THE CASCADING EFFECTS OF INVASIVE SITKA BLACK-TAILED DEER (ODOCOILEUS HEMIONUS SITCHENSIS) ON THE SOILS, PLANTS AND FUNGAL COMMUNITIES OF HAIDA GWAII

by

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B.Sc., University of Washington, 2011

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Forestry)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

December 2018

The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

The cascading effects of invasive Sitka black-tailed deer (*Odocoileus hemionus sitchensis*) on the soils, plants and fungal communities of Haida Gwaii

submitted by Dylan Thomas Mendenhall in partial fulfillment of the requirements for the degree of Master of Science in Forestry

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Abstract

In Haida Gwaii, the invasion of Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) has decreased the abundance and diversity of plant communities, but little is known about their indirect effects on the activity, abundance, diversity and structure of soil fungal communities. In a natural experiment, I compared the edaphic properties, understory plants and soil fungi of deer-invaded and non-invaded islands. Soils on deer-invaded islands had significantly higher penetration resistance, higher phosphate concentrations and lower pH than soil on non-invaded islands. Understory plants on deer-invaded islands had significantly lower cover-abundance, higher species evenness and significant shifts in community composition, largely driven by differences in the abundances of *Gaultheria shallon*, *Polystichum munitum*, *Lonicera involucrata* and *Menziesia ferruginea*. Ergosterol, a biomarker of fungal biomass, was similar between deer-invaded and non-invaded islands. Illumina sequencing revealed significant differences in the species evenness and composition of the soil fungal community, but similar relative abundances of arbuscular mycorrhizal fungi, ectomycorrhizal fungi, ericoid mycorrhizal fungi and saprotrophic fungi between non-invaded and deer-invaded islands. Differences in the fungal community were significantly correlated with the cover-abundance of understory plants, the depth of the organic horizons, pH and concentrations of ammonium. In a greenhouse bioassay, *Thuja plicata* seedlings grown in living soils from deer-invaded islands had significantly lower chlorophyll fluorescence values (F_v/F_m), an indicator of plant stress, than seedlings grown in living soil from non-invaded islands, but there were no significant differences in the biomass, root:shoot ratios or the inoculum potential of arbuscular mycorrhizal fungi and dark septate endophytes in *Thuja plicata*. There were no significant differences in the biomass, root:shoot ratios, F_v/F_m values or inoculum potential of ectomycorrhizal fungi in *Tsuga heterophylla* grown in soils from deer-invaded and non-invaded islands. Fluorescence-based enzyme assays reveal significantly higher phosphatase activity, lower β-glucosidase and lower cellobiohydrolase activity in soils from deer-invaded islands. Through changes in the physiochemical soil properties and understory plant abundance, Sitka black-tailed deer initiate a cascade of indirect effects on the activity, diversity and composition of the soil fungal community.

Lay Summary

In this study, I investigated the effects of invasive deer on the soils of Haida Gwaii by comparing the plants and soil fungi of invaded and non-invaded islands. I measured plant abundance, soil nutrients, fungal biomass, and used genetic techniques to identify fungal species. I grew trees in the soil to determine the amount of symbiotic fungi colonizing their roots. I also measured the activity of soil enzymes involved with decomposition and nutrient acquisition. I found that invasive deer were associated with differences in the composition of the fungal community. Cedar trees grown in soil from deer-invaded islands had greater stress than those grown in soil from non-invaded islands. Soils from deer-invaded islands had greater enzyme activity associated with nutrient acquisition and less enzyme activity associated with decomposition. In conclusion, invasive deer indirectly alter the soil fungi of Haida Gwaii.

Preface

This thesis represents original, unpublished work by Dylan Thomas Mendenhall. I was responsible for developing the research questions, analyzing the data, and writing the manuscript. The experimental design and field protocols of this study were co-created by Dr. Sue Grayston, Catch Catomeris and me. Soil samples and field data were collected by Dr. Sue Grayston, Dr. Jean-Louis Martin, Catch Catomeris, Dr. Simon Chollet, Morgane Maillard, Maria Continentino, Yonadav Anbar and me. Soil moisture was measured by Maria Continentino, Yonadav Anbar and me. pH was determined by Catch Catomeris, Morgane Maillard and me. Ammonium, nitrate and ergosterol concentrations were determined by the Analytical Chemistry Laboratory of the BC Ministry of Environment and Climate Change Strategy. Phosphate concentrations were determined by me. The results of Chapter 4 were presented at the Society for Ecological Restoration Western Canada Conference in 2018. DNA extraction, initial PCR, DNA quantification and DNA purification were performed by me. Secondary PCR and Illumina sequencing was performed by Dr. Sunita Sinha at the University of British Columbia Sequencing and Bioinformatics Consortium. Dr. David Levy-Booth assisted with the processing of sequencing data through the PIPITS bioinformatics pipeline. The greenhouse bioassay was conducted by Ruby Pyke, Jonathan Zajonc and me. Chlorophyll fluorescence data, microscopy and biomass data were collected by me. The enzyme assays were designed by me, based on methods developed by Beth Brockett. The enzyme assays were conducted by Kate Kourbatova and me.

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List of Abbreviations and Symbols

abbr.	abbreviation
AM	arbuscular mycorrhizae
ANOVA	analysis of variance
bp	base pairs
BP	before present
C	carbon
Ca	calcium
CAP	canonical analysis of principal coordinates
Cl	chlorine
DNA	deoxyribose nucleic acid
DSE	dark septate endophytes
EcM	ectomycorrhizae
ErM	ericoid mycorrhizae
F_m	maximum fluorescence
F_v	variable fluorescence
g	gram
HPLC	high-performance liquid chromatography
IQR	interquartile range
K	potassium
L	liter
m	meter
M	molarity (moles per liter)

Pa	pascal
mol	mole
N	nitrogen
NA	not applicable
NH_4	ammonium
NO_3	nitrate
OTU	operational taxonomic unit
P	phosphorus
PCoA	principal coordinates analysis
PCR	polymerase chain reaction
PERMANOVA	permutational analysis of variance
pH	power of hydrogen
PO_4	phosphate
PSII	photosystem II
db-RDA	distance-based redundancy analysis
SE	standard error
vs	versus
α	alpha
±	plus or minus
˚C	degree Celsius
%	per cent

Acknowledgements

I acknowledge that my research was conducted on the traditional, ancestral and unceded territories of the X̲aayda (Haida) and xʷməθkʷəy̓əm (Musqueam) peoples.

My supervisor Dr. Sue Grayston and committee members Dr. Melanie Jones, Dr. Suzanne Simard provided invaluable guidance and critical feedback during the length of my studies. Dr. Kern Ewing, Dr. Sarah Reichard**,** Dr. David Levy-Booth and Dr. Tim Philpott provided further mentorship, criticism and the occasional praise. Dr. Sunita Sinha patiently supported me through the Illumina sequencing process. Dr. Robert Guy advised me on my greenhouse experiment and kindly allowed me to borrow his laboratory equipment. Dr. Robyn Irvine provided logistic support in the field.

Maria Continentino, Yonadav Anbar, Yifei Shao, Ruby Pyke and Jonathan Zajonc provided meticulous assistance and plain old hard work in the field, laboratory and greenhouse. The success of our fieldwork also depended on the knowledge, skill and discernment of our boat captains, Bruce Woodburn and Paul Rosang. Haawa.

Our horticulture greenhouse staff, including Melina Biron and Jimmy Sung, helped keep my seedlings alive through their thoughtful care and attention. Charlie and Lola provided further integrated pest management support and kept me company on many a cold winter's day.

Thank you to the former and current members of the Belowground Ecosystem Group who provided additional guidance and support, including, Dr. Mina Momayyezi, Dr. Shannon Guichon, Dr. Monika Gorzelak, Dr. Brian John Pickles, Anya Reid and Amanda Asay.

