*Peromyscus leucopus*) may have less than 5% fungi (Maser et al 1978). Since fungi can make up a substantial portion of a rodents' diet understanding the variation in what truffle species are found and their abundance can give insight into rodent diversity and community composition (North et al. 1997).

While it is known that common New England woodland rodents do ingest truffles (Whitaker 1962, Maser et al. 1978), the presence and abundance of these truffles and how they relates to environmental variables in New England is not as well understood as it is in the Pacific Northwest of the United States and Australia. Similarly, how the amount of fungus changes within a small mammals diet across a season is also not well understood for the New England region. This project investigated the relationships between ectomycorrhizal fungi, forest composition, and small mammals in northern New England forests. Truffles were excavated in

the Bartlett Experimental Forest and their biomass and richness compared to nine habitat variables. Within the same area small mammals were live captured and the fecal pellets of three common species, the southern red backed vole (*Myodes gapperi*), the woodland jumping mouse (*Napaeozapus insignis*), and the eastern chipmunk (*Tamias striatus*) were examined for the presence of fungal spores.

METHODS

Study Area and Small Mammal Trapping Grids

The Bartlett Experiment Forest (BEF) is located centrally in the greater White Mountain National Forest, New Hampshire. BEF covers 2,600 acres and ranges in elevation from 680 ft. to 3,000 ft. The climate is humid continental, with warm summers and cool winters, and around 50 inches of precipitation deposited evenly throughout the year. Dominant tree species are hemlock (*Tsuga canadensis*), American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), sugar maple (*Acer sacchrum*), balsam fir (*Abies balsamea*), yellow birch (*Betula alleghaniensis*) and red spruce (*Picea rubens)* with spodosol being the dominant soil type. Twelve small mammal trapping grids were established in the Bartlett Experimental Forest during the summer of 2013. These grids are evenly distributed across the three main forest types, Hardwood (n = 4), Softwood (n = 4), and Mixed (n = 4). Grids are 1h in extent and contain an 8 x 8 array of 64 stations, spaced 15 meters from one another.

Truffle Sampling

Using a systematic random sampling approach, a total of 16 stations per grid were identified for truffle sampling, with 2 stations chosen along each of the 8 trap lines. To allow for resampling each station was then subdivided into 4 truffle plots 2m from the center of the station in the four cardinal directions (North, East, South, and West). Per trapping station, each

subdivided plot was randomly assigned a sampling month (June, July, August, or September). This allowed for 64 truffle plots per grid and 256 truffle plots per forest type.

Truffle plots were demarcated by placing a metal scribe in the center of the plot and scribing a 4m² circle around it. Leaf litter was then removed from within the circle. The first 10cm of soil was raked with a 4 tinged garden cultivator and broken apart by hand to feel and collect truffles. Truffles were then photographed, identified to species, dried at 60˚C, and weighed to the nearest 0.01 gram (Lehmkuhl et al. 2004, North and Greenberg 1998). Truffles were identified to species using three keys: *Key to spores of the Genera of Hypogeous Fungi of the North Temperate Forests* by Castenallo et al 1989, A *Field Guide to North American Truffles by* Trappe et al 2007, and *Ascomycete Fungi of North America* by Beug et al 2014. Representative samples were sent to be verified by a leading truffle biologist.

Vegetation and Habitat Survey

Trees within 5m of a trapping station center were identified to species and the diameter at breast height (DBH) was measured. If a tree was < 1 inch in diameter it was excluded from this analysis. Tree diversity and species-specific basal area were calculated at the station level. Within 2m of a trapping station center the volume of all downed coarse woody debris (CWD) was measured. Leaf litter depth was measured at 2 points 0.5m from the center of the station and averaged. ArcGIS v 10.1 was used to calculate slope at each trap station by using a 10m resolution digital elevation model from the National Elevation Dataset (Gesch et al. 2002). Streams and wetlands were spatially mapped within 30m of the trapping grid and georeferenced within ArcGIS v 10.1. These were then combined with the National Hydrologic Layer (Simley and Carswell Jr 2009) and distance to nearest stream was calculated at the station level.

