THE ROLE OF SCIURIDS AND MURIDS IN THE DISPERSAL OF TRUFFLE-FORMING ECTOMYCORRHIZAL FUNGI IN THE INTERIOR CEDAR-HEMLOCK BIOGEOCLIMATIC ZONE OF BRITISH COLUMBIA

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The College of Graduate Studies

(Biology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Okanagan)

January 2012

Abstract

Ectomycorrhizal fungi form an integral tripartite relationship with trees and rodents whereby the fungi provide nutritional benefits for the trees, the trees provide carbohydrate for the fungi, and the rodents feed on the fruit bodies produced by the fungi and then disperse the fungal spores in their feces. When forests are harvested, new ectomycorrhizae must form. It has been assumed that dispersal beyond the root zone of surviving trees happens by way of animals dispersing the spores in their feces, but the importance of particular animal taxa to fungal spore dispersal into disturbed areas in the Interior Cedar Hemlock Biogeoclimatic zone of British Columbia has not previously been investigated. This study observed the occurrence and prevalence of hypogeous fruit bodies (truffles) of ectomycorrhizal fungi, and fungal spores in the feces of a range of rodent species. Truffles were excavated and sciurids (squirrels, chipmunks) and murids (mice, voles) were trapped on sites in a 7 to 102-year chronosequence, as well as unharvested sites adjacent to 7- and 25-year-old sites. The average truffle species richness in soil did not change significantly over the chronosequence. *Rhizopogon* species were present at all sites and treatments. Deer mice (*Peromyscus maniculatus*) and yellow-pine chipmunks (*Tamias amoenus*) were the most commonly trapped rodents across all site ages and were also the most likely to move between harvested and unharvested areas. Red-backed voles (*Clethrionomys gapperi*), red squirrels (*Tamiasciurus hudsonicus*), and flying squirrels (*Glaucomys sabrinus*) were also studied, but were trapped in much lower numbers and rarely, if ever, were detected moving between harvested and adjacent mature sites. However, all animal taxa studied carried fungal spores in their feces. Spores of *Rhizopogon* spp.

and *Hysterangium separabile* were the most frequently consumed by all the animals studied. Because deer mice and chipmunks were the most likely to move between mature and harvested sites and they frequently carried fungal spores in their feces, they are likely the most important mammals for dispersal of ectomycorrhizal fungal spores in this area. This study highlights the importance of small mammal conservation when forest management is considered.

Preface

This thesis is based on an experimental design by Dr. Daniel Durall and Dr. Karl Larsen. Dr. Dan Luoma and Joyce Eberhart aided in collection and identification of truffle fruiting bodies. I was responsible for choosing the specific study sites, determining the setup of the sites for trapping, collecting truffles and fecal samples, and identifying spores in fecal samples.

Some of the work presented in Chapter 2 in regards to identifying *Rhizopogon vinicolor* and *R. vesiculosis* has been published: Luoma, D.L., Durall, D.M., Eberhart, J.L., Sidlar, K. 2011. Rediscovery of the vesicles that characterized *Rhizopogon vesiculosis*. Mycologia. 103: 1074-1079.

This work was conducted with approval from the UBC Animal Care Committee under the guidelines of the Canadian Council for Animal Care (CCAC, certificate number A07-0265-R001).

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Acknowledgements

I'd like to thank my supervisors, Dr. Dan Durall and Dr. Karl Larsen for their help and support through this process, as well as my committee members Dr. Melanie Jones and Dr. Jason Pither for their revisions and help with statistical analyses. Dr. Dan Luoma and Joyce Eberhart were invaluable for fungal identification and collection.

The Forest Science Program and the Natural Sciences and Engineering Research Council provided funding without which this project would not have been possible.

I also extend my gratitude to Adam Collins and Matthew Weiss, my field assistants who survived getting up at the crack of dawn for weeks on end to trap mammals. Lab assistants Valerie Ward, Dan Salloum, Julianne Beam, Kenny Dalen-Gryckiewicz, and Natasha Lukey saved me from many all-nighters in the lab by working very diligently. Lori Philips, Jeremy Bougoure, and Jennifer Walker provided invaluable advice for lab work. I'd also like to thank Dr. Leonard Hutchison, whose passion in the field of mycology inspired me to continue in the field after my years at Lakehead University.

1 Introduction

Healthy forests are a complex combination of biotic and abiotic components including plants, animals, fungi, and bacteria, which all interact with the environment to form an intricate system of interacting organisms. As these organisms have evolved together for millions of years, different types of symbioses have developed, many of which are essential to the organisms involved. One of the important interactions in temperate forests is the relationship between trees, ectomycorrhizal (ECM) fungi, and rodents, whereby trees provide fungi with carbohydrates, fungi provide mineral nutrients to trees, and rodents use the fungal fruiting bodies as food. The latter results in longer distance dispersal for the fungi than would otherwise be possible without the mammal vector. When forests are disturbed by natural or human activities, this delicate balance may be upset. This close-knit connection and its recovery after disturbance is the subject of this thesis.

Ectomycorrhizal symbiosis

Ectomycorrhizae form when fungal hyphae associate with roots of vascular plants, and grow within the cell walls of epidermal and cortical root cells but do not physically contact or penetrate the plasma membrane (Peterson and Massicotte, 2004). This symbiosis is beneficial to the fungus as it receives organic carbon from the plant. The plant symbiont benefits as the hyphae extend beyond the zone of nutrient depletion around the roots and increase the nutrient-absorbing surface of the root system thereby increase the availability of poorly-mobile nutrients such as phosphorus (Jones *et*

al.1991; Thomson *et al.,* 1994). The hyphae can also excrete hydrolytic enzymes that release soluble nitrogen from soil organic matter (Lucas and Casper, 2008). Some ECM fungi can be important in water transport, especially those that form highly differentiated rhizomorphs, such as *Rhizopogon* spp. (Eggerton-Warburton *et al.,* 2007). Finally, ectomycorrhizae may also help to protect plants from pathogens (Branzanti *et al*., 1991) and heavy metal toxicity (Wilkinson *et al*, 1995).

Truffles

Many ECM fungi form sexual fruiting bodies: epigeous fruiting bodies are known as mushrooms, while hypogeous fruiting bodies are known as truffles. Truffles evolved from mushrooms as an adaptation to harsh environmental conditions or physical barriers to emergence such as hard, dry ground in arid climates or a hard frost layer in cold environments (Trappe, 1998; Thiers 1984). Most of the fungi involved in truffle-forming ECM relationships are found in both the Basidiomycota (e.g., *Rhizopogon* and *Gautieria*), commonly known as ‘false truffles’ and Ascomycota (eg., *Elaphomyces* spp. and *Tuber* spp.), known as 'true truffles' (Halling, 2001). There are also a few cases of Glomeromycota forming truffle-like structures (e.g., *Endogone*), though they form arbuscular mycorrhizae rather than ectomycorrhizae.

Distribution and occurrence

Truffles are found worldwide (see reviews by Tóth and Barta, 2010; Tedersoo *et al*., 2010), including the northern temperate regions in North America, Asia, and Europe; Australia and New Zealand, southern South America; and, less abundantly, in Africa and tropical regions. Several factors influence the prevalence and taxa of mycorrhizae in an area. In Australia, climatic factors such as temperature and available moisture, as well as microhabitat factors such as leaf litter and soil fertility, affect distribution of truffles (Claridge *et al.*, 2000). Several studies have correlated truffle presence with fallen decaying wood (Gomez *et* al., 2003; Claridge *et al.,* 2000; Waters *et al.* 1997, etc.). Amaranthus *et al.* (1994) also noted that mature forests tend to have more truffles than plantations. Some truffle-forming species are host-specific on a particular tree species (e.g., *Truncocolumella citrina* on *Pseudotsuga menziesii* (Halling, 2001)) or may be a generalist and occur on a wide variety of tree species (e.g., *Rhizopogon* spp. form associations with many species of *Tsuga, Pinus, Abies, Pseudotsuga, Larix, Quercus*, etc. (Molina and Trappe, 1994). Some species have been found to be positively or negatively correlated with each other, but these are likely the result of habitat preference rather than species interaction (Jumpponen *et al*, 2004). Many individual species show seasonal trends to fruiting, peaking in spring or fall (Colgan *et al.*, 1999; Luoma *et al.,* 1991). Although few studies have examined truffle communities in winter, both Colgan *et al*. (1999) and North *et al.* (1997) found truffles in sub-zero temperatures in winter, although the species richness and biomass was lower than in any other season.

Response to thinning and forest harvesting

Several studies have reported on the effects of disturbance, such as wildfire and various kinds of timber harvesting, on ECM forest communities. Even when the below-ground ECM community is not directly disturbed by these events, they are affected indirectly by changes in the physical composition of the above-ground ecosystem (Visser, 1995; Jumpponen *et al.*, 1999; Jones *et al*., 2003; Twieg *et al.*, 2007). Colgan *et al.* (1999) found that standing crop biomass of truffles was lower in thinned stands than in the control, but that some species responded differently: abundance of *Gautieria* spp. and *Hysterangium* spp., etc. declined in thinned stands, whereas diversity and productivity of *Melanogaster* species were higher in thinned stands than controls. *Tuber*, *Truncocolumella*, and *Elaphomyces* species have also been found to decrease with increased thinning (Gomez *et al.* 2003). Luoma *et al.* (2004) studied varying degrees of green tree retention and found that truffle production declined in all harvesting retention levels, regardless of whether the retention was dispersed (remaining trees occurring singly) or aggregated (remaining trees in groups).