My research received funding from the following organizations: Natural Sciences and Engineering Research Council of Canada, AmeriCorps and UBC Faculty of Forestry. My research was also supported through in-kind support from Parks Canada, and the BC Ministry of Forests.

Thank you to my family and friends for your strength, love and kindness.

To my father, Philip Stephen Mendenhall,

who taught me to follow my heart

with integrity and respect.

Chapter 1 – Introduction

1.1 Research Overview

Hyperabundant herbivore populations are an issue of global concern due to their impacts on the abundance, diversity and structure of plant communities (Côté et al., 2004; Fuller and Gill, 2001; Leopold et al., 1947). When the populations of top predators are reduced or removed from the landscape, herbivores populations increase beyond the carrying capacity of their ecosystems (Côté et al., 2004; Leopold et al., 1947). Through the direct consumption of plants, hyperabundant herbivores initiate a cascade of indirect effects on other organisms including amphibians (Beschta and Ripple, 2009), birds (Chollet et al., 2014) and invertebrates (Allombert et al., 2005). Herbivores can also reach hyperabundant densities when they are introduced into a non-native range where they are released from predation (Golumbia et al., 2008; J. Terborgh et al., 2001; Wood et al., 2015). Wildfires, logging operations and hunting practices can also contribute to overabundant population densities of deer (Leopold et al., 1947; Martin and Baltzinger, 2002). As an example of a terrestrial trophic cascade, when mountain lions became rare in Zion National Park in Utah, black-tailed deer (*Odocoileus hemionus*) reached population densities as high as 30 deer per km^2 whereas historical records indicated there were fewer than 4 deer per km^2 prior to the loss of effective predation (Ripple and Beschta, 2006). Increased browsing intensity by the black-tailed deer greatly reduced the regeneration of cottonwood trees, resulting in the degradation of habitat for amphibians, butterflies and lizards, whose populations subsequently declined (Ripple and Beschta, 2006). The cascading effects of aboveground herbivores extend to the belowground components of terrestrial ecosystems (Bressette et al., 2012). While aboveground herbivores have more direct interactions with the plants which they consume, they can indirectly influence soil microbial communities by i) regulating the quantity of organic matter returned to the soil via the physiological responses of plants to herbivory, through changes in growth, biomass allocation and root exudation, ii) altering the quality of organic soil inputs through their urine and dung, or by inducing physiological changes in the concentration of nutrients and defensive compounds in plant foliar tissue, and iii) altering quantity or quality of plant litter through the long-term changes in the functional composition of plants (Bardgett and Wardle, 2003).

Although there exists a substantial body of knowledge on the effects of aboveground herbivores on the symbioses of arbuscular mycorrhizae as a physiological interaction mediated through plants (Gehring and Whitham, 1994), there has been less research done on the effects of herbivores on soil fungi at a community scale (Gehring and Whitham, 2002). Furthermore, most research on the effects of

herbivores on soil fungi has focused on the abundance and diversity of mycorrhizal fungi in grasslands, pastures and temperate deciduous forests. There is comparatively little information on how aboveground herbivores in temperate conifer forests more broadly affect the abundance, diversity and composition of soil fungal communities.

The archipelago of Haida Gwaii provides a unique opportunity to study the effects of hyperabundant herbivores on the soil fungi of temperate conifer forests. Though native to North America, Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) are a non-native species on Haida Gwaii (Golumbia et al., 2008). The invasion of Sitka black-tailed deer has devastated the understory plant communities (Stockton et al., 2005) of Haida Gwaii. While there has been extensive research on the effects of the invasive deer on the diversity and abundance of aboveground organisms such as trees (Stroh et al., 2008) songbirds (Chollet et al., 2014) and invertebrates (Allombert et al., 2005), there remains a dearth of knowledge on how Sitka black-tailed deer affect soil fungal communities of Haida Gwaii. To improve our understanding of how terrestrial herbivores indirectly affect the abundance, diversity and structure of soil fungi communities, this thesis presents the results of a natural experiment in which I compared soils from islands on Haida Gwaii invaded or not invaded by Sitka black-tailed deer. This chapter reviews literature on the cascading effects of terrestrial herbivores on plant communities, soil properties, microbial activity and soil fungi. Finally, the research question and objectives of this thesis are presented along with an outline of the subsequent research chapters.

1.2 Background

1.2.1 The Functions and Diversity of Soil Fungi

Soils are a living body on the surface of the earth, integrating the biological, hydrological, atmospheric and geological processes of terrestrial ecosystems. Although soil biota represent only a small fraction of the total mass of soil, they have a disproportionately large effect on the ecological processes and functions governed by the soil. Microorganisms such as fungi, bacteria and archaea are responsible for the decomposition and transformation of organic matter, as well as the cycling and mobilization of nutrients (Lladó et al., 2017). Soil microorganisms also form many symbiotic relationships with plants (Lladó et al., 2017), with nitrogen-fixing bacteria and mycorrhizal fungi responsible for over 75% of the nitrogen and phosphorus acquired by plants in temperate and boreal forests (van der Heijden et al., 2008). Although bacteria are also involved in the decomposition of organic matter, saprotrophic fungi tend to dominate in the upper horizons of temperate forest soils

owing to their ability to produce a diverse range of extracellular enzymes capable of breaking down the complex forms of organic matter produced by trees (Lladó et al., 2017), with the fungal/bacterial biomass ratio generally decreasing with depth (Baldrian, 2016). In contrast, pathotrophic fungi exploit the tissues of living plants and animals as a food source and contribute to the succession and structuring of plant communities (Klironomos, 2002; van der Heijden et al., 2008). Mycorrhizal fungi form associations with plants that vary along a mutualism-parasitism gradient (Johnson et al., 1997), but generally result in positive effects on the growth and survival of their plant hosts by facilitating the uptake of nutrients and water from the soil (Tedersoo et al., 2014; van der Heijden et al., 2015). Temperate and boreal forests are characterized by the high abundance of ectomycorrhizal (EcM) fungi (Baldrian, 2016). Other mycorrhizal functional groups include arbuscular mycorrhizal fungi, which are generally accepted to be obligate symbiotrophs (Bago and Bécard, 2002; van der Heijden et al., 2015), and ericoid mycorrhizal (ErM) fungi, which specialize in forming associations with ericaceous plants (Brundrett, 2004). Dark septate endophytes (DSE), are a phenotypically defined group of fungi whose ecological function is not fully understood (Baldrian, 2016). DSE have been observed to colonize the roots of plants and tend to be associated with greater nutrient uptake and positive growth responses in their hosts (Newsham, 2011). Due to the apparent lack of specialized structures for nutrient exchange, some researchers do not yet classify DSE as true mycorrhizal fungi (van der Heijden et al., 2015), but they may be regarded as functionally equivalent to the more commonly recognized mycorrhizal fungi (Newsham, 2011). Facultative mycorrhizal fungi, such as EcM or ErM fungi, retain varying capacities to produce extracellular enzymes involved in the decomposition of organic matter, however, the degree to which these fungi are able to subsist on organic matter as free-living organisms remains controversial (Baldrian, 2009). Ectomycorrhizal fungi, for example, have far fewer copies of genes involves in the production of cellulolytic and ligninolytic enzymes compared with saprotrophic basidiomycetes (Baldrian, 2009). Because of the interspecific variation in the capacities of fungi to decompose organic matter, shifts in the diversity and composition of fungal communities can indirectly affect rates of soil decomposition (van der Wal et al., 2015). For example, early-successional cord-forming ectomycorrhizal fungi such as *Cortinarius* spp. are associated with lower rates of carbon sequestration than ascomycetous ericoid mycorrhizal fungi (Clemmensen et al., 2015). Shifts in the abundance, diversity and composition of soil fungal communities also have important implications for plant succession, as feedbacks between plants and soil fungi contribute to the structuring of plant communities (Klironomos, 2002; van der Heijden et al., 1998). Rare plant species are generally associated with negative soil-plant feedbacks and abundant plant species are generally associated with positive soil-plant feedbacks

(Klironomos, 2002). Thus, by altering the abundance, diversity and structure of soil fungal communities, aboveground herbivores have the potential to influence important soil processes such as nutrient cycling, decomposition, carbon sequestration and the interactions between plants and soil biota.