Small Mammal Trapping and Fecal Analysis

Small mammals were surveyed using Sherman live traps during six trapping sessions over the summer of 2014 (2x June, 2x July, and 2x August). Trapping procedures were certified by the UNH IACUC (Protocol#120708). Each grid was surveyed once per month for 4 consecutive days. When an individual was captured for the first time it was identified to species, marked with a unique identifier, and released at the point of capture. Five to ten fecal pellets were collected from the Sherman trap and placed in an Eppendorf vial. Multiple fecal samples were only taken for an individual if that individual was caught during a subsequent month. To prevent contamination traps were then cleaned before being reset. Only fecal pellets from southern red backed voles (*Myodes gapperi*), woodland jumping mice (*Napaeozapus insignis*), and eastern chipmunks (*Tamias striatus*) were used in this analysis.

Fecal pellets were freeze dried and weighed. Individual samples were then ground to homogenize a sample. A subset of 0.01g – 0.04g was placed in KOH for 5 minutes to clear cell walls. The sample was then washed through a 120µl screen with deionized water to separate course and fine fractions. The total volume of fine fraction was measured and 100µl was placed and spread on a microscope slide. Once it was dry, a drop of viskol and a drop of Iodine solution were added to the slide, and left to dry overnight. A coverslip was secured over the sample using 200µl of Permount. Using a compound microscope at 400x magnification 25 random fields of view were examined per slide. In each field of view spores were counted and identified to genus.

Data Analysis

Biomass of individual truffle species were combined at the station level and analyzed using multiple regression models against 9 habitat variables (leaf litter depth, volume of CWD, slope, distance to nearest stream, basal area of beech, basal area of red maple, basal area of hemlock, basal area of red spruce, and tree diversity) in JMP v.11. Truffle richness was

calculated at the station level and analyzed using the same habitat variables. Beech, red maple, hemlock, and red spruce were chosen because they were the four most abundant tree species across all grids.

Due to time constraints only fecal samples from 3 grids (hardwood $n = 1$, mixed $n = 1$, and softwood $n = 1$) were analyzed for this study. These three grids were trapped at the same time and were representative of the three main forest types. ANOVAs were used to test differences in the richness of truffle genera found within a small mammal's diet across the three trapping periods (June, July, and August) in JMP v.11. If significance was found, Tukey post-hoc test was used to test for significance between months. Samples were compiled across grids to have a large enough sample size for analysis.

RESULTS

During the sampling period, 6,326 individual truffles were collected across 5 genera *(Elaphomyces, Genea, Hydnotrya, Rhizopogon,* and *Tuber*). *Elaphomyces* had the greatest number of individual truffles totaling at 6,222 followed by *Tuber* ($n = 71$), *Hydnotrya* ($n = 29$), *Rhizopogon* ($n = 2$) and *Genea* ($n = 2$). Within *Elaphomyces* the species *Elaphomyces verruculosus* was the most common truffle ($n = 5{,}338$). Only two *Elaphomyces* truffles were not able to be identified to species. Total number of truffles by species and forest type are shown in Table 1. July was the most productive month for truffles totaling 2,420 individuals, followed by August ($n = 1{,}498$), June ($n = 1{,}214$) and September ($n = 1{,}194$).

Only seven species had abundances high enough to include in the multiregression models (*Elaphomyces americanus, Elaphomyces anthracinus, Elaphomyces leveillei, Elaphomyces spinoreticulatus, Elaphomyces verruculosus, Hydnotrya cubispora,* and *Tuber shearii*). Basal area of hemlock had the largest impact on biomass with a significant positive relationship for

three species of truffles (*E. anthracinus*, Est = 0.016, t-ratio = 3.46, *P* = 0.0007, *E. leveillei*, Est = 0.149, t-ratio = 3.96, *P* = 0.0001 and *E. verruculosus* Est = 1.032, t-ratio = 3.74, *P* = 0.0002) and for truffle richness (Est = 0.022, t-ratio = 4.29, *P* < 0.0001). Volume of coarse woody debris was significant for the biomass of *T. shearii* (Est = 2.738, t-ratio = 5.6, *P* < 0.0001) and for truffle richness (Est = 28.47, t-ratio = 3.09, *P* = 0.002). Tree diversity was the only variable not significant for any truffle species. All other variables were only significant with one truffle species and are listed in Table 2. Model R^2 values ranged from 0.255 – 0.045 with habitat explaining the most variance for truffle richness.