Mature trees have been shown to act as refugia for ECM fungi, including truffle-forming species, allowing them to colonize surrounding trees and seedlings and thereby playing an important role in forest succession (Hagerman and Durall, 2004). Cline *et al*. (2007) found that seedlings planted within 6 m of mature Douglas fir (*Pseudotsuga menziesii*) had higher ECM species richness and diversity than those seedlings planted more than 16 m from mature trees. Further, Luoma *et al.* (2006) reported that in the 25 months after harvest with retention trees, there was a 50 % decline in ECM types up to 25 m from retained trees; in areas farther than 5 m from a tree, there was a shift in ECM

community structure, showing that retention trees are necessary to retain ECM diversity to recolonize areas where trees have been removed. Kranabetter (1999) examined retained paper birch (*Betula papyrifera*) trees in clearcut harvested areas in the Interior of British Columbia. Seedlings next to refuge trees in clearcuts had the same ECM species richness as seedlings next to birch trees in mature forests; this study and others give a strong indication that retaining mature trees in harvested areas can help retain the ECM fungal diversity of mature forests (Luoma *et al*., 2004; Luoma *et al.*, 2006; Jones *et al.*, 2008).

Chronosequence studies

Chronosequence studies are used to examine ecosystems that change over time too slowly to easily accommodate the timelines of a scientific study. By choosing different-aged forest types with otherwise similar environmental conditions, extrapolations can be made about the manner in which forests change over a long period of time. Care must be taken in interpreting the results of chronosequence studies, however, because factors other than age, including the nature of disturbance and climate, may vary among sites. There is a lack of chronosequence studies examining truffles, though several studies have examined root tips or mushrooms in forest succession.

Last *et al.* (1987) reviewed data from several studies in different geographical regions and categorized ECM fungi into early or late successional stages of forests, with early-stage fungi having characteristics of r-selection (Grime 1974), including rapid

growth, wide host ranges, and the ability to colonize seedlings, while those categorized as late-stage fungi exhibited S-selection (stress-tolerant, slow growth, high rates of nutrient retention), a higher degree of host specificity, and were only able to colonize mature trees. The fungal species present in forests change over time as the trees mature and above-ground vegetation composition changes. Density of ectomycorrhizae increases with stand age when the forest is young, but levels off in stands that are 30 to 40 years old (Palfner *et al*., 2005). Visser (1995) sampled root tips across a chronosequence of jack pine forest sites disturbed by wildfire and added the category "multi-stage" to describe those ECM fungal species that colonized seedlings or younger trees in regenerating stands but remained prevalent as the trees matured. However, Twieg *et al.* (2007) sampled a chronosequence of mixed birch and Douglas-fir stands and found that such simplified categories were insufficient to describe the complex relationships that occurred in such stands: young stands were dominated by host-specific species while in older stands, host generalists became more common. Waters *et al*. (1997) found particular truffle-forming fungal species associated with old growth forests (over 200 years old) and others associated with mature (over 100 years old). Although these studies considered the age of the stand and tree hosts, Jumpponen *et al.* (1999) hypothesized that habitat characteristics and fungal life history strategies are more important in mycorrhizal fungal succession than the age of the host. Tweig *et al*. (2007) noted that an increase in ECM diversity in the first twenty-five years of succession could be related to the canopy not being closed; higher leaf area in an open canopy would allow for more carbon to be allocated to below-ground resources including ectomycorrhizae.

Several chronosequence studies of ECM communities have specifically considered regeneration after wildfire. Bruns *et al*., (2002) found that, after wildfire, *Suillus pungens* genets were common, but small, and different from those found before the fire; *Amanita francheti*, though common before the fire, was not found afterwards. This is an important difference that indicates some species are capable of regenerating from resistant propagules in the soil while others must be dispersed by spores. Other studies have confirmed that burning reduces live roots in the soil and significantly reduces the ECM richness (Smith *et al.*, 2004), requiring regeneration of fungi from spore banks, undamaged material, vegetative growth through the soil, or fresh spores moving in from another location.

Mycophagy

Mycophagy is generally rare in vertebrates, with the exception of Class Mammalia. It is practiced by a wide range of mammals including grizzly bears (Mattson *et al*., 2002), armadillos (Nouhra *et al*., 2005), hogs and pigs (Hohmann and Huckschlag, 2005; Genard *et al*., 1998); squirrels and chipmunks (Jacobs and Luoma, 2008), mice and voles (Katarzyte and Kutorga, 2011), deer, elk, and caribou (Launchbough and Urness, 1992); wallabies (Claridge *et al*., 2001), and potoroos (Mcilwee and Johnson, 1998; Claridge and Cork, 1994; Claridge *et al.*, 1992); however, except for humans, it is rare among primates (Hanson *et al*., 2003). The that mammals utilize fungi as a food source varies widely from opportunistic to a major part of their diet.

A variety of methods are used by animals to locate fungi. Many mycophagists, such as deer and caribou, eat epigeous fungi and locate them by sight alone (Launchbaugh and Urness, 1992). To ensure that mycophagous animals are able to locate the truffles, hypogeous fungi have evolved chemical attractants to assist mycophagists in finding them (Pyare and Longland, 2001). Dimethyl sulphide is commonly produced by hypogeous fungi (Bellina-Agostinone *et al.*, 1990), including the Perigord black truffle, which is highly prized by humans (Talou *et al.*, 1990). Although most animals who consume truffles find them by these chemical attractants, Northern flying squirrels (*Glaucomys sabrinus*) may be able to remember the location of truffles and return to the same location from one year to the next (Pyare and Longland, 2001). Even though truffle abundance tends to peak in one season, fecal studies have shown that many mammals use them year-round as a food source (North *et al*., 1997), indicating that animals keep caches of truffles on which they depend on when other food items are scarce.

Mycophagy may also be essential for long-distance dispersal of truffle-forming fungi. Truffles produce their fruiting bodies underground, and therefore cannot depend directly on wind for dispersal as mushrooms do; instead, animals eat them and pass the indigestible fungal spores in their feces. Frank *et al*. (2009) only found mycorrhizal root tips within 5 m of a parent tree canopy when other trees were not nearby, thereby limiting the potential spread of the fungus by mycelial growth. However, they did find rodent inoculums as far as 35 m from trees, thereby allowing the species to be dispersed much farther than would be possible without mammals. Colgan *et al*. (2002)

showed that in some cases, spores are more viable and germinate with higher frequency after they have passed through the digestive tract of a small mammal.

Nutrition

Although fungi often make up a large part of the diet of many small mammals, they cannot comprise the entire diet because they are limited in some nutrients. Many studies show that fungi are not a good source of nitrogen as the nitrogen is of low digestibility (Claridge *et al*., 1999; Mcilwee and Johnson, 1998; Claridge and Cork, 1994; Cork and Kenagy, 1989). In a study involving ground squirrels (*Spermopohilus saturatus*), Cork and Kenagy (1989) noted that most of the nitrogen was found in the indigestible spores and peridium of the fungi, but animals may be able to survive with truffles as a major food source because they are easy to find and the foraging costs are very low. Cheung (1997) concluded that mushrooms and truffles are a good source of dietary fiber for humans, so it seems likely that they may play this role in the diet of other mammals. Many mushrooms that are considered edible by humans are high in aluminum, calcium, iron, potassium, magnesium, and phosphorus (Dursun *et al*., 2006). Consequently, despite their low available nitrogen levels, animals may use them as a source of essential minerals. Bozinovic and Munoz-Pedreros (1995) also suggest that water, vitamins, and minerals are the reasons that animals choose to eat fungi. In a study by D'Alva *et al*. (2007), two species of *Peromyscus* were offered fungal fruit bodies, oats, and rodent chow. Most of the animals, when given the choice, ate fungi followed by rodent chow and oats, showing that both truffles and non-truffle food may

be important in maintaining adequate nutrition. Small mammals may also be able to survive on low-quality food by lowering their basal metabolic rate; this causes food to be in the digestive tract for a longer period of time and, therefore, can be digested more efficiently (Veloso and Bozinovic, 1993). Zabel and Waters (1997) found that northern flying squirrels, when given the choice of eight natural foods (including fungi, lichens, bryophytes, and seeds), chose the truffles *Gautieria monticola* and *Alpova trappei* more often than *Gymnomyces abeitis* truffles, fir seeds, and certain types of lichen.

Small mammal mycophagists in Central Interior British Columbia

Most mycophagy studies in British Columbia have been conducted in coastal forests, so mycophagy has not been well studied among sciurids (squirrels, chipmunks) and murids (mice, voles) in the Interior of British Columbia. However, given that studies on the same or closely related mammal species in Alaska (Pyare *et al.*, 2002), Washington (North *et al.,* 1997; Lehmkuhl *et al*., 2004), and Oregon (Jacobs and Luoma, 2008), have confirmed their major role in mycophagy, they could be expected to fill a similar, important niche in interior British Columbia forest ecosystems. The northern flying squirrel is the species perhaps the most strongly linked to mycophagy in northern North American forest ecosystems; however, several other potential mycophagistic sciurids and murids were also studied in this thesis as their roles in mycophagy in this area have not previously been examined extensively.

Northern flying squirrels (*Glaucomys sabrinus*)

Flying squirrels are strictly nocturnal (Maser, 1998) and, although they have relatively large home ranges, varying from 3.7 - 4.2 ha (Witt, 1992), they usually travel less than 100 m in a night (Ransome and Sullivan, 2002). They live in areas with large decaying trees (Holloway and Malcolm, 2007; Maser, 1998, Carey 1995). The presence of spruce trees and snags also indicates good habitat for flying squirrels (Gabel *et al.* 2010; Holloway and Malcolm, 2007; Holloway and Malcolm 2006), and the presence of dense shrub cover is needed for a habitat to support a high abundance of flying squirrels (Carey, 1995).

Flying squirrels are known to be highly mycophagous and depend on fungi as a food source. Mitchell (2001) found that although flying squirrels in West Virginia consume tree buds, lichens, and hypogeous fungi in the spring, in the fall they consume more epigeous fungi along with hypogeous fungi and beechnuts. The winter and summer diet of flying squirrels in Alberta includes both epigeous and hypogeous fungi. The winter diet includes hypogeous species of the genera *Elaphomyces, Gautieria, Hymenogaster, Hysterangium,* and *Rhizopogon* (Currah *et al*. 2000). In addition to those species, Gabel *et al.* (2010) also noted *Geopora* in the diets of flying squirrels in South Dakota.