1.2.2 Effects of Herbivores on Plant Communities

Deer and other large terrestrial herbivores directly affect the growth and survival of plants through the consumption of their leaves, stems and other aboveground tissues (Côté et al., 2004). Over time, herbivores can alter the composition of plant communities by preferentially consuming palatable species, thus increasing the relative abundance of less palatable or herbivore-tolerant species (Leopold et al., 1947). In extreme cases, hyperabundant populations of deer can reduce the abundance of their food sources to such an extent that they overwhelm the carrying capacity and become susceptible to severe malnutrition or starvation (Côté et al., 2004; Leopold et al., 1947). Selective grazing by herbivores interferes with competition between plant species which may increase or decrease the overall diversity of plant communities and drive long-term changes in forest succession, resulting in the replacement of dominant tree species (Côté et al., 2004). For example, in a forest in Pennsylvania, increasing densities of white-tailed deer (*Odocoileus virginianus*) were associated with a decrease in the abundance of *Rubus* spp., birch (*Betula* spp.), American beech (*Fagus grandifolia*) and red maple (*Acer rubrum*), while plant species avoided by deer or tolerant of deer browsing, such as grasses and sedges, increased in abundance (Horsley et al., 2003). In the same study, the plant species richness of plants was negatively correlated with increasing densities of white-tailed deer (Horsley et al., 2003). A separate study in the deciduous forests of Pennsylvania compared plots inside and outside of 60-year old deer exclosures and found that plots exposed to browsing by white-tailed deer had lower cover-abundance and species richness of shrubs and herbaceous plants compared with plots in the exclosures (Goetsch et al., 2011). A natural experiment in Venezuela found tree seedling density to be lower on islands with high herbivore densities compared with mainland areas where predators reduce herbivore pressure (John Terborgh et al., 2001). In Japan, overabundant populations of sika deer (*Cervus nippon*) have altered the structure and composition of forests by reducing the regeneration of trees while increasing the abundance of unpalatable plant species such as ragwort (*Senecio cannabifolius*),bracken fern (*Pteridium aquilinum*) and grasses (Takatsuki, 2009). The effects of terrestrial herbivores on the structure of plant communities can be more complex than simple one-to-one interactions with the plants they consume. In boreal forests of Quebec dominated by balsam fir (*Abies balsamea*) and white birch (*Betula papyrifera*), the

species richness of herbaceous plants was significantly higher in herbivore exclosures than in areas exposed to grazing by white-tailed deer, which multivariate analysis revealed to be the result of facilitation by plant species tolerant of deer browsing, both inside and outside of the exclosures (Beguin et al., 2011).

1.2.3 Effects of Herbivores on Soil Properties

Aboveground herbivores can alter the physical and chemical properties of soils through a variety of direct and indirect pathways. Herbivores directly interact with the soil in two ways: i) through the physical process of walking on its surface which may compact the soil over time, and ii) by contributing nutrient inputs to the soil through their urine and dung. However, through their effects on the abundance, diversity and composition of plant communities, aboveground herbivores can indirectly affect the properties of soils by regulating the quantity, quality and composition of organic matter returned to the soil (Bardgett and Wardle, 2003). At the scale of individual plants, herbivores can induce changes in the growth, biomass allocation and root exudation of plants, as well as the concentrations of nutrients and defensive compounds in plant tissues as a physiological response to herbivory (Bardgett and Wardle, 2003), which can either increase or decrease rates of decomposition (Wardle and Bardgett, 2004). For example, increasing grazing intensity by deer and goats in a New Zealand forest was positively correlated with the decomposition rate of the plant foliage of several species, likely by stimulating plants to concentrate nitrogen and phosphorus in their leaves, resulting in lower lignin:N and lignin:P ratios in the litter (Wardle et al., 2002). In an Alaskan taiga ecosystem, browsing by moose caused a reduction in the C:N ratios in the leaves of *Salix alaxensis*, resulting in faster rates of litter decomposition (Kielland et al., 1997). Similarly, the C:N ratios of soils in a Mongolian grassland tended to be higher in areas where livestock were excluded for ten years, although the difference was not significant (Yong-Zhong et al., 2005). Though herbivores can directly stimulate physiological changes in the quality of plant foliar tissues through grazing, they reduce the overall quality of plant litter entering the soil on a community scale. By preferentially consuming plants that are more palatable (Leopold et al., 1947), aboveground herbivores can drive changes in the functional composition of plant communities resulting in the dominance of plant that produce poorer quality litter (Bardgett and Wardle, 2003). For example, by depleting the plant community of species that are high in foliar nitrogen, aboveground herbivores can decelerate rates of nutrient cycling as the C:N ratio of leaf litter rises (Côté et al., 2004). In a grassland ecosystem in northeastern China, grazing by sheep and cattle was associated with increases in

the C:N ratio of leaf litter and slower rates of decomposition compared with ungrazed sites (Wang et al., 2018). Herbivores can also stimulate the production of defensive compounds in plant leaves that slow their rate of decomposition. For example, in a greenhouse experiment, the leaves of cottonwood (*Populus deltoides*) damaged by spider mites decomposed approximately 50% more slowly than the leaf litter of unaffected plants, likely due to higher concentrations of phenolic compounds in the damaged plants (Findlay et al., 1996).

As a result of the counteracting processes by which aboveground herbivores can alter the quantity and quality of litter entering the soil, deer and other large herbivores are often associated with neutral or negative effects on total soil organic matter content. For example, in deciduous forests browsed by white-tailed deer (*Odocoileus virginianus*) in Wisconsin and Michigan, there was no significant difference in soil organic matter between the inside and outside of 10 to 20 year old herbivore exclosures (Sabo et al., 2017). Likewise, in the grasslands of Yellowstone National Park, grazing by ungulates had no effect on total soil carbon (Tracy and Frank, 1998). In Turkey, forested areas exposed to browsing by red deer (*Cervus elaphus*) had lower soil carbon content compared to ungrazed areas (Kumbasli et al., 2010). The net effect of herbivores on rates of carbon sequestration and litter accumulation depends on the balance between counteracting processes that either increase or decrease the quantity and quality of litter entering the soil.

Aboveground herbivores can also alter the quantity and quality of soil organic matter more directly through inputs from their urine and dung (Bardgett and Wardle, 2003). When forage material is relatively high in nitrogen, ungulates such as deer excrete the majority of the excess nitrogen in their urine in the form of urea which rapidly transforms into plant available ammonium through hydrolysis (Hobbs, 1996). The feces of ungulates contains both organic and inorganic forms of nitrogen, with lower C:N ratios than plant litter (Hobbs, 1996). When the amount of nitrogen deposited into the soil through urine and dung exceeds losses of nitrogen through the consumption of plant biomass, herbivores may result in net increases in the total nitrogen content of the soil (Augustine et al., 2011). Applications of dung from deer, sheep and cattle to a pasture in New Zealand resulted in higher concentrations of phosphate and nitrate in the soil compared with untreated control plots (Williams and Haynes, 1995). Likewise, soils from inside 18-year old herbivore exclosures in Virginia had significantly lower nitrate and phosphorus concentrations than in soils from areas exposed to deer browsing (Bressette et al., 2012). However, a study comparing islands with varying densities of herbivorous red howler monkeys (*Alouatta seniculus*) found increasing monkey densities to be associated with decreases in nitrogen concentrations

in the soil (Feeley and Terborgh, 2005). Aboveground herbivores may contribute to net losses of nitrogen from an ecosystem by inducing greater production of urease (Frank and Evans, 1997), resulting in higher rates of ammonia volatilization (Douglas A. Frank and Groffman, 1998; McInnes et al., 1986). Alternatively, local nitrogen losses may be the result of spatially heterogenous patterns in the urine and fecal inputs of aboveground herbivores. For example, on a landscape scale, herbivores can redistribute the nitrogen and phosphorus in an ecosystem by consuming foliar nutrients in one location and then migrating elsewhere before depositing those nutrients back into the soil (Augustine et al., 2011; Singer and Schoenecker, 2003). Because of the complex counteracting processes between the direct and indirect effects of herbivores on the quantity and quality of organic matter entering the soil, their effects on soil nutrients are often site specific and difficult to predict.