Fecal pellets from 24 *Myodes gapperi*, 33 *Napaeozapus insignis*, and 43 *Tamius striatus* were examined. *T. striatus* had the highest richness of truffles within its diet (*n* = 14), followed by *M. gapperi* (*n* = 11), and *N. insignis* (*n* = 8) (Table 3). Spore richness within the diet of *M. gapperi* did not significantly differ across trapping periods (*n* = 22, df = 2, *F* = 1.99, *P* = 0.16) (Figure 1). Richness in the diet of *T. striatus* significantly increased across trapping periods (*n* = 43, df = 2, *F* = 4.30, *P* = 0.02) (Figure 1). Post-hoc Tukey tests revealed a significant difference between the months of June and August (STDE = 0.482, *p* = 0.017), but not June and July (STDE = 0.444, *P* = 0.376) or July and August (STDE = 0.409, *p* = 0.141). Richness in the diets of *N. insignis* did significantly change across trapping periods (*n* = 32, df = 2, *F* = 3.77, *P* = 0.03) (Figure 1) although post-hoc Tukey tests did not reveal any significant differences among months (June – July, STDE = 0.282, *P* = 0.062, July – August, STDE = 0.252, *P* = 0.070, June – August, STDE = 0.282, *P* = 0.953).

DISCUSSION

Basal area of eastern hemlock had a positive impact on truffle richness and the biomass of three truffle species. The remaining eight habitat variables were found to be significant in two

or fewer models. Over a hundred species of ectomycorrhizal fungi are reported to form symbiotic relationships with various hemlock species (Kropp and Trappe 1982). My findigns suggest that in northern New England forests hemlock may play a critical role in truffle abundance and richness, but the amount of hemlock is on the decline. The introduction of the invasive hemlock woolly adelgid (*Adelges tsugae*) has led to declines in the amount of hemlock in southern New England and is expected to kill large numbers of the tree as the insect spreads north (Orwig and Foster 1998). Hemlock declines in northern New England forests could be especially problematic for the abundance and diversity of truffles since other truffle rich tree species, as in pines (*Pinus spp*) and oaks (*Quercus spp*) (Trappe et al 2009), are less abundant.

Overall, the presence of the four dominant tree species and five habitat variables explained only a small portion of the variance found within truffle species biomass and truffle richness, R^2 values ranged from 0.045 - 0.255. Instead, biomass in this system may be structured by large scale environmental factors such as temperature and precipitation (Mehus 1986) or microhabitat soil characteristics as in the amount of carbon or iron present (Castrignano et al. 2000). Future studies in similar forest types across a physical gradient would be needed to tease apart which of these factors are influencing truffle biomass.

Fungal richness in the diets of *Tamias striatus* increased significantly throughout the summer season. This increase can be attributed to either, increases in fungal biomass towards the end of the summer and the diet changing to compensate for this, or that other resources have decreased toward the end of the summer and *T. striatus* are forced to consume more fungi (North et al 1997). Conversely, the amount of fungi in the diet of *Myodes gapperi* did not change throughout the summer season. This can be attributed to *M. gapperi* being a fungal generalist and consuming what is available consistently throughout the season (Orrock and Pagels 2002). There

was a significant change in the diet of *Napaeozapus insignis* over the summer season, although directionality was not determined, most likely due to small sample size. It was noticed however that *N. insignis* rely heavily on Glomalean fungi (Table 3). This matches what was found in previous studies (Orrock et al 2003), and may mean that individuals of *N. insignis* only consumes other fungal types when its preferred food source, Glomalean fungi, is limited.