The amount of fungi consumed by flying squirrels is quite variable, even within one location. Vernes *et al.* (2004) found that flying squirrels in New Brunswick consumed 35-95% more taxa of fungi in summer than in other seasons. They found up to six different spore types in a single sample, indicating that the animals use a variety

of taxa when other common food sources might be scarce. Gabel *et al*. (2010) also found that between 79% and 98% of flying squirrel samples examined contained fungal spores, though the percentage varied from year to year.

Red squirrels (*Tamiasciurus hudsonicus*)

Red squirrels (*Tamiasciurus hudsonicus*), although a conifer-cone specialist, are also ominivorous, eating a variety of fungi, flowers, berries, insects, and even other vertebrates (Fisher, 2000). Vernes *et al*. (2004), studying flying squirrels in New Brunswick, reported that the amount of fungi that make up their diet varies by season and year; however, they can collect large food caches (Fisher, 2000), so they may be able to eat some types of food, including truffles, when they are not typically available. Red squirrels in old-growth habitat consistently consume *Elaphomyces* spp. and *Hysterangium* spp. among others, with fungal species richness in fecal samples highest in the summer months (Vernes *et al*., 2004). The abundance of red squirrels tends to decline in recently harvested stands, and, like flying squirrels, their abundance is correlated with snags and large trees, especially conifers (Holloway and Malcolm, 2006). They are known to travel more than 900 m to search for new territory (Larsen and Boutin, 1994), so it is possible for them to also explore large areas in search of food, including fungi.

Yellow-pine chipmunks (*Tamias amoenus*)

Chipmunks are strictly diurnal (Maser, 1998). They have fairly large home ranges that may overlap, though breeding female chipmunks may become territorial (Nagorsen, 2005). They are known to explore areas far from their home range in search of food, including fungal fruiting bodies (Maser, 1998). Eastern chipmunks (*Tamias striatus*) are known to prefer habitats with an abundance of woody debris and declining trees (Holloway and Malcolm, 2006). In terms of habitat, Sullivan *et al*. (2009) found 2.3-3.4 times more chipmunks in young stands compared to older stands, and Klenner and Sullivan (2009) found them primarily at clearcut sites. Zwolak (2009), in a meta-analysis, found that yellow-pine chipmunk abundance generally increased after forest harvesting. Few studies have closely examined the fungal diets of chipmunks, but both Cazarez *et* al (1999) and Jacobs and Luoma (2008) found that more than 99% of the closely-related *T. siskiou* and *T. townsendii* chipmunks examined in Oregon had been consuming truffles, including *Rhizopogon* spp., *Geopora cooperi*, and *Hysterangium separabile*. The species in this study, *Tamias amoenus*, caches seeds, but not fungi over winter (Kuhn and Vander Wall, 2008; Kuhn and Vander Wall, 2009). The diets of yellow-pine chipmunks have not been previously examined for fungal consumption.

Southern red-backed vole (*Clethrionomys gapperi*)

Southern red-backed voles forage both during day and night. They have a home range of 60-70 m (Gillis and Nams, 1998). Their response to clearcut logging varies:

Gitzen *et al*. (2007) found their response was unpredictable following clearcutting and green tree retention, and Tallmon and Mills (2004) found the density of voles did not change between the edges and interior of remaining forests. In contrast to the previously mentioned studies, Sullivan *et al.* (2009) noted that red-backed voles had significantly higher abundance in old-growth stands than younger thinned stands. The closely related species, *C. californicus,* was found to prefer mature forests over clearcuts, but could also be found in clearcuts, even when mature forest habitat was available (Hayes *et al*., 1986).

Several studies have shown fungi to be important to the diets of red-backed voles, though the animals have not been shown to cache fungi to eat when fresh truffles are not available (Fisher *et al*., 2000). Maser *et al.* (1978) found that red-backed voles in Oregon coniferous forests regularly consumed more than five hypogeous fungal taxa, which was more than the other mammals they studied (chipmunks, meadow voles, and mice). *Rhizopogon* and *Gautieria* are important food sources for voles: Jacobs and Luoma (2008) found *Rhizopogon* spores in 97.7% of fecal samples of voles in Oregon and *Gautieria* in 16.7% of samples; Cazares *et al*. (1999) found *Rhizopogon* spores in 99.9% of vole fecal samples and *Gautieria* in 33% of fecal samples from Oregon.

Deer mice (*Peromyscus maniculatus*)

Deer mice live in a wide range of habitats, with home ranges of 1.4 – 1.9 ha (Maser 1998). Their abundance has been found to increase after clearcutting (Sullivan *et al.*, 2009; Gitzen *et al*., 2007). Deer mice forage almost exclusively by smell and

rarely by sight (Maser 1998) and may even climb trees in search of food (Fisher *et al.*, 2000). They eat a variety of foods and, although they have been noted to consume fungi, their fungal consumption is limited in both abundance and richness (Maser *et al*., 1978; Pyare and Longland 2001b). Because most studies have shown them to consume only few fungi, there is little information available about the type of fungi that they consume.

Objectives

The overarching objective of my research was to investigate whether mycophagist scurids and murids in Interior British Columbia are moving truffle spores from mature forests into adjacent disturbed areas.

My specific objectives included the following:

1. To describe changes in the truffle community over time by sampling a chronosequence of sites ranging from 7 to 102-years-old.
2. To identify potentially important small mammal dispersers of hypogeous fungi by identifying fungal spores from rodent feces from across the chronosequence.
3. To identify the relative importance of different rodents in moving fungal spores from mature to disturbed areas.

My predictions were as follows: older stands (67 and 102-years-old) would have higher truffle richness than younger stands, and members of the genus *Rhizopogon*

would be commonly found at all site ages. Flying and red squirrels would only be found in older (67 and 102-year-old) mature sites, whereas voles and mice would be more common at the younger disturbed sites (5 and 25 years old). Chipmunks would be common throughout all age classes and treatments. I also predicted that flying and red squirrels wouldl have higher fungal spore richness in their feces than would chipmunks and mice.

Overview

To address the objectives listed above, a chronosequence of sites disturbed by forest harvesting or wildfire (approximately 7, 25, 67, and 102 years ago) were used, as well as unharvested areas adjacent to the 7- and 25-year-old sites. Three replicates were used for each age. In both the spring (May-July) and fall (September-October) of 2008, rodents were trapped for three days and nights on all plots and tagged, and their feces were collected for examination of fungal spores. On the same plots and during the same seasons as the mammals were trapped, truffles were collected. In Chapter 2, "Community structure and richness of truffle-forming species across a 100-year chronosequence," I examine the change in truffle communities found over time. In Chapter 3, "ECM fungal spores in rodent feces in a chronosequence of mixed temperate forests," I present fungal spore data from various rodent species caught across the chronosequence. Chapter 4 contains my general conclusions and discusses my findings in the context of forest management. It also describes possible future

research that could further add to the body of knowledge of the dispersal of truffle-forming fungi by rodents in the Interior of British Columbia.

2 Community structure and richness of truffle-forming species across a 100-year chronosequence

Synopsis

Truffles are fungal fruit bodies that have evolved to be hypogeous in order to avoid arid conditions or frost (Trappe 1998). Unlike mushrooms, they cannot disperse their spores in wind; thus they have evolved volatile chemicals that attract small mammals, which subsequently ingest them and deposit their indigestible spores through their feces (Pyare and Longland 2001). Truffle-forming fungi form ectomycorrhizal (ECM) associations with a diversity of vascular plants. In this mutualistic symbiosis, the fungi provide the plant access to organic phosphorus, organic nitrogen, water, and other nutrients (Eggerton-Warburton et al. 2007), as well as protection from drought, disease, parasites (Branzanti et al.1999), and heavy metal toxicity (Wilkinson et al. 1995). In return, the plants provide a source of organic carbon to the fungus.

Hypogeous ectomycorrhizae are known to produce their fruit bodies coinciding with available moisture availability (Newbound et al 2010), and hence often fruit only for a short period during certain seasons (Colgan et al 1999; States and Gaud 1997). Several studies have shown that truffle abundance is correlated with moisture levels. Luoma and Frenkel (1991) studied habitats in Oregon with varied moisture levels and found that mesic forests had the highest biomass of hypogeous fungi and also noted that many individual species showed strong seasonal trends with peaks of production in spring or summer. Microhabitats that allow accumulation of moisture are also known to

be correlated with truffle fruit bodies. For example, Amaranthus *et al*. (1994) found ten times the number of truffles in coarse woody debris than in soil in mature forest.

Major above-ground disturbances in the form of clearcutting and wildfire can drastically alter the species richness and abundance of ectomycorrhizae (Jumpponen *et al*. 1999; Visser, 1995; Twieg *et al*. 2007). Areas disturbed by clearcutting often have fewer mycorrhizae than undisturbed forests (Durall *et al.* 2005) and forests that are simply thinned can have lower truffle abundance and diversity than old-growth forests (Carey *et al*., 2002; Colgan *et al.,* 1999). Waters *et al*. (2007), examining Californian old-growth and mature fir stands, found that truffle production in 100-year-old stands had returned to a state similar to that which existed before stand-replacement wildfire.