Aboveground herbivores are also associated with changes in soil pH. One potential mechanism for herbivores to indirectly alter soil pH is through their preferential browsing of more palatable plant species, leading to long term functional shifts in the composition of plant communities. Because foliar pH is a species-driven trait that can be maintained by plants independent of their environmental conditions (Cornelissen et al., 2011), differences in the pH of plant leaves contributes to the interspecific variation in rates of leaf litter decomposition (Cornelissen et al., 2006). Therefore, changes in the functional composition of plant communities due to selective grazing may result in net increases or decreases in the pH of litter entering the soil, depending on the species being consumed by herbivores. Alternatively, aboveground herbivores can more directly alter soil pH through their urine deposition. Following the hydrolysis of urea, the primary form of nitrogen found in mammalian urine, soil pH temporarily rises but begins to decline during the subsequent nitrification process (Haynes and Williams, 1992).

Previous research has revealed aboveground herbivores to have inconsistent effects on soil pH, with results varying between different ecosystem types and species of herbivore, possibly due to contrasting mechanisms by which herbivores alter soil pH. A study in the Scottish Highlands comparing soils inside and outside of 24-year old herbivore exclosures found significantly higher pH in plots exposed to browsing by red deer (*Cervus elaphus*, Harrison and Bardgett, 2004). Similarly, grasslands in China grazed by sheep and cattle had significantly higher pH in soils from the grazed areas compared with ungrazed areas (Wang et al., 2018). The opposite effect was found in a study on grassland soils in Uganda where soils from grazed plots had lower pH than where herbivores were excluded for 24 years (Hatton and Smart, 1984). Likewise, a study based in Turkey found soil pH to be lower in forested areas

exposed to herbivory by red deer (Kumbasli et al., 2010). Using a different methodology, a study comparing islands with varying densities of red howler monkeys (*Alouatta seniculus*) found increasing herbivore densities to be associated with decreased soil pH (Feeley and Terborgh, 2005). Other studies have not found soil pH to be significantly affected by aboveground herbivores. A study on 7-year old herbivore exclosures in NW Patagonia found no difference in soil pH between plots exposed to invasive deer and plots in the exclosures (Relva et al., 2014). Similarly, a study on 10-year old and 20-year old herbivore exclosures in forests of Wisconsin and Michigan found no significant difference in the pH between soils inside and outside of the exclosures (Sabo et al., 2017).

An additional way that aboveground herbivores directly affect belowground soil properties is through their physical trampling of the soil. Large terrestrial herbivores tend to increase soil compaction as measured through differences in bulk density, penetration resistance or soil horizon depth. A study based in Wisconsin and Michigan compared soils exposed to white-tailed deer (*Odocoileus virginianus*) with soils inside of 10-20 year old herbivore exclosures and found that soils outside of the exclosures had significantly higher penetration resistance (Sabo et al., 2017). The same study found significantly thinner E horizons in soils outside of herbivore exclosures, but no significant difference in the depth of the A horizon (Sabo et al., 2017). Similarly, forested areas exposed to browsing by red deer (*Cervus elaphus*) in Turkey had higher soil bulk densities compared with unexposed areas (Kumbasli et al., 2010). Soils in areas exposed to grazing by cattle have also been found to be have higher bulk densities compared with ungrazed areas (Sharrow, 2007). In contrast, a study in Patagonia examining soils inside and outside of 7-year old exclosures for invasive deer (*Cervus elaphus*, *Dama dama*) found no significant differences in bulk density (Relva et al., 2014), suggesting that legacy effects on soil compaction may continue for years after herbivore removal. However, a study in Indiana found soil compaction significantly decreased after excluding white-tailed deer for as little as 2 years (Shelton et al., 2014).

1.2.4 Effects of Herbivores on Soil Fungal Communities

Aboveground herbivores can influence soil fungal communities through their direct and indirect effects on plant communities and the edaphic properties of an ecosystem. Shifts in the composition of plant communities can alter soil fungal communities through a variety of mechanisms, including: i) differences in the quantity and chemical composition of leaf and root litter between different plant species, ii) interactions between living plant roots and the biotrophic or saprotrophic soil organisms in the rhizosphere, and iii) changes to soil microclimates and other abiotic properties (Kardol and De Long,

2018). Most of the previous research on herbivore-fungi interactions has focused on the effects of aboveground herbivores on mycorrhizal fungi. Because mycorrhizal fungi and aboveground herbivores compete with each other for the resources produced by plants, terrestrial herbivores can indirectly affect the abundance, diversity and composition of mycorrhizal fungal communities by altering the abundance and diversity of their plant hosts (Gehring et al., 2002). A thorough review by Gehring & Whitham (2002) identified six general patterns in the interactions between aboveground herbivores and mycorrhizae:

1. Aboveground herbivores are associated with a reduction in mycorrhizal root colonization and a shift in the community composition of mycorrhizal fungi.
2. Mycorrhizae affect herbivores both negatively and positively.
3. Interactions between mycorrhizae and aboveground herbivores are conditional on abiotic factors affecting plant stress.
4. Impacts of mycorrhizae on herbivores differ between arbuscular mycorrhizae and ectomycorrhizae.
5. Generalist herbivores are affected more strongly by mycorrhizae than specialist herbivores.
6. Effects of aboveground herbivores on mycorrhizae are likely to have a greater magnitude than the effects of mycorrhizae on aboveground herbivores.

The mechanisms for these patterns remain unclear and likely depend on several contextual factors. Herbivores tend to have negative effects on the abundance of mycorrhizal fungi likely by limiting the carbon that plants allocate belowground, which may shift the structure of mycorrhizal communities towards species with lower carbon demands (Gehring and Whitham, 2002). Mycorrhizal fungi can benefit herbivores by increasing the nutrient density in plant foliage or by stimulating overall photosynthesis, allowing plants to compensate for biomass lost through grazing (Gehring and Whitham, 1994). However, mycorrhizal fungi also increase the carbon efficiency of water and nutrient uptake in plants, thereby enhancing their capacity for producing antiherbivore compounds (Gehring and Whitham, 1994). Because plant species vary in their tolerance to herbivory and their dependence on mycorrhizal fungi, the effects of herbivores on mycorrhizal fungi likely depend on the plant species involved (Gehring and Whitham, 1994). Furthermore, there may be thresholds in the amount of defoliation that plants can tolerate before their capacity to provide carbon to their mycorrhizal symbionts is reduced (Gehring and Whitham, 1994). As a result, the effects of aboveground herbivores on mycorrhizal fungi may vary along a gradient of grazing intensity.