Each small mammal species found higher truffle richness than what was found during truffle surveys (Table 3). This can be attributed to small mammals having a heightened sense of smell and being able to find ripe truffle species (Frank et al. 2006). These findings may suggest that small mammal fecal microscopy may give a more accurate estimate of truffle and fungal richness in an area than truffle surveys, although some taxonomic resolution may be lost. Only two of the 6,326 truffle acquired during surveys were not identified to species level, whereas most spores found within fecal pellets could only be identified to genus. Fecal microscopy also does not give an accurate representation of the biomass of each truffle species or genera, rather it provides the proportional representation of food types consumed. To gain an accurate understanding of the abundance and diversity of ectomycorrhizal fungi within an area it is crucial to use both sampling methods.

The symbiotic interactions between ectomycorrhizal fungi, tree species, and small mammal species are complex. More information is needed to understand the environmental and microhabitat factors that influence truffle abundance and diversity especially within New England Forests. Understanding these factors will allow for better predictions for how changes in forest composition will affect truffle abundance and diversity, and the cascading effects this will have on small mammal populations and overall forest health.

AKNOWLEDGEMENTS

I would like to thank Mariko Yamasaki for providing access to the Bartlett Experimental Forest. I would also like to thank Andrew Uccello, Chris Burke, Karrah Kwasnik, and others for assisting in data collection. I would like to thank Daniel Luoma (OSU) for help with methods development, James Trappe (OSU) for assistance with truffle identification, and PhD student Ryan Stephens for providing valuable insight throughout the entirety of the project. This project was funded through a Summer Undergraduate Research Fellowship by the Hamel Center. Further Support was provided by the NH Agricultural Experiment Station (NH00072-M).

TABLES AND FIGURES

Table 1: Counts of truffle species across the three forest cover types.

	Hardwood	Mixed	Softwood
Elaphomyces			
americanus	69	85	88
anthracinus	8	45	5
leveillei	11	222	215
maculatus	1		
spinoreticulatus	55	34	44
unidentified		2	
verruculosus	300	2115	2923
Genea			
brachytcheca		2	
Hydnotrya			
cubispora	7	10	11
tulasnei	1		
Rhizopogon			
truncatus		1	
vulgaris			1
Tuber			
lyonii	1		1
shearii	7	11	51
Total Count	460	2527	3339
Richness	10	10	9

Table 2. Multiregression estimates for nine habitat variables for the seven most common truffle species and truffle richness. **Bolded** estimates denote significant *p* values: * < 0.05, ** < 0.01, *** < 0.001. Model R^2 values are

	Intercept	Leaf Litter Depth	Volume of Coarse Woody Debri	Slope	Distance to Stream	Beech Basal Area	Red Maple Basal Area	Hemlock Basal Area	Red Spruce Basal Area	Tree Diversity	R^2 of Model
E. americanus	1.802	-0.207	7.746	0.036	-0.003	-0.005	0.029	-0.004	***-0.057****	-0.061	0.061
E. anthracinus	0.146	0.048	8.167	-0.023	1.45E-05	-0.006	0.001	***0.016****	-0.015	-0.119	0.113
E. leveillei	-1.595	***1.724****	-19.573	-0.150	0.006	-0.156	***-0.102****	***0.149****	-0.104	0.497	0.152
E. spinoreticulatus	-0.417	-0.002	-7.181	0.081	***-0.005****	***0.051****	-0.006	0.011	-0.019	0.424	0.055
E. verruculosus	32.692	0.074	279.826	1.077	0.011	-0.918	-0.394	***1.033****	-0.154	-17.001	0.19
H. cubispora	-0.012	0.002	-0.103	0.001	3.17E-05	-0.0005	0.0005	0.0001	-0.0004	0.007	0.045
T. shearii	0.019	-0.005	***2.738****	-0.0003	-3.25E-05	0.0003	0.0001	1.26E-05	0.0002	-0.009	0.16
Truffle Richness	0.668	0.052	***28.470****	***0.063****	-0.001	-0.015	0.010	***0.022****	***-0.029****	0.080	0.255