Several studies have documented the succession of mycorrhizal fungi through forest seral stages. Some studies have classified fungi as early- and late-stage successors (Last *et al.* 1987). Some fungi, such as those in the genus *Rhizopogon*, are common across a large range of forest ages (Tweig *et al.*, 2007), while others may be associated with certain forest stages. Visser (1995), studying epigeous fungi, also added a “multi-stage” category for those fungi that were present across a broad age range of forests. However, Tweig *et al.* (2007) found these labels were insufficient to describe the complexity of the relationship of mycorrhizal fungi to their associate trees through long-term succession. Jumpponen *et al.* (1999) studied primary and secondary succession on a glacial front and hypothesized that predictions of fungal communities based on host age and physiology were too simplistic and that fungal life history strategies and habitat characteristics were much more important to fungal succession.

It is clear that fungal succession occurs as forests age, though the changes that occur are variable and complex.

Mature trees can act as refugia for ECM fungi following clearcutting. Cline *et al* (2005) planted seedlings less than 6 m and greater than 16 m from mature Douglas-fir trees. They found that root systems of seedlings near the mature trees had higher species richness and diversity of ECM fungi than seedlings that were planted farther away. In addition, they found that ECM fungal communities on roots of seedlings near trees were similar to that of the mature trees, while seedlings planted farther from mature trees had ECM fungal communities more similar to greenhouse seedlings. Luoma *et al.* (2006) found similar results with mycorrhizae from soil cores 8-25 m from mature trees isolated through forest harvesting. They found a 50% decline in the number of ECM morphotypes in soil cores taken more than 5 m from a mature tree, and they noted a shift in ECM fungal community structure. In studying mycorrhizal genets after wildfire, Bruns *et al*. (2002) found that *Suillus pungens* recolonized a bishop pine (*Pinus muricata*) stand quickly with numerous small genets, while *Amanita francheti* was absent after wildfire, indicating that it likely required dispersal by spores and could not survive a major above-ground disturbance . These results indicate the importance of available mature trees to retain mycorrhizal inoculum in the soil, but stress their inability to spread beyond a few meters on their own without connecting to living trees. It is this longer-distance dispersal for which animal vectors appear essential, although the effectiveness of this mechanism will be limited by home range size and the movement patters of particular animals

The purpose of this study was to examine the truffle communities present within forest stands originating from forest harvesting or wildfire, across a 5- to 100-year chronosequence. Although several studies have characterized truffle communities in Pacific Northwest forests, few studies have examined truffle communities in the interior of British Columbia. For comparison, I also collected data on mature stands located adjacent to each of the relatively young forest stands in this chronosequence. I predicted that truffle species richness would be lower in the 5- and 25-year old disturbed sites than the older and mature sites. I further predicted that sporocarps of the genus *Rhizopogon* would be commonly found throughout the study sites.

Methods

Study sites

Sites were chosen (Tweig *et al.* 2007; see Table 2.1) within the Interior Cedar Hemlock Biogeoclimatic Zone in the southern interior of British Columbia; this area has long, dry summers and winters with high precipitation (Lloyd *et al.*, 1998). Despite the zonation name, the sites chosen were dominated by Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.)). These sites comprised a chronosequence, with three sites from each age category averaging 7, 25, 67, and 102 years-old being sampled (exact ages of sites are in Table 2.1, see map Figure 2.1). In the case of 7 and 25-year-old sites, one 30 x 30 m plot was placed in the disturbed area and a similar plot was placed in the adjacent mature (more than 65 years) area, each as close as possible to the edge without overlapping the other habitat type. The mature areas were chosen to represent pre-harvest communities, which could act as sources for spores transported

into clearcuts. In the 67-year-old age class, only one plot was sampled per site because the disturbance was caused by forest fire and thus there were no easily discernable boundaries between the pre- and post-disturbance forest. In the 102-year-old old sites, the two plots per site were located approximately 30 m from each other, a comparable distance to the plots in disturbed and adjacent plots at the younger age classes.

Table 2.1: Site characteristics (from Twieg *et al*., 2007).

Site	Age (yr)	Stand initiation	ICH variant*	Elevation (m)	Latitude/Longitude	Map point (Fig 2.1)
Alone	8	clearcut	mw2	600	N 50º 32’ 43” W 118º 52’ 49”	A
Birch City	6	clearcut	mw2	750	N 50º 38' 48" W 118º 45' 36"	B
White Lake	7	clearcut	mw3	700	N 50º 53' 51" W 119º 16' 27”	C
No Map	23	clearcut	mw2	550	N 50º 36' 15" W 118º 40' 47"	D
Stand Release Control	25	clearcut	mw2	900	N 50º 43' 04" W 119º 06' 54"	E
Zappa	27	clearcut	mw2	650	N 50º 36' 34" W 118º 39' 47"	F
Baldry	65	wildfire	mw2	700	N 50º 34' 03" W 118º 50' 50"	G
Mara	73	wildfire	mw2	600	N 50º 39' 28" W 119º 03' 49"	H
Rocky Road	63	wildfire	mw2	800	N 50º 41' 55" W 118º 46' 07"	I
4 Wheel Drive	105	wildfire	mw2	550	N 50º 36' 47" W 118º 50’ 26"	J
Across the Road	100	wildfire	mw2	600	N 50º 37' 25" W 118º 46' 06"	K
Bobby Burns Parking	103	wildfire	mw2	750	N 50º 27' 17" W 118º 49' 30"	L

*based on Lloyd *et al*, 1998

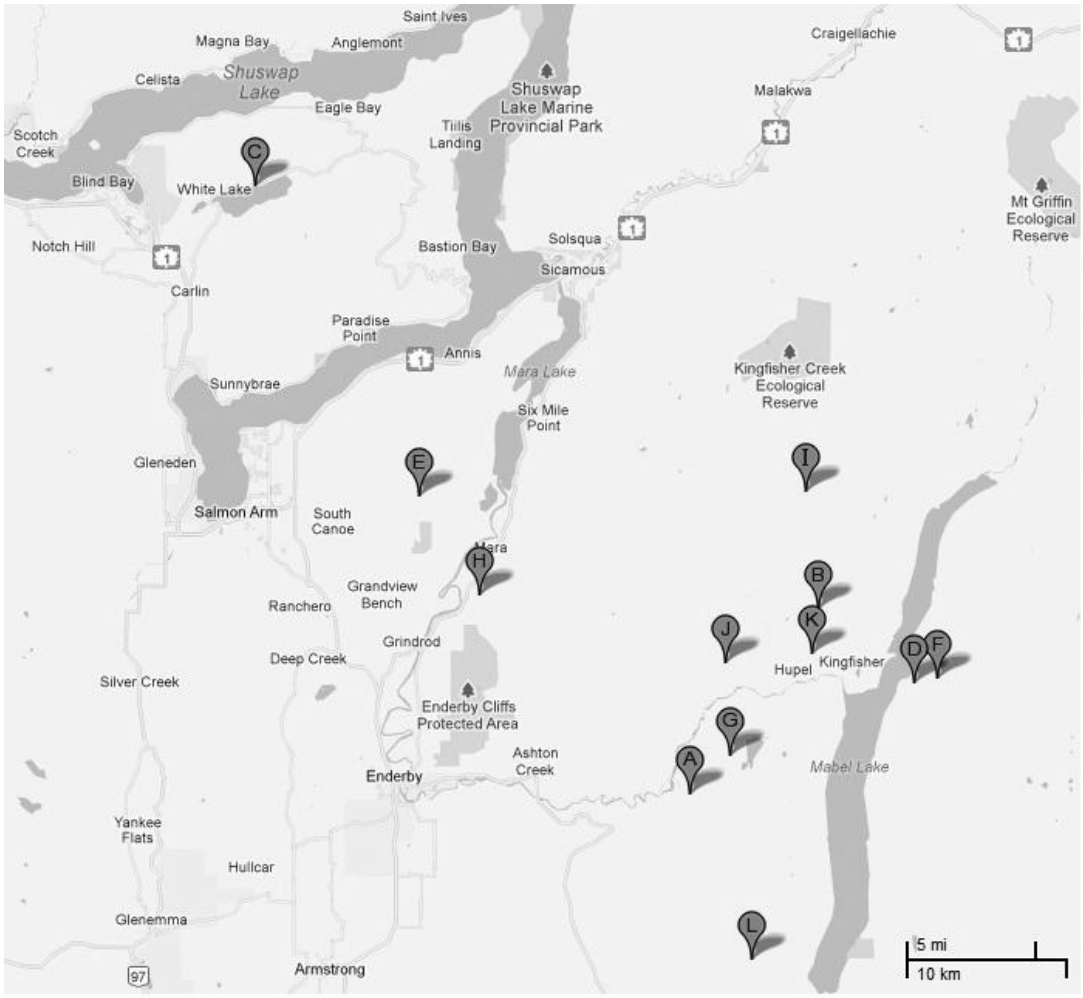

Figure 2.1: Map of study sites. Letters match sites found in Table 2.1. (©Google 2011)

Truffle collection and identification

Truffles were collected on each of the 12 sites in both the spring (late June/early July) and fall (late September/early October) of 2008. Truffles were located by scratching at the ground with truffle forks in areas within the plots where truffles were likely to be present (i.e., under large trees, near coarse woody debris etc.). Three person-hours were allocated on each 30 m by 30 m plot for finding truffles. Collected truffles were placed in wax bags and then oven-dried prior to identification.

Truffles were identified by morphological characteristics alone whenever possible. Restriction-fragment length polymorphism analysis was used to distinguish *Rhizopogon vinicolor* from *R. vesiculosis* because morphologic characteristics alone are insufficient to distinguish these two species. DNA was extracted from fruit bodies in 400 µL of QIAGEN AP1 lysis buffer and shaken with a ceramic bead for 45 seconds at 6.5 m s^{-1} DNA was isolated according to the manufacturer's protocol with the QIAGEN DNEasy 96 Plant Extraction Kit (QIAGEN, Valencia, California). Samples were then diluted 10-fold with ultrapure deionized water. The ITS region of rDNA was amplified using the primers ITS-1F and ITS-4 (Gardes and Bruns, 1993). For the PCR, there was an initial 30-second denaturation at 94 °C, followed by 35 cycles (93 °C for 35 seconds, 55 °C for 53 seconds, 72 °C for 30 seconds). The PCR products were then viewed on a 2% agarose gel stained with SYBR-Safe (Invitrogen Canada Inc., Burlington, Ontario). Restriction digests of the PCR products using the enzyme AluI was then performed to produce restriction fragment-length polymorphisms (RFLPs) to differentiate between *R. vesiculosis* and *R. vinicolor* (Kretzer *et al*. 2003).