Previous research has found aboveground herbivores to have minimal effects on the abundance of soil fungi. The biomass or abundance of fungi can be evaluated directly through the observation of spore density, hyphal length, or the relative abundance of root colonization, as well as indirectly through the measurement of molecular biomarkers that have been found to correlate with fungal biomass such as ergosterol (Montgomery et al., 2000) or the phospholipid fatty acids, 18:2ω6,9 and 18:1ω9 (Frostegård et al., 2011). Reducing the grazing intensity of sheep in a UK pasture was associated with increases in fungal hyphal length and fungal biomass in the soil (Bardgett et al., 1993). However, a 3-year study of pasture soils in Texas using phospholipid fatty acids analysis found no significant difference in fungal biomass between grazed and ungrazed areas (Acosta-Martínez et al., 2010). Similarly, in the grasslands of Yellowstone National Park, grazing by elk and deer had no effect on total microbial biomass, inclusive of both fungal and bacterial biomass (Tracy and Frank, 1998). In a tallgrass prairie in Kansas, the abundance of hyphae was significantly greater in plots exposed to grazing by cattle but only during one of the two years studied (Eom et al., 2001). However, phospholipid fatty acid analysis of soils in a tundra heath ecosystem in Finland revealed significantly lower fungal:bacterial biomass ratios in the 6-year old herbivore exclosures compared with areas grazed by reindeer, but there was no significant difference in total fungal biomass (Stark et al., 2015). Similarly, phospholipid fatty acid analysis of soils in a Scots pine (*Pinus sylvestris*) forest in Finland showed no difference in total fungal biomass or the fungal:bacterial biomass ratios between grazed areas and ungrazed areas where reindeer had been excluded for 30 to 80 years (Stark et al., 2010). Several studies have found that herbivory by sheep, elk and bison is associated with a significant decrease in the spore density of arbuscular mycorrhizal fungi (Antoninka et al., 2015; Mendoza et al., 2011; Murray et al., 2010). In contrast, an herbivore-exclosure study in a tallgrass prairie in Kansas found that grazing by cattle (*Bos taurus*) was associated with a significant increase in the colonization of arbuscular mycorrhizal fungi in the roots of grasses (Eom et al., 2001). The effects of herbivory on the root colonization rates of ectomycorrhizal fungi remain unclear, but a study on the effects of browsing by moose (*Alces alces*) and snowshoe hare (*Lepus americanus*) in the Alaskan taiga found a small but significant increase in ectomycorrhizal root colonization of willow trees (*Salix* spp.) where herbivores were excluded for 4 years compared with the roots of trees exposed to herbivory (Rossow et al., 1997), which may suggest that herbivores have contrasting effects on the root colonization of arbuscular mycorrhizal fungi in grasslands compared with ectomycorrhizal fungi in forest ecosystems.

Plants in stressful conditions are predicted to severely decrease their resource allocation to mycorrhizal fungi in response to herbivory, whereas plants with low environmental stress may be able

to sustain relatively high levels of root colonization (Gehring and Whitham, 2002). For example, grazing by caribou in a montane tundra ecosystem in Finland was associated with higher AM fungal root colonization in *Solidago virgaurea* growing in alkaline, fertile soils, but lower AM colonization in areas with acidic infertile soils (Ruotsalainen and Eskelinen, 2011). Root colonization by dark septate endophytes followed a similar pattern and was lower when *Solidago virgaurea* was exposed to grazing in the acidic soils (Ruotsalainen and Eskelinen, 2011), suggesting that environmental abiotic thresholds may influence the effect of large herbivores in mycorrhizal communities. Plants exposed to grazing by herbivores may not be able to sustain mutualisms with root endophytes as well when growing in relatively stressful conditions.

Changes in the abundance of mycorrhizal spores, hyphae and other structures can affect the capacity of soils to inoculate plants with mycorrhizal fungi. Most research has found terrestrial herbivores to be associated with decreases in the mycorrhizal inoculum potential of arbuscular mycorrhizal fungi. In the tropical rainforests of northeast Australia where terrestrial vertebrates had been excluded for 3 years, soils had lower diversity and abundance of AM spores (Gehring et al., 2002). In the same study, maize grown in soils from the exclosure plots had less colonization by AM fungi and lower shoot biomass (Gehring et al., 2002). Similarly, in a semi-arid savannah in Kenya where ungulate herbivores were excluded for 15 years, maize grown in soils outside the exclosures had greater colonization by AM fungi than maize grown in soils inside the herbivore exclosures (Petipas and Brody, 2014). However, most studies find large terrestrial herbivores such as sheep, elk and deer to be associated with decreases in the root colonization of AM fungi (García et al., 2012; Wallace, 1987) or decreases in the spore density of AM fungi (Antoninka et al., 2015; Mendoza et al., 2011; Murray et al., 2010). In New Zealand, *Melicytus ramifloru* seedlings grown in soils from inside 15-year old herbivore exclosures had greater biomass and root colonization by AM fungi than seedlings grown in soils from areas exposed to grazing by invasive ungulates (Kardol et al., 2014). After excluding deer for 18 years in forests dominated by oak (*Quercus* spp.) and hickory (*Carya* spp.) in Virginia, *Zea mays* grown in soils from inside the herbivore exclosures had greater colonization by AM fungi than plants grown in soil from outside the exclosures compared with outside, although the difference was not statistically significant (Bressette et al., 2012).

Less is known about the effects of herbivores on the inoculum potential of ectomycorrhizal fungi and dark septate endophytes. In a steppe ecosystem of Argentina, higher grazing intensity by sheep was associated with increased colonization by DSE in the roots of two grass species (García et al., 2012). In

beech forests (*Lophozonia menziesii*) of New Zealand, non-native elk (*Cervus elaphus*) dispersed viable spores of ectomycorrhizal fungal in their dung, which were able to successfully colonize the roots of non-native *Pinus contorta* and facilitate their invasion (Wood et al., 2015). However, while mycophagous animals may enhance the dispersal of ectomycorrhizal spores, browsing by herbivores does not necessarily increase the inoculum potential of EcM fungi in the soil. Browsing by moose (*Alces alces*) and snowshoe hare (*Lepus americanus*) was associated with decreased root colonization of EcM on willow (*Salix* spp.) and balsam poplar (*Populus balsamifera*) in an early successional taiga ecosystem in the US state of Alaska (Rossow et al., 1997).

Aboveground herbivores tend to have minimal effects on the species richness or diversity of mycorrhizal fungal communities. An herbivore-exclosure study in a tallgrass prairie in Kansas found that grazing by cattle (*Bos taurus*) was associated with a significant decrease in the diversity of arbuscular mycorrhizal fungi and a shift in species composition (Eom et al., 2001). In Yellowstone National Park, the species richness of arbuscular mycorrhizal fungi was significantly higher in grassland areas exposed to grazing by elk (*Cervus elaphus*) and bison (*Bison bison*) compared to soils inside herbivore exclosures (Murray et al., 2010). However, most studies have not found the species richness or evenness of arbuscular mycorrhizal fungi to be affected by aboveground herbivores (Antoninka et al., 2015; Burke et al., 2011; Valyi et al., 2015). However, in a tropical rainforest in Australia characterized by its emergent conifer trees (*Agathis atropurpurea*), 3 years of excluding a variety of small vertebrate mammals such as the long-nosed bandicoot (Perameles nasuta) and the red-legged pademelon (*Thylogale stigmatica*) resulted in significantly lower species richness and diversity of arbuscular mycorrhizal fungi in the soil, suggesting that aboveground herbivores can play an important role in the structuring of mycorrhizal fungal communities (Gehring et al., 2002). A study on the effects of white-tailed deer (*Odocoileus virginianus*) on the soils of a temperate hardwood forest dominated by oak (*Quercus alba*, *Quercus rubra*), sugar maple (*Acer saccharum*) and beech (*Fagus grandifolia*) found no significant effects of grazing on the diversity and species richness of ectomycorrhizal fungi. However, excluding deer for 4.5 years resulted in a small but significant increase in the species evenness of ectomycorrhizal fungi (Burke et al., 2011). Overall, these results suggest that while aboveground herbivores have the potential to affect fungal diversity, they are more likely to contribute to subtle shifts in the species composition of fungal communities through their effects on the biotic and abiotic conditions of an ecosystem.