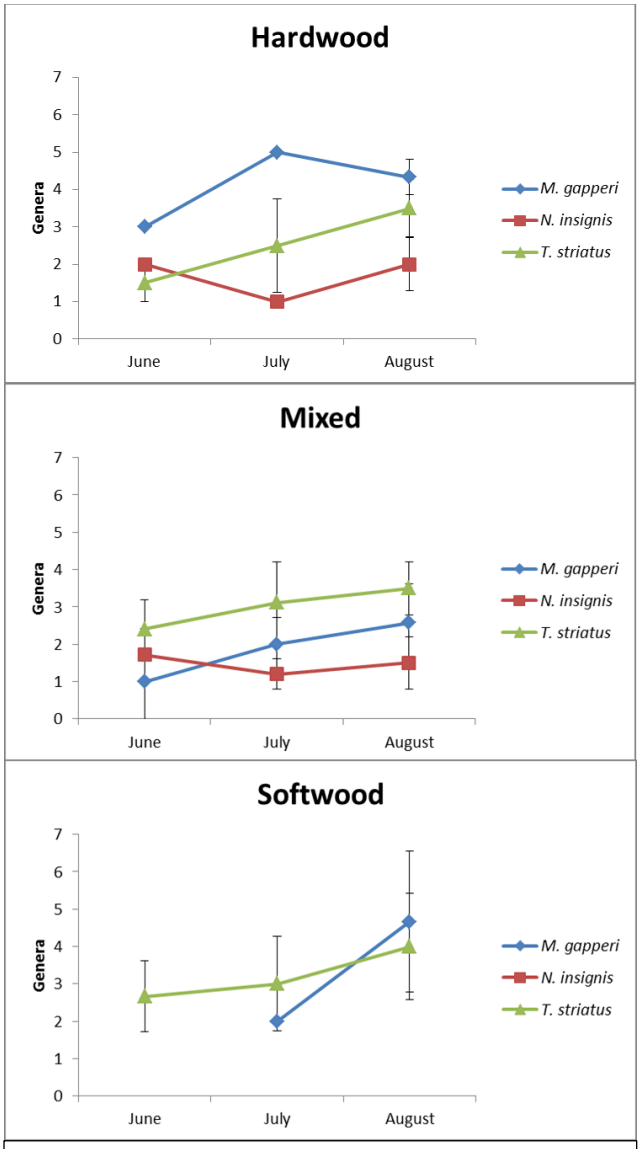

Figure 1: Average truffle richness within fecal samples of three small mammal species: *Myodes gapperi*, *Napaeozapus insignis*, and *Tamias striatus* across three trapping periods (June, July, and August). Error bars represent one standard deviation from the mean.

Table 3: Proportions of fungal genera found within the fecal pellets of *Tamias striatus, Napaeozapus insignis,* and *Myodes gapperi.*

	T. striatus	*N. insignis*	*M. gapperi*
Elaphomyces	0.18	0.18	0.19
Glomus	0.22	0.61	0.30
Hysteranguim	0.11	0.02	0.10
Melanogaster	0.05	0.04	0.04
Rhizopogon	0.03	0.02	0.03
Gymnomyces	0.16	0.06	0.08
Octavianina	0.06	0.04	0.08
Russulaceae	0.04		0.03
Cortinarius	0.04		0.01
Hydnotrya	0.05		0.11
Hymenogaster	0.02		
Gauteria	0.02		
Chamonixia	0.01		
Leucophleps	0.02		
Leucogaster		0.02	
Tuber			0.03

SOURCES

Beug, M. W., A. E. Bessette, A. A. Bessette. 2014. Ascomycete Fungi of North America. Austin Texas: University of Texas Press, Print.

Castellano, M. A., J. M. Trappe, Z. Maser, C. Maser. 1989. Key to the Spores of Hypogeous Fungi of North Temperate Forest with Special Reference to Animal Mycophagy. Eureka California: Mad River Press, Print.