Statistical analysis

Analysis of variance (ANOVA) was used to test for differences in species richness between treatments; Excel 2007 (Microsoft Office, 2007) was used for this calculation, with the significance level $\alpha = 0.05$, and with the null hypothesis that all treatments have similar means. The treatments used were: 7- and 25-year-old disturbed, adjacent to 7 and 25-year-old disturbed, 67-year-old, and two groups of the 102-year old plots (the two plots from each site were randomly split to balance the design), for a total of six treatments. Normality of the data was tested with the

Kolmogorov-Smirnov test. EstimateS (Colwell 2009) was used to calculate first-order Jackknife species accumulation to test for completeness of sampling.

Results

A total of 202 truffles were collected, representing 18 species from 11 genera (Table 2.2). Because truffle production is ephemeral and the available sampling time was limited, the two seasons of truffle data were combined. *Rhizopogon villosulus*, *R. vesiculosus*, and *R. vinicolor* were all found at least once in each age/treatment combination. *Truncocolumella citrina* and *Hysterangium separabile* were found in all sites over 7 years old. *Tuber* sp. was only found once, in a 5-year-old clearcut site. *Elaphomyces granulatus* and *Hymenogaster* cf. *sublilacinus* were found on only the 102-year-old sites.

Truffle species richness (depicted in Figure 2.2) did not differ by age/treatment combination ($F = 1.27$, $df = 5, 12$; $p = 0.34$, $n = 3$). However, the first-order Jackknife estimator, which was used to determine if sampling effort was sufficient to adequately sample the richness, did not reach an asymptote (Figure 2.3).

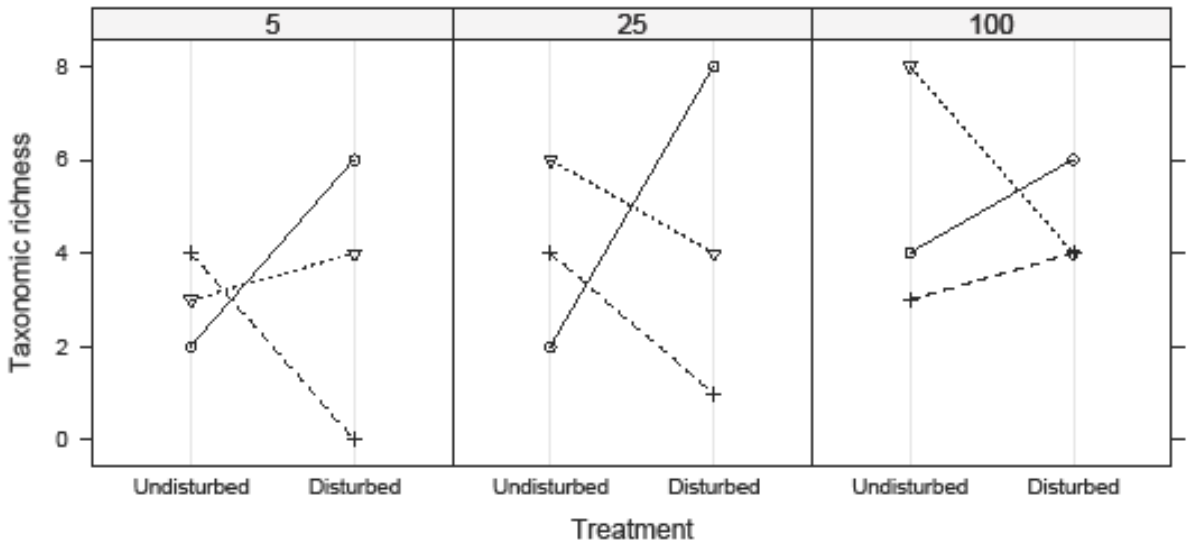

Figure 2.2: Truffle species richness in different aged sites. Lines connect paired plots.

Table 2.2: Truffle species found in each age/treatment combination, including total richness for each age/treatment combination and the average richness and standard deviations for the sites. n=3

	7-year		25-year		67-year	102-year	
Species	**Disturbed**	**Adjacent**	**Disturbed**	**Adjacent**		**A**	**B**
Elaphomyces granulates							+
Elaphomyces muricatum				+	+	+	
Gautieria monticola			+		+	+	+
Geopora cooperi					+		
Hymenogaster cf. *sublilacinus*							+
Hysterangium separabile		+	+	+	+	+	+
Hysterangium coriaceum	+		+		+	+	+
Leucogaster rubescens			+				
Pyrenogaster atrogleba			+	+			+
Rhizopogon salebrosus			+	+			
Rhizopogon subsalmonius				+			
Rhizopogon truncatus				+			
Rhizopogon vesiculosus	+	+	+	+	+	+	+
Rhizopogon villosulus	+	+	+	+	+	+	+
Rhizopogon vinicolor	+	+	+	+	+	+	+
Sarcosphaera eximia		+					
Truncocolumella citrina		+	+	+	+	+	+
Tuber sp	+						
Total Richness	**5**	**6**	**10**	**10**	**9**	**8**	**10**
Average richness ± standard error	3.5 ± 2	4.3 ± 0.67	3 ± 1.15	6 ± 1	6 ± 1	5 ± 1.53	5 ± 0.67

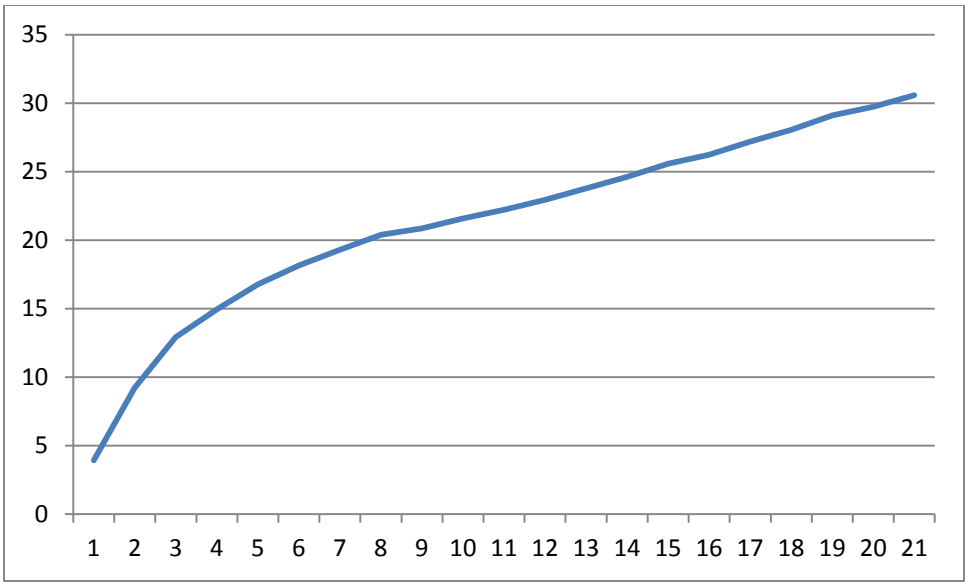

Figure 2.3: First-order Jackknife richness estimator.

Discussion

In contrast to my prediction, this study showed no change in truffle species richness over time. This sharply contrasts to Visser (1995) and Twieg *et al*. (2007), who used different methods (epigeous sporocarps and root tips, respectively) in very different conifer ecosystems (northern Alberta and central interior British Columbia, respectively) and yet still found truffle richness to increase over time. Izzo *et al*. (2005) studied a mixed conifer forest in the Sierra Nevada range in California and found hypogeous species to be the dominant class of fungi on those roots. However, all of these studies used different sampling methods (epigeous sporocarps, soil cores; molecular techniques and morphotyping of roots, feces, and truffles, respectively), and the sampling and identification methods for ECM communities are known to affect results (Jonsson *et al*., 1999; Durall *et al.,* 1999).

Two main patterns emerged from the truffle presence data collected. First, *Elaphomyces* species were not found in stands disturbed less than 25 years ago. North *et al*. (1997) also found that *Elaphomyces* species were only found in sites at least 70 years old. The same study also found *Leucogaster rubescens* more commonly in older stands; by contrast, the only occurrence of *L. rubescens* in this study was in the 25-year-old disturbance class. Second, several species of *Rhizopogon* were found throughout the chronosequence, from the youngest to the oldest forests. North *et al.* (1997) found that members of the Rhizopogonaceae were more common at younger clearcut stands, whereas in this study, as I predicted, *Rhizopogon vesiculosis, R, vinicolor,* and *R.* villosulus were found in all age/treatment combinations. Similarly, Tweig *et al*. (2007), studying ectomycorrhizae on clearcut and wildfire sites, found that *Rhizopogon* spp. were the most commonly encountered truffle-forming ECM fungi in every stand age, but also found them to be more dominant in the younger sites than the older ones. Smith *et al*. (2005) also noted that root tips with *Rhizopogon* spp were common both before and after fire and were abundant across study sites in Oregon. Buscardo *et al.* (2010) found that fire could increase or decrease the presence of *Rhizopogon* on root tips in a Mediterranean open forest, depending on the species: *R. luteolus* decreased whereas *R. roseolus* increased in abundance after major wildfire. As *Rhizopogon* spores are known to persist in spore banks and may be viable for decades (Bruns *et al*, 2009), the genus may be especially important in recolonizing areas that have been altered by major above-ground disturbances.