Aboveground herbivores can also influence the abundance and diversity of soil fungi through the dispersal of their spores (Johnson, 1996). Arbuscular mycorrhizal fungi are especially limited in their

dispersal ability, with their hyphae growing through the soil at rates between 1 and 3.2 m/yr (Powell, 1979). Through the consumption of fungal fruiting bodies, mycophagous animals can assist in the dispersal of mycorrhizal fungi by depositing viable spores in their dung (Gehring et al., 2002; Mangan and Adler, 2000; Reddell et al., 1997). For example, in the temperate forests of Argentina, invasive ungulates such as elk (*Cervus elaphus)*, fallow deer (*Dama dama*) and wild boar (*Sus scrofa*) were found to disperse viable spores of ectomycorrhizal fungi which were able to successfully inoculate a non-native tree, promoting its invasion (Nuñez et al., 2013). In the temperate forests of New Zealand, non-native elk were capable of dispersing viable spores of non-native ectomycorrhizal fungi which were able to successfully colonize the roots of two invasive tree species, shore pine (*Pinus contorta*) and Douglas-fir (*Pseudotsuga menziesii,* Wood et al., 2015). However, the invasive ungulates were not found to disperse viable fungal inoculum for native trees (Wood et al., 2015). Similarly, a study from the Pacific Northwest of the United States found black-tailed deer (*Odocoileus hemionus*) to be capable of dispersing viable spores for some species of ectomycorrhizal fungi, which were then able to colonize the roots of pioneer plants on early successional sand dunes (Ashkannejhad and Horton, 2006).

In summary, aboveground herbivores have the potential to influence the abundance, diversity and activity of soil fungal communities. Most of the previous research on the effects of aboveground herbivores and soil fungi has focused on mycorrhizal fungi in grasslands, temperate deciduous forests or intensively managed pasture systems. There remains a paucity of knowledge on how hyperabundant terrestrial herbivores such as deer affect soil fungi in temperate conifer forests. Further still, there is a need for greater information on the mechanisms through which aboveground herbivores affect the abundance, diversity and composition of soil fungal communities. Extensive research has investigated the effects of herbivores on the relative abundance of mycorrhizal fungi in the roots of plants as a physiological response to grazing, but few studies have explored the effects of herbivores on mycorrhizae at the community or population scale. Lastly, while there exists a generous body of knowledge on the effects of herbivores on arbuscular mycorrhizal fungi, there is little information on their interactions with other functional groups such as ectomycorrhizal fungi, ericoid mycorrhizal fungi and dark septate endophytes.

1.2.5 Effects of Herbivores on Soil Microbial Activity

Microbial activity can be investigated through measurements of the enzymes produced by microorganisms or by directly measuring processes associated with microbial activity such as soil

respiration, organic matter decomposition and nitrogen mineralization. By depositing more labile forms of carbon, nitrogen and other nutrients, herbivores can stimulate soil microbial activity (Bardgett and Wardle, 2003) resulting in the acceleration of nutrient mineralization rates and the decomposition of organic matter (Hobbs, 1996). However, the opposite process also occurs, as terrestrial herbivores indirectly influence soil substrates is through long-term shifts in the functional composition of plant communities towards species producing leaf and root litter more recalcitrant to decomposition (Bardgett and Wardle, 2003). The palatability of plant foliage is affected by similar ecophysiological traits that govern rates of litter decomposition. Thus palatable species tend to have higher concentrations of nitrogen and lower concentrations of lignin (Grime et al., 1996; Wardle et al., 2002). Thus, by selectively consuming plants that are more palatable, herbivores may indirectly decrease the nitrogen and increase the lignin in organic soil substrates, resulting in slower decomposition rates and reduced soil microbial activity (Bardgett and Wardle, 2003; Grime et al., 1996). For example, soil respiration rates were lower inside herbivore exclosures in an Alaskan taiga ecosystem browsed by moose (Kielland et al., 1997). Excluding red deer for 14 years in the Scottish Highlands resulted in increased nitrate and ammonium concentrations and higher microbial C:N ratios, likely the result of higher nitrogen mineralization rates in the exclosures (Harrison and Bardgett, 2004b). Generally, terrestrial herbivores are predicted to stimulate soil microbial activity in relatively fertile ecosystems dominated by short-lived and fast-growing plants by consuming a large proportion of plant biomass which is then returned to the soil in highly labile forms of organic matter (Stark et al., 2015; Wardle et al., 2004). In contrast, herbivores are hypothesized to suppress microbial activity in relatively infertile ecosystems that are dominated by long-lived and slow-growing plants by selectively consuming the more palatable species, shifting the plant community towards species with poor quality litter (Bardgett and Wardle, 2003; Wardle et al., 2004).

The potential activity of extracellular enzymes can be quantified as an indicator of different soil processes associated with microbial activity. The decomposition of organic matter is facilitated by various hydrolytic and oxidative enzymes. Cellulose is decomposed through the hydrolysis of its β-1,4-glycosidic bonds by endocellulases such as endo-β-1,4-glucanase, and exocellulases such as cellobiohydrolase, producing substrates of smaller length such as cellobiose (Lynd et al., 2002). β-glucosidase is involved in the final stage of cellulose decomposition and catalyzes the hydrolysis of the glycosidic bonds of cellobiose or at the terminal end of longer oligosaccharides (Allison et al., 2008; Lynd et al., 2002), producing glucose which can be taken up directly by microbial cells (Philpott, 2018; Sternberg et al., 1977). β-glucosidase is produced by microorganisms in part as a response to the availability of labile substrates (Turner et al., 2002), making its activity a useful indication of the quality

of organic matter and overall microbial activity. As the final limiting step for soil microorganisms to acquire carbon from cellulose decomposition, β-glucosidase activity tends to strongly correlate with the microbial fraction of total soil carbon (Turner et al., 2002) and soil respiration rates (Liang et al., 2015; Merino et al., 2016). More complex forms of organic matter such as lignin are decomposed in part by oxidative enzymes such as phenol oxidase and peroxidase (Sinsabaugh, 2010). Enzymes involved in the mineralization of nutrients include phosphatase, urease and β-1,4-N-acetylglucosaminidase (NAG). Phosphatases mineralize phosphate groups bound to organic substrates and are produced by both plant roots and soil microorganisms (Olander and Vitousek, 2000). Urease catalyzes the hydrolysis of urea, resulting in the production of ammonia which then rapidly volatilizes into the atmosphere if it is not incorporated into the soil by further reacting with water to form ammonium (McInnes et al., 1986; McNaughton et al., 1997). NAG is a nitrogen-acquiring enzyme involved in the decomposition of chitin, a biopolymer found in the cell walls of fungi (Ueno and Miyashita, 2000). Because of the specificity of the substrates and products of enzymatic reactions, the measurement of soil enzymes is a useful tool for investigating the effects of aboveground herbivores on soil microbial activity.

One of the mechanisms by which aboveground herbivores can influence soil enzyme activity is through changes in the quantity and quality of organic substrates (Allison and Vitousek, 2005) through shifts in the functional diversity of plant communities (Bardgett and Wardle, 2003; Wardle and Bardgett, 2004). Plant species vary in the functional traits of their leaf litter, resulting in variable rates of decomposition (Zukswert and Prescott, 2017). Thus, changes to the diversity and composition of plant communities can alter the quality and composition of organic substrates entering the soil through leaf and root litter (Bardgett and Wardle, 2003), which in turn can induce or suppress the activity of extracellular soil enzymes (Hernández and Hobbie, 2010; Maltz et al., 2017). For example, in a study comparing the interacting effects of plant diversity and habitat fragmentation on soil enzyme activity in the coastal shrublands of California, rates of leaf-litter decomposition and enzyme activity were lower in the soils of smaller habitat fragments with low plant diversity (Maltz et al., 2017). As a result, β-glucosidase and cellobiohydrolase activity was positively correlated with plant species richness (Maltz et al., 2017). The production of β-glucosidase is particularly sensitive to changes in quantity or quality of organic substrates (Turner et al., 2002). By altering the diversity and composition of plant communities, herbivores can induce or suppress the activity of soil enzymes through changes in soil substrates.