Castrignano, A., P. Goovaerts, L. Lulli, G. Bragato. 2000. A geostatistical approach to estimate probability of occurrence of *Tuber melanosporum* in relation to some soil properties. Geoderma 98: 95 - 113

Cázares, E., Trappe, J. M. (1994). Spore dispersal of ectomycorrhizal fungi on a glacier forefront by mammal mycophagy. Mycologia 86: 507-510

Dighton, J., J. M. Poskitt, D. M. Howard. 1986. Changes in occurrence of basidiomycete fruit bodies during forest stand development: with specific reference to mycorrhizal species. Transactions of the British Mycological Society 87: 163-171

Frank, J. L., S. Barry, D. Southworth. 2006. Mammal mycophagy and dispersal of mycorrhizal inoculum in Oregon white oak woodlands. Northwest Science 80: 264-273

Gesch, D., M. Oimoen, S. Greenlee, C. Nelson, M. Steuck, D. Tyler. 2002. The national elevation dataset. Photogrammetric engineering and remote sensing 68:5-11.

Kropp, B. R., J. M. Trappe. 1982. Ectomycorrhizal fungi of *Tsuga heterophylla*. Mycologia. 74: 479 - 488

Lehmkuhl, J. F., L. E. Gould, E. Cazares, D. R. Hosford. 2004. Truffle abundance and mycophagy by northern flying squirrels in eastern Washington forests. Forest Ecology and Management 200: 49-65.

Maser, C., J. M. Trappe, R. A. Nussbaum. 1978. Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests. Ecological Society of America 59: 799-809.

Maser, Z., C. Maser, J. M. Trappe. 1985. Food habits of the northern flying squirrel (*Glaucomys sabrinus*) in Oregon. Canadian Journal of Zoology 63: 1084-1088

Mehus, H. 1986. Fruit body production of macrofungi in some north Norwegian forest types. Nordic Journal of Botany 6: 679-702.

North, M., J. Greenberg. 1998. Stand conditions associated with truffle abundance in western hemlock/Douglas fir forests. Forest Ecology and Management 112: 55-66

North, M., J. M. Trappe, J. Franklin. 1997 Standing crop and animal consumption of fungal sporocarps in Pacific Northwest forests. Ecology 78: 1543-554

Orrock, J. L., J. F. Pagels. 2002. Fungas consumption by the southern red-backed vole (*Clethrionomys gapperi*) in the southern Appalachians. The American Midland Naturalist 147: 413-418

Orrock, J. L., D. Farley, J. F. Pagels. 2003. Does fungus consumption by the woodland jumping mouse vary with habitat type or the abundance of other small mammals? Canadian Journal of Zoology 81: 753-756

Orwig, D. A., D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. Journal of the Torrey Botanical Society. 125: 60-73

Simley, J. D., W. J. Carswell Jr. 2009. The national map—hydrography. U.S. Geological Survey Fact Sheet 2009-3054. http://pubs.usgs.gov/fs/2009/3054/pdf/FS2009-3054.pdf.

Simard, S. W., K. J. Beiler, M. A. Bingham, J. R. Deslippe, L. J. Philip, F. P. Teste. 2012 Mycorrhizal networks: mechanisms, ecology, and modelling. Fungal Biology Reviews 26: 39-60.

Trappe, M., F. Evans, J. Trappe. 2007. A Field Guide to North American Truffles. New York: Ten speed Press, Print.

Trappe, J. M., R. Molina, D. L. Luoma, E. Cazares, D. Pilz, J. E. Smith, M. A. Castellano, S. L. Miller, M. J. Trappe. 2009. Diversity, Ecology, and Conservation of Truffle Fungi in Forests of the Pacific Northwest. United States Department of Agriculture USA

Whitaker, J. 0. Jr. 1962. Endogone, Hymenogaster, and Melanogaster as small mammal foods. American Midland Naturalist 67: 152-56.