Contrary to many previous studies, this study shows no difference in truffle richness between very young (7-year-old) and mature (102-year-old) sites. This may

indicate that forests in this area are more resilient than previously thought and forest harvesting may not be a major barrier to fungal community regeneration, and also that information obtained from one forest type may not be applicable to other, even similar, forest systems. This also shows that small mammals in the vicinity of forest harvesting still have access to truffles as a food source after this type of disturbance, thereby allowing natural ecological processes to continue. However, some truffle-forming fungi are known to be ephemeral in their sporocarp formation (States and Gaud 1997; Yamada and Katsuya, 2001), so is possible that many more truffles might have been found with more extensive sampling. The lack of an asymptote in the species accumulation curve also indicates the need for further sampling before rigidly interpreting the truffle richness results. Further studies examining the same sites over a longer period of time and throughout the year could shed more light on the way that these fungal communities change over time.

3 Consumption and dispersal of fungi within a community of mycophagous rodents in a chronosequence of mixed temperate forests

Synopsis

Small mammals are an integral part of a complicated ecosystem triad involving ectomycorrhizal (ECM) fungi and trees. Ectomycorrhizal fungi form mutualistic symbiotic associations with the roots of vascular plants and aid the plants in acquisition of nutrients and water (Jones *et al.*, 1991; Thompson *et al.*, 1994; Eggerton-Warburton *et al.*, 2007), as well as protection from heavy metals (Jones *et al*., 1988; Wilkinson *et al*., 1995) and pathogens (Brazanti *et al*., 1991). In return, the plant symbiont provides the fungus with organic carbon in the form of photosynthates. Many ECM fungi form their fruiting bodies underground, thus disabling them from dispersing their spores in the wind. As an alternative form of dispersal, they produce chemical attractants that allow small mammals to find them (Pyare and Longland, 2001). Once eaten, the indigestible spores are deposited in the mammals' feces, potentially at a great distance from the original source. Passing through the digestive tract of a mammal often renders the spores more viable than they had been (Colgan and Claridge 2002). This tripartite relationship between trees, fungi, and animals is important in forest ecosystems as it benefits all taxa involved (Johnson, 1996, Halling, 2001)

A number of small mammal species (all members of the Order Rodentia) native to the interior of British Columbia are known to consume fungi. *Glaucomys sabrinus* (northern flying squirrel) is known to depend on the fruiting bodies of fungi for a large part of their diet (Fisher *et al*., 2000). Another arboreal sciurid, the North American red

squirrel (*Tamiasciurus hudsonicus*), tends to consume truffles when other food sources are limited, and/or will incorporate fungi into its food caches (Fisher *et al.*, 2000). Chipmunks (*Tamias* and *Eutamias* spp.) are common in North America and are known to consume truffle-forming fungi (Jacobs and Luoma 2008), though they have not been found to cache fungi (Kuhn and Vander Wall, 2009; Kuhn and Vander Wall, 2008). Similarly, *Clethrionomys gapperi* (western red-backed voles) are known to consume, but not cache fungi (Fisher, 2000). Deer mice (*Peromyscus maniculatus*) are not commonly thought of as being mycophagists, although they have been known to consume fungi on occasion (Pyare and Longland, 2001). In a comparative study, Maser *et al*. (1978) studied the digestive tracts of more than 400 individual mammals in Oregon and found that sciurids (chipmunks and squirrels), cricetids (deer mice), and microtids (voles) were common consumers of a wide range of hypogeous and epigeous fungi.

The densities (and potential importance) of many known mycophagic rodents vary across forest types and habitats. Holloway and Malcolm (2006) found that the densities of flying and red squirrels in Ontario were significantly lower in stands that had been harvested 3-10 years previously when compared to stands that were unharvested. They also noted a strong positive relationship between squirrel populations and large spruce and hardwood trees and snags. This relationship, however, has not been seen across the entire range of northern flying squirrels (Wheatley, 2010). Red-backed voles are rarely found in young forest stands, being more associated with mature forests (Tallmon and Mills, 2004). Deer mouse populations are known to increase following forest harvesting (Gitzen *et al*., 2007). The potential for mammals to spread ECM

fungal spores from mature into harvested forest areas could therefore be very important in re-introducing fungi into disturbed areas.

Some hypogeous fungal species, such as those in the genus *Rhizopogon*, are consumed by many mycophagists in many geographical regions, including across North America (Claridge *et al*., 1999; Currah *et al.,* 2000; Lehmkuhl *et al.*, 2004, etc.) and Australia (Claridge and Cork, 1994, Bell and Adams, 2004). Others, such as those in the genera *Gautieria* and *Geopora,* are consumed more frequently in some areas, and more frequently by generalists such as northern flying squirrels than animals that opportunistically eat truffles (e.g., deer mice) (Mitchell, 2001; Lehmkuhl *et al*., 2004; Jacobs and Luoma, 2008).

As a truffle community changes because of forest harvesting and subsequent regeneration, so likely does the diet of the small mammals that depend on truffles as a food source. North *et al.* (1997) studied the effect of forest harvesting on truffle biomass and noted that clearcut areas in Washington had higher biomass of *Rhizopogon vinicolor* and *R. subcaerulescens*, as well as *Truncolomella citrina* and *Melanogaster tuberiformis*, than in natural and old growth stands. Carey *et al.* (2002) found that the diets of flying squirrels in Washington were much more diverse in un-thinned compared to thinned forest stands and, with the exception of *Rhizopogon*, all fungal species found were more often in fecal pellets from animals occupying unthinned rather than thinned forests. However, *Elaphomyces*, which was by far the most common ectomycorrhizal genus studied, was significantly lower in the young stands than the mature or old growth stands.

The communities of truffle-forming fungi that exist after forest harvesting likely depend on one or more factors: fungi in neighboring mature areas propagating vegetatively through the soil, a spore bank left in the soil of the harvested area, surviving mycorrhizal fungi on the roots of standing live trees, and mycophagous animals spreading the spores from adjacent mature areas into the disturbed area. Ectomycorrhizal fungi can propagate asexually through vegetative hyphal growth, sclerotia, or conidia, or sexually through meiotic spores that are dispersed by animals or wind. Some species can use several methods while others rely on only one or the other. Cline *et al*. (2005) found that seedlings planted within 6 m of mature Douglas-fir trees had higher ectomycorrhizal species richness and diversity than those planted more than 16 m away. Furthermore, Luoma *et al.* (2006) found that soil cores taken from more than 5 m from a mature tree left in a clearcut had a 50% decline in the number of ectomycorrhizal fungi present, compared to the number of ectomycorrhiza found before the harvest. These results indicate the importance of nearby mature trees to act as refugia for ectomycorrhizal fungi. However, Bruns *et al.* (2002) found that *Amanita francheti* did not recover after wildfire, unlike *Suillus pungens*; this highlights the importance of spore dispersal for recolonization for some fungi.

The objectives of this study were to: 1) determine the presence of mycophagous small mammals in a chronosequence ranging from 7 to 102 years old; 2) determine the fungal spore composition in the feces of rodents in a chronosequence ranging from 7 to 102 years-old; and 3) determine the small mammals that are implicated in moving truffle inoculum from undisturbed to harvested areas. I conducted this work within the Interior Cedar-Hemlock biogeoclimatic zone of southern interior British Columbia (Pojar *et al*.,

1987), an area that is often disturbed by wildfire and forest harvesting, and where little research has assessed the role of mycophagists in forest management. I predicted that red and flying squirrels would be present only in the 65- and 102-year-old sites and in the mature areas of the other age classes. Chipmunks were expected to be present throughout all age classes and treatments. I further predicted that *Rhizopogon* truffles would be consumed most widely across all mammal species and forest ages. Chipmunks and deer mice would be more likely to cross from mature to harvested areas than red and flying squirrels.

Methods

Site selection

I selected study sites from a previous study (Twieg *et al.*, 2007) in the Interior Cedar Hemlock Biogeoclimatic zone of southern interior British Columbia (Pojar *et al.*, 1987) (see Table 3 in Chapter 2). Sites were selected based on the last time they had experienced a major disturbance: forest harvesting, either approximately 7 or 25 years prior to my study, or a wildfire 67 years prior. Sites that had not experienced a major disturbance in over 102 years were also selected as mature forests. In the 7- and 25-year-old sites, paired plots were chosen both in the harvested area and in the adjacent mature forest; these plots were located as close as possible to the forest edge to document movements of small mammals between the disturbed and adjacent sites, but at least 30 m apart. At the 67-year-old sites, only one plot was used as the edges of disturbance were not readily discernable. At the 102-year-old age class, adjacent plots

were also established nearby in the same forest type to serve as a comparison to the younger disturbed paired plots. Three replicate sites of each age class were used.

Small mammal trapping

During the spring (late May to early July) and fall (early September to mid-October) of 2008, small mammals were trapped using Tomahawk (Tomahawk Live Trap LLC Tomahawk, WI) and Longworth-style (Rogers Manufacturing, West Kelowna, BC) live-traps. For sampling mice and voles, 20 of the latter style of traps were placed in an approximate 30 m x 30 m grid across each plot, while Tomahawk traps targeting sciurids were strategically placed in areas within the plots most likely to heighten trapping success, i.e., on running logs, under large trees, near middens, etc. Tomahawk traps were baited with peanut butter, oats, and raisins; Longworth-style traps were baited with oats, raisins, and apple. Three days before trapping began, traps were locked open with bait to acclimatize animals to them, thus increasing trapping success. Traps were then set for three consecutive days on each site. Traps were first set at dusk to target nocturnal animals (flying squirrels, voles, mice). These traps were checked at dawn and the animals processed. Subsequently, traps were left open for three hours to trap diurnal animals (red squirrel, chipmunk, mice). All animals caught were ear-tagged with a No. 1 size small animal tag (National Band and Tag Co, Newport KY), and feces present in the trap were collected; squirrel, chipmunk, and vole feces were stored both in 70% ethanol and dry in envelopes; deer mouse feces were only stored in ethanol to mitigate the risk from airborne Hanta virus particles. To prevent transmission of disease between sites, at the end of each trapping session,

Longworth-style traps were cleaned in 10% bleach and Tomahawk traps were sprayed with 80% alcohol.