However, changes in the diversity and composition of organic substrates often have complex non-additive effects on the activity of soil enzymes (Hernández and Hobbie, 2010), which may explain

why previous studies have found inconsistent relationships between the presence of aboveground herbivores and the activity of soil enzymes such as phosphatase, β-glucosidase and cellobiohydrolase. For example, there were no significant differences in the potential enzyme activities of phosphatase, β-glucosidase and cellobiohydrolase in soils inside and outside of 4.5 year old herbivore exclosures in a hardwood forest browsed by white-tailed deer (*Odocoileus virginianus*) in Pennsylvania (Burke et al., 2011). Excluding reindeer for 10 years from sub-arctic meadows resulted in a small but statistically significant 4.6% increase in potential phosphatase activity in soils inside the herbivore exclosures but no significant difference in potential β-glucosidase activity (Francini et al., 2014). A three-year study of an integrated cropping-livestock system found alkaline-phosphatase activity to be significantly lower in soils from grazed pastures compared with an ungrazed pasture, but only in one out of the three years studied (Acosta-Martínez et al., 2010). The same study found no significant differences in β-glucosidase activity between the soils of grazed and ungrazed pastures (Acosta-Martínez et al., 2010). Likewise, in tundra heaths grazed by reindeer (*Rangifer tarandus*), there was no significant difference in β-glucosidase activity in between soils inside and outside of 6-year old herbivore exclosures (Stark et al., 2010). In an 18-year post-fire chronosequence, β-glucosidase was significantly lower in the areas grazed by elk (*Cervus canadensis*) compared with soils inside herbivore exclosures, but there was no consistent pattern for phosphatase (Stritar et al., 2010). In an unusual case, urease and alkaline phosphatase activity were significantly higher in soils from 10-year old herbivore exclosures than in grazed areas of a Mongolian grassland ecosystems severely degraded by livestock grazing (Yong-Zhong et al., 2005). Overall, these studies demonstrate that while soil enzyme activity can be influenced by the presence of aboveground herbivores, the effects on specific enzymes are often subtle and site specific.

While soil enzymes can be induced through changes in the quantity or quality of their respective substrates, the production of phosphatase and other hydrolytic enzymes can also be limited by nitrogen availability (Allison and Vitousek, 2005). As fertilization experiments have demonstrated, nitrogen deposition in organic soil horizons is associated with increases in the activity of carbon-acquiring enzymes and decreases in nitrogen-acquiring enzymes (Allison et al., 2008; Saiya-Cork et al., 2002). Long-term nitrogen fertilization in an *Acer saccharum* forest in Michigan resulted in increased activity of β-glucosidase and phenol oxidase in the litter layers, but decreased the activity of β-1,4-N-acetylglucosaminidase (Saiya-Cork et al., 2002). Similarly, in an Alaskan boreal forest, nitrogen fertilization stimulated the activity of β-glucosidase activity while suppressing the activity of NAG (Allison et al., 2008). Therefore, as an additional mechanism, aboveground herbivores may induce or

suppress the activity of extracellular enzymes indirectly through changes in the concentration of soil nitrogen.

1.2.6 Methods of Studying Herbivore-Soil Interactions

Studies on herbivore-soil interactions use a wide variety of experimental designs for evaluating the effects on large terrestrial herbivores on belowground ecosystems. Natural experiments, which exploit spatial variability in the ecological effects of herbivores (Côté et al., 2004), vary in their design and can include evaluations of gradients in herbivore population density (Feeley and Terborgh, 2005; Horsley et al., 2003; Terborgh et al., 2001), gradients in grazing intensity (Smet and Ward, 2006) or gradients in the length of time since initial colonization by herbivores (Allombert et al., 2005; Vila et al., 2005, 2004b). Island comparisons can serve as ideal natural experiments when predators are restricted to nearby mainland areas, resulting in islands with isolated herbivore populations (Feeley and Terborgh, 2005), or when islands themselves vary in the presence or absence of an herbivore (Allombert et al., 2005). Natural experiments involving island comparisons, however, can be limited by confounding effects that may be correlated with the presence or density of herbivores on a given island, or by autocorrelation between samples due to pseudoreplicated sampling designs (Hurlbert, 1984). Island comparisons may also be influenced by patterns of biodiversity associated with the area or isolation of an island (MacArthur and Wilson, 1967), such as relationships between island size and plant species richness (Feeley and Terborgh, 2005).

Manipulative experiments often compare the presence or absence of herbivores in paired plots through the construction of herbivore exclosures in areas that were previously exposed to grazing (Burke et al., 2011; Goetsch et al., 2011). An alternative method is the use of controlled gradients in grazing intensity or population density (García et al., 2012; Horsley et al., 2003). Because herbivore exclosure studies use statistically independent replicates in which the explanatory variable is assigned randomly or systematically to each plot, experiments using herbivore exclosures are statistically more robust than natural experiments. The use of paired samples between plots that were ecologically similar to one another prior to the construction of the exclosures further strengthens the explanatory power of these experiments. However, herbivore exclosures suffer from severe limitations that make them inferior to island comparisons in many ways. Species that were already present in an exclosure prior to its construction may continue to occupy or modify niches, thereby preempting the establishment of other species (Fukami, 2015). Without enough time for significant successional change to have occurred,

herbivore exclosures may be temporally limited by their prior conditions – a phenomenon known as priority or legacy effects (Weidlich et al., 2016). Thus, manipulative experiments using herbivore exclosure are testing the effect of removing an herbivore, whereas natural experiments such as island comparisons are generally testing the effect of introducing an herbivore (Chollet et al., 2014). As small patches within a larger matrix grazed by ungulates, herbivore exclosures are also spatially limited by the biotic and abiotic conditions of their environment – edge effects. Herbivore exclosures vary considerably in their size but tend to be relatively small, with diameters ranging between 2 m (Chen et al., 2013), 10 m (Sabo et al., 2017), 14 m (Burke et al., 2011) and 25 m (Martin and Baltzinger, 2002), although some can also be as large as 2 ha in area (Goetsch et al., 2011). In the study of soil fungi, smaller exclosures may be particularly influenced by the edge effects of the surrounding fungal community. For example, ectomycorrhizal genets, which range in size from less than 1 m to more than 40 m in diameter (Beiler et al., 2010), could potentially extend from the surrounding soil and underlay an entire herbivore exclosure. The successional development of communities within herbivore exclosures is also limited by the ability of organisms to disperse to the exclosures through the surrounding matrix, presumably influenced by the excluded herbivores.

In summary, natural experiments such as island comparisons are typically used to evaluate the effects of introducing aboveground herbivores to large ecosystem patches, while manipulative experiments involving herbivore exclosures are used to test the effects of removing herbivores from small ecosystem patches.

1.3 Sitka Black-Tailed Deer on Haida Gwaii

Haida Gwaii, an archipelago located approximately 50 km off the mainland coast of British Columbia, is the historical, ancestral and unceded territory of the Haida Nation. The lowland temperate rainforests of Haida Gwaii are dominated by mixed stands of western hemlock (*Tsuga heterophylla*), Sitka spruce (*Picea sitchensis*) and western redcedar (*Thuja plicata*), with minor contributions of Alaska yellow cedar (*Cupressus nootkatensis*), shore pine (*Pinus contorta*), red alder (*Alnus rubra*), mountain hemlock (*Tsuga mertensiana*), and Pacific yew (*Taxus brevifolia*) (Banner et al., 2014; Pojar et al., 1991). The understory on most islands of Haida Gwaii, however, is sparsely developed due to browsing by invasive deer, with the forest floor dominated by mosses and other bryophytes (Banner et al., 2014; Chollet et al., 2013). Sitka black-tailed deer were first introduced to Haida Gwaii at the north end of the

archipelago in 1878 and quickly spread south across Graham Island and Moresby Island (Foster, 1963; Golumbia et al., 2008). Further introductions of Sitka black-tailed deer by the BC Game Commission and the Royal Canadian Navy continued until 1925 (Golumbia et al., 2008; Sharpe, 1999). By the middle of the 20th century, Sitka black-tailed deer had colonized nearly every island of the archipelago (Golumbia et al., 2008). Recent population densities of Sitka black-tailed deer were estimated to range from 13 to 30 deer per km^2 (Martin and Baltzinger, 2002), often higher than the population densities of this species in its native range in Alaska and British Columbia (Sharpe, 1999). A recent deer eradication program on Ramsay Island by Parks Canada yielded estimates of 25 deer per km^2 (R. Irvine, pers. comm. 2018). To date, out of the hundreds of islands in the archipelago, there are only 8 known islands that Sitka black-tailed deer have not colonized, comprising a total areas of less than 50 ha (Golumbia et al., 2008), including the islands of Agglomerate, Lost, Low, South Low and the Tar Islands. Several of these non-invaded islands are present in the regions of Laskeek Bay and the northeast part of the Gwaii Haanas National Park Reserve, National Marine Conservation Area Reserve, and Haida Heritage Site, where previous studies have used natural experiments to explore the effects of Sitka black-tailed deer on aboveground organisms such as the birds, vascular plants and bryophytes of these forest ecosystems. The effects of the deer invasion on the plant community has cascading effects of other organisms. For example, islands invaded by Sitka black-tailed deer have higher abundance and diversity of bryophytes than non-invaded islands (Chollet et al., 2013), likely by removing competition from the higher plants that are consumed by the deer (Stockton, 2004; Stockton et al., 2005). For example, the abundance and diversity of songbirds is significantly lower on deer-invaded islands, particularly for species that nest on the ground or in the forest understory (Chollet et al., 2016, 2014). The diversity and abundance of insects are also lower on deer-invaded islands (Allombert et al., 2005).

While the effects of invasive deer on the aboveground subsystem has been extensively studied on Haida Gwaii, there has been little research on how Sitka black-tailed deer may directly or indirectly affect the belowground elements and processes of these forests. As previous research on the aboveground-belowground interactions of ungulate herbivory has focused on grasslands, savannahs and temperate deciduous forests, the close proximity of several non-invaded and deer-invaded islands in the regions of Gwaii Haanas and Laskeek Bay provides an opportunity to expand our knowledge of how aboveground herbivores affect the abundance, diversity and composition of soil fungal communities in temperate conifer forests. This thesis presents a natural experiment in which I compared the soil physiochemical properties, understory plants and soil fungi on islands invaded or not invaded by Sitka black-tailed deer.

1.4 Research Question and Objectives

How do the invasive Sitka black-tailed deer affect the abundance, diversity and composition of the soil fungal communities on Haida Gwaii? To address this fundamental research question, several investigative objectives were developed which guided the design and implementation of the experiment.

Objective 1: to determine the physical and chemical properties of soils on islands invaded or not invaded by Sitka black-tailed deer, including the depth of the organic horizons, penetration resistance, pH, and the concentrations of ammonium, nitrate and phosphate, in Gwaii Haanas and Laskeek Bay.

Objective 2: to determine the abundance, diversity and composition of the understory plant community on islands invaded or not invaded by Sitka black-tailed deer in Gwaii Haanas and Laskeek Bay.

Objective 3: to determine the biomass, diversity and composition of the soil fungal communities on islands invaded or not invaded by Sitka black-tailed deer in Gwaii Haanas and Laskeek Bay.

Objective 4: to determine the growth response (biomass, root:shoot ratios), stress response (chlorophyll fluorescence) and inoculum potential (root colonization by arbuscular mycorrhizal fungi, dark septate endophytes and ectomycorrhizal fungi) in *Thuja plicata* and *Tsuga heterophylla* seedlings grown in soil from non-invaded and deer-invaded islands in the regions of Gwaii Haanas and Laskeek Bay.

Objective 5: to determine the extracellular activities of phosphatase, β-glucosidase and cellobiohydrolase in soils from islands invaded or not invaded by Sitka black-tailed deer in the regions of Gwaii Haanas and Laskeek Bay.

1.5 Thesis Outline

In Chapter 2 of this thesis, I describe the design of this natural experiment, including a description of the study area in the archipelago of Haida Gwaii and the general survey and sampling methods used in the field.

In Chapter 3, I describe the physical and chemical properties of the soils on deer-invaded and non-invaded islands (Objective 1). These edaphic properties are then used later in Chapter 5 as potential explanatory variables for the shifts in the fungal community structure.

In Chapter 4, I then describe how the cover-abundance, diversity and composition of the plant community varies between deer-invaded and non-invaded islands (Objective 2). I then identity individual plant species that are significant indicators for the presence or absence of Sitka black-tailed deer, which I use as potential explanatory variables for differences in the fungal community described in the next chapter.

In Chapter 5, I begin to address the fundamental research question by describing how the biomass, diversity and composition of soil fungal communities differ between deer-invaded and non-invaded islands (Objective 3). In addition to comparing the relative abundance of soil functional groups such as ectomycorrhizal and ericoid mycorrhizal fungi, I provide exploratory analysis of potential explanatory variables among the cover-abundance and diversity metrics of the plant community, as well as the physiochemical soil properties described in the previous chapters. I also discuss taxonomic differences between in the fungal communities of deer-invaded and non-invaded islands at both broad and fine scale phylogenetic resolutions.

In Chapter 6, I present the findings of a greenhouse bioassay used to determine the inoculum potential of three major function groups of fungi: ectomycorrhizal fungi, arbuscular mycorrhizal fungi and dark septate endophytes. I discuss how differences in the abiotic and biota properties of the soils from deer-invaded and non-invaded islands affect the growth response, stress response and mycorrhizal root colonization of two species of conifer trees, *Tsuga heterophylla* and *Thuja plicata* (Objective 4).

In Chapter 7, the analysis shifts towards an assessment of how potential enzyme activity varies between the soils of deer-invaded and non-invaded islands. I present the findings of an experiment in which I measured the potential activities of enzymes involved in nutrient acquisition and soil decomposition (Objective 5).

Finally, in Chapter 8, I conclude the thesis by summarizing the findings of this study, discussing the limitations of this research and proposing new hypotheses for further research.

CHAPTER 2 – Study Area and Experimental Design

2.1 Study Area

2.1.1 Islands

To determine the effects of Sitka black-tailed deer on the soils, plants and fungi of Haida Gwaii, a natural experiment was designed comparing deer-invaded islands with non-invaded islands in two regions: Laskeek Bay (N 52.85°, W 131.60°) and the Gwaii Haanas National Park Reserve, National Marine Conservation Area Reserve, and Haida Heritage Site – henceforth referred to as Gwaii Haanas (N 52.62°, W 131.43°). This thesis presents data from 27 plots sampled on 3 islands in Gwaii Haanas, and 22 plots sampled on 6 islands in Laskeek Bay (Table 2.1). Although Lost Island is within the bounds and jurisdiction of Gwaii Haanas, for the purposes of this research, Lost Island was considered part of the Laskeek Bay region due to its proximity to the other islands of Laskeek Bay and their shared bedrock type (Brown, 1968).

Table 2.1: Study site characteristics, including area in hectares (ha), number of plots, latitude, longitude and estimated deer population density at time of sampling.

Region	Island	Abbr.	Area (ha)	Plots	Latitude	Longitude	Deer per km^2
Gwaii Haanas	Agglomerate	