Microscopic morphology

Fecal samples were dried in a drying oven at 65 °C for 12 to 14 hours. They were then weighed and rehydrated with a mixture of equal parts lactic acid, Melzer's reagent, and glycerol equal to ten times the weight of the feces. They were allowed to rehydrate overnight and then were crushed with a pipette tip. Thirty μl of the spore suspension was applied to each of two microscope slides and covered with a glass coverslip. The slides were sealed with several layers of clear nail polish.

For each slide, five fields were chosen randomly and examined at 400x total magnification, for a total of ten fields per sample. In each field, the spores found were identified to the lowest possible taxonomic level using current literature (Castellano *et al.*, 1989; Jacobs *et al*., 2007), and the presence of each spore type was recorded.

Results

I trapped a total of 343 individual animals across all sites (Table 3.1). Spring and fall captures were combined for analysis because there were no patterns evident in the number of individuals trapped between spring and fall seasons, and the main focus of the study was to examine the community of truffles available rather than the truffles available during a specific time frame. Flying squirrels were only trapped on sites 25 years old and older. Red squirrels were only found on the unharvested sites. Chipmunks were common across all sites and disturbances, but were slightly more

common on the younger sites. Red voles were captured infrequently or not at all on some treatments. Deer mice were the most common across all site ages and treatments.

For each species trapped (except red and flying squirrels, which were never caught on adjacent plots), I documented cases where individual animals were trapped in both types of neighbouring habitat, indicating potential for spore transmission (Table 3.2). Chipmunks were trapped on both plots more often than the other animals, but deer mice also moved between plots frequently. Red-backed vole individuals were caught more than once only in the 25-year-old sites, and of those five individuals, only one was caught on both harvested and adjacent mature plots.

Table 3.1: Number of individuals of each animal species trapped in each forest type.

	Flying squirrels	Red squirrels	Chipmunks	Red-backed voles	Deer mice
7-year-old disturbed	0	0	19	1	28
Adjacent to 7-year-old disturbed	0	1	12	0	25
25-year-old disturbed	2	0	10	6	27
Adjacent to 25-year-old disturbed	2	0	10	6	27
67 years	5	0	5	3	13
102 years*	2	1	4	0	17

Note: some individuals were captured in both disturbed and adjacent sites and they were counted as individuals on each plot

*one of each pair of plots on the 102-year-old forest stands were chosen at random

Table 3.2: Numbers of individual animals that were captured on both harvested (7- or 25-year old) and adjacent mature neighbouring stands. At the 102-year old sites, both plots were in mature stands.

Age	Species	# caught more than once	# that were recaptured in the adjacent plot	% that were recaptured in the adjacent plot
7	Deer mice	38	7	18 %
	Chipmunks	14	9	64 %
25	Deer mice	35	4	11 %
	Chipmunks	12	7	58 %
	Red-backed voles	5	1	20 %
102	Deer mice	24	8	33 %
	Chipmunks	9	6	67 %
	Red squirrels	2	0	0

A total of 403 fecal samples were examined, representing 251 individual animals. There were 129 samples that did not contain spores, although 18 of these had remnants of hyphae. The remaining samples (n= 274, ≅ 68%) contained fungal spores. All animal species consumed *Rhizopogon*-like spores more than any other fungal species (Table 3.3). Chipmunks and deer mice consumed *Hysterangium* species less frequently than other fungal taxa. Deer mice consumed all fungal species found, though some fungal species were recorded in only a single fecal sample. *Rhizopogon*-like spores, *Geopora cooperi*, and *Glomus macrocarpa* were found in feces of animals in all stand ages and treatments (Table 3.4). *Gautieria monticola* was not found in either the harvested or adjacent mature area of the youngest sites. Spores of *Hysterangium separabile* were found in feces from all age classes and treatments except for the plots adjacent to the 7-year-old cut sites. *Pyrenogaster atrogleba*, *Elaphomyces granulatus*, *Tuber* spp., *Hymenogaster sublalicilus*, and *Lactarius tomentosus* did not exhibit any

patterns in their presence in feces in the different site types, and often were recorded only once per site/treatment combination.

Table 3.3: The percentage of individuals, trapped on all ages of sites, whose feces contained the specified fungal spores. Multiple samples from the same individual were considered one sample

Animal Species	***Rhizopogon*-like spores**	***Geopora Cooperi***	***Hysterangium separabile***	***Gautieria monticola***	***Pyrenogaster atrogleba***	***Glomus macrocarpa***	***Elaphomyces granulates***	***Tuber* spp.**	***Hymenogaster sublalicilus***	***Lactarius tomentosus***	***Leucogaster rubescens***
Glaucomys sabrinus (n=13)	92 %	8 %	62 %	31 %	0	0	0	0	15 %	8 %	8 %
Tamiasciurus hudsonicus (n=5)	80 %	40 %	60 %	20 %	20 %	0	0	20%	0	0	0
Tamias amoenus (n=78)	76 %	14 %	3%	1%	0	8 %	1 %	4 %	1 %	3 %	0
Clethrionomys gapperi (n=13)	85 %	8 %	54%	15%	0	8 %	8 %	0	0	8 %	0
Peromyscus maniculatus (n=141)	58 %	18 %	2%	1%	1 %	15 %	1 %	2 %	1 %	1 %	1 %

Table 3.4: The percentage of individuals (all animal species combined) that consumed each fungal species in different forest types; three replicates of each site type are combined.

	***Rhizopogon*-like Spores**	***Geopora cooperi***	***Hysterangium separabile***	***Gautieria monticola***	***Pyrenogaster atrogleba***	***Glomus Macrocarpa***	***Elaphomyces granulatus***	***Tuber* spp.**	***Hymenogaster sublalicilus***	***Lactarius tomentosus***	***Leucogaster rubescens***
7-year-old disturbed (n=47)	55 %	21 %	4 %	0	0	17 %	0	0	2 %	4 %	0
Adjacent to 7-year-old (n=35)	54 %	11 %	0	0	3 %	9 %	3 %	6 %	0	0	3 %
25-year-old clearcut (n=50)	82 %	20 %	10 %	8 %	0	6 %	0	8 %	2 %	0	2 %
Adjacent to 25-year-old (n=27)	70 %	19 %	19 %	4 %	0	15 %	4 %	0	4 %	4 %	0
67-year-old fire (n=31)	84 %	6 %	23 %	10 %	0	13 %	3 %	0	3 %	3 %	0
102 years old (n=60)	62 %	15 %	7 %	2 %	2%	10 %	0	2 %	0	3 %	0

Discussion

Animal captures and movement

Capturing success for the different animal species was generally consistent with my prediction, with the exception of two flying squirrels caught on 25-year-old sites. In a meta-analysis, Holloway and Smith (2011) noted that flying squirrels are usually found in mature, unharvested forests. Snags and cavities common in those forests may be very important to flying squirrel populations. The presence of snags combined with the close proximity to mature forest may account for these squirrels being found at the relatively young sites. Neither red squirrels nor flying squirrels individuals were caught on both harvested and mature sites. The low number of captures (13 flying squirrels, 5 red squirrels) makes it difficult to make conclusions about their potential movement and spore dispersal. The number of red-back voles caught was also very low (13 individuals), and only one was found on both adjacent plots. Studies have shown that trapping success for voles can be highly variable by year (Sullivan *et al*., 1999; Tallmon and Mills, 2004; Pearce and Venier, 2005), so these capture rates may not accurately reflect potential captures that could happen over time.

As predicted, chipmunks were abundant at all forest ages and their adjacent forests. I also found evidence of chipmunks moving between undisturbed and disturbed plots of all stand ages. Klenner and Sullivan (2009) found chipmunks primarily at clearcut sites, and Sullivan *et al*. (2009) found far more chipmunks in young compared to older sites, so these results are not surprising and reflect the species preference for recently harvested areas.

I also predicted that deer mice would be more common at younger disturbed sites than older ones; however, I found deer mice from all ages of sites and from all adjacent undisturbed sites with no clear preference towards a particular site age. Studies have reported that deer mice are more abundant within clearcut areas in the Douglas-fir forests of British Columbia (Klenner and Sullivan 2009; Sullivan *et al*. 2009). Although the abundance of deer mice caught on the disturbed and adjacent plots in this study were very similar, only 18% and 11% of individual animals caught more than once were found on both the disturbed and adjacent mature plots for the 7- and 25-year-old sites, respectively. This suggests that although these animals did make movements from the mature into disturbed areas, they apparently did this less frequently than chipmunks. The low number (one third) of deer mice that moved between adjacent plots without major differences in microhabitat at the 102-year-old sites suggests that the distance to travel between plots may be more of a factor than the actual clearcut treatment on the site.

Spores in rodent feces

As predicted, *Rhizopogon* spp., found in more than 75% of fecal samples from every mammal species in this study, were the most widely-consumed fungi across all animal taxa studied. Reported frequencies of particular fungal species present in animal diets vary considerably throughout the literature (e.g., Currah *et al.*, 2000; Lehmkuhl *et al.*, 2004; Jacobs and Luoma, 2008). This may be due to several factors, including subspecies of animals, different forest types allowing for different types of fungi, and seasonal differences in animals' diets captured by different studies.

Northern flying squirrel had the highest percentage of individuals (92%) with *Rhizopogon* spp. spores in their feces of all the animal taxa studied. Other studies have reported high frequency of occurrence of northern flying squirrels carrying spores of *Rhizopogon* spp. (Cazares *et al.* 1999; Lehmkuhl *et al.* 2004; Jacobs and Luoma 2008). Contrary to my results, some studies have reported low frequency of occurrence of flying squirrels carrying *Rhizopogon* spp. spores or have not found them at all in these animals (Currah *et al*. 2000; Mitchell 2001; Carey et al. 2002). For example, Mitchell (2001) did not detect *Rhizopogon* in the diet of *Glaucomys sabrinus fuscus* (West Virginia Northern Flying Squirrel). *Geopora cooperi* was noted in only one flying squirrel sample in this study. Other studies did not report finding *G. cooperi* at all (e.g. Carey *et al*., 2002; Mitchell, 2001), or at low frequencies (1.1% in Jacobs and Luoma, 2008; 3.4% in Lehmkuhl *et al.,* 2004). More than half of flying squirrel samples had *Hysterangium separabile* spores. This is similar to Jacobs and Luoma (2008) who found 62.4% of flying squirrels had *Hysterangium* spp. spores in their feces, but higher than Cazarez *et al.* (1999) who reported finding these spores in 18-22% of fecal samples and other studies that found it rarely (Currah *et al*., 2000; Carey *et al.*, 2002; Lehmkul *et al*. 2004).

Gautieria monticola was found in one third of flying squirrel samples in this study. The frequency with which it has been found in other areas varies widely: Jacobs and Luoma (2008) found it in 79.4% of flying squirrel samples, while Lehmkuhl *et al*. (2004) found it in only 16.4% and Carey *et al.* (2002) in 13% of samples. *Elaphomyces granulatus* was absent from flying squirrel feces in this study. Mitchell (2001) found *Elaphomyces* in about half of all *G. sabrinus fuscus* fecal samples in West Virginia, while Jacobs and Luoma (2008) found it in less than 2% of flying squirrels in Oregon. In

my study, *Hymenogaster* was found in only one or two samples of flying squirrel feces, which was similar to a previously published study (Mitchell 2001). *Tuber* is rarely found in any mycophagist feces, as evidenced by low numbers from other studies as well: 0.9% of samples of flying squirrels (Lehmkuhl *et al*., 2004; Jacobs and Luoma, 2008). *Leucogaster rubescens*, though found in only 1 flying squirrel sample in the present study, was found in 67.2% of flying squirrel samples by Jacobs and Luoma (2008).

Rhizopogon spp. spores were found in 80% of red squirrels fecal samples in this study. This is in contrast to Currah *et al* (2000), who did not find *Rhizopogon* spp. spores in red squirrels feces. More than half of the red squirrel fecal samples collected contained *Hysterangium separabile* spores, though other studies found only trace amounts of this fungal species in red squirrel feces (Currah *et al*., 2000).

Three quarters of chipmunk (*Tamias amoenus*) fecal samples obtained contained *Rhizopogon* spp. spores. Cazarez *et al.* (1999) and Jacobs and Luoma (2008) found that more than 99% of *T. siskiyou* and *T. townsendii* in Oregon had *Rhizopogon* spores in their feces. Only 14% of chipmunk individuals in this study had *Geopora cooperi* spores in their feces. Jacobs and Luoma (2008) found a similar frequency (21%) in closely related chipmunk species. In my study, only a single chipmunk individual had *Hysterangium separabile* spores in its feces; this is in contrast to Cazarez *et al.* (1999) who found that about one fifth of *T. siskiyou* fecal samples contained *H. separabile* spores. *Tuber* spp. spores were rarely found in chipmunks in this study and *Leucogaster rubescens* spores were not found at all. Jacobs and Luoma (2008) found *Tuber* spp. spores in only 3.7% of the chipmunk fecal samples they examined, but found *Leucogaster rubescens* in almost half of the chipmunks sampled.

Rhizopogon spp. spores were found in 85% of red-back vole samples in this study. Similarly, Cazares *et al*. (1999) reported red-backed voles had 98-100% frequency of *Rhizopogon* spores in their feces. One individual vole consumed *Geopora cooperi* in this study; several studies have not reported finding *G. cooperi* in vole diets (e.g. Carey *et al*., 2002; Mitchell, 2001) but others have reported frequencies as high as 42% (Jacobs and Luoma, 2008). Half of the vole fecal samples examined in this study had *Hysterangium separabile* spores; Cazarez *et al*. (1999) found about one-fifth of voles carried *H. separabile* spores in their feces and Jacobs and Luoma (2008) found a much higher frequency (62.4%) in their study. *Tuber* spp. and *Leucogaster reubescens* spores were not found in voles in this study; low frequencies were found in other studies (2.6% for *Tuber* spp. and 17.7% for *L. rubescnens* in Jacobs and Luoma, 2008).

Role of animals in spore dispersal

The relatively high abundance and their ubiquity in different ages of forest combined with the rather high frequency of spores in their feces, makes chipmunks potentially one of the most important spore-dispersers in the ecosystem. Chipmunks (and deer mice) may be underappreciated in their potential to disperse truffle-forming fungal spores in this forest ecosystem. Individual voles, chipmunks, and deer mice were detected moving between clearcut and adjacent mature forests, showing potential for them to carry spores from mature refugia into areas where the ectomycorrhizal fungal community has been disturbed by human activities. Individual chipmunks were detected shifting habitats more often than not over the 3-day trapping periods, suggesting that their role in dispersing spores into disturbed areas may need to be

examined further. However, deer mice may also play a similar, prominent role: though I detected less than one third of these animals moving between clearcut and adjacent areas, their relatively large numbers at most sites makes them potentially important in spores dispersal.

Studies have shown that trapping success for voles can be highly variable by year (Sullivan *et al*., 1999; Tallmon and Mills, 2004; Pearce and Venier, 2005), so their ability to move spores of hypogeous fungi will vary from year to year and is unlikely that the potential would be consistent with respect to a specific age of forest. Red squirrels and flying squirrels were not caught frequently or repeatedly enough in this study, so conclusions about their ability to disperse spores across habitat boundaries cannot be made.

In conclusion, my work reveals that chipmunks, and, to a lesser degree, deer mice, move between disturbed (young forest) and mature forest stands and can distribute spores of a variety of fungal species between these two different habitats. They are abundant at all forest ages studied. Across all forest ages, small mammals are moving fungal spores in their feces, and *Rhizopogon* spp. spores are the most common across all rodent species studied. All told, this suggests that the conservation of small mammal populations in these heavily-impacted forest ecosystems is potentially very important to the healthy regeneration of forest ecosystems.

4 Conclusion

General analysis

This study has shown no change in truffle richness over time since the last major disturbance, which is inconsistent with other similar studies by Twieg *et al.* (2007), Visser (2007), and Izzo *et al*., (2005). Similar to other chronosequence studies (Amaranthus *et al.*, 1994; Twieg *et al.,* 2007), *Rhizopogon* species were found commonly throughout all age classes and treatments, illustrating the resilience of the genus.

Flying squirrels, one of the animals most commonly studied for their ability to disperse truffle spores, and red squirrels, were not captured in high enough numbers to make conclusions about their ability to disperse spores. Voles have potential to move spores, but because their populations fluctuate, this potential varies by year and is likely inconsistent between forest ages and treatments. Deer mice are very common in these forests and they do consume fungi, though they seem to specialize in a few species (*Rhizopogon* spp, *Geopora cooperi*, *Glomus macrocarpa*) with some exceptions. Because these animals have rather small ranges, the potential for them to disperse a significant amount of fungal spores is limited. Chipmunks, however, are shown in this study to be perhaps the most important spore-dispersing rodents in the area. The combination of their abundance, the richness of the fungal species represented in their feces, and their willingness to cross from mature into harvested areas makes them prominent dispersers of truffle-forming fungal spores.

Overall significance of research and applications

To my knowledge, this is the first study to trap mycophagous rodents both in harvested and adjacent mature areas, thus demonstrating the capacity of these animals for spreading fungal spores from one area into another. Because truffles cannot distribute their spores in wind as mushrooms do, they require a mycophagist to aid in digging up the truffles and dispersing them. It was found that chipmunks (*Tamias hudsonicus*) and deer mice (*Peromyscus maniculatus*) have the potential to play important roles as spore-dispersers in this area. This highlights the importance of managing forests in such a way to consider habitat for these animals as they can he helpful in maintaining fungal diversity in disturbed areas.

Study limitations

The funding and logistical constraints of this project only allowed for one year of data to be collected, and similarly, sampling was limited to only a few days each in the spring and fall. Being able to examine patterns in truffles and animal abundances for a longer period of time and throughout the seasons would have beneficial, as some studies have shown that truffles are formed ephemerally at certain times of the year (Colgan *et al.*, 1999; Luoma *et al.,* 1991) and the diets of small mammals also can change during the year (North *et al*., 1997). Since some truffle-forming fungi fail to produce fruit bodies every year, multiple years of study in the same region would yield a more complete picture of the truffle diversity and their presence in the diets of rodents.

Also, as many small mammal populations fluctuate over the years, more long-term sampling could deliver a clearer picture of rodent populations in the area.

Other limitations of this study are the identification of fungal spores and most fruit bodies using morphological techniques only, as well identifying only truffles as epigeous fungi were not collected. Also, collecting root tip samples and using molecular techniques to identify fungal species both from root tips and from fecal samples would allow a more complete and specific picture of the entire ectomycorrhizal community: more species could have been identified to a lower taxonomic level and fungal species that were not fruiting during the limited duration of the study may have also been identified.

Future directions

My research indicates the need for further research of mycophagous rodents in the interior British Columbia forests. The importance of chipmunks, in particular, may have been overlooked as a spore-dispersing animal in the area. Further research with more extensive truffle sampling and longer sampling seasons could shed more light on truffle communities and also better account for variation in the seasonal tendencies of truffles in the area and in different age classes. The combination of more research into mycophagists and the truffle communities they feed on could lead to better forest management practices through an improved understanding of the elements important to complete forest ecosystems.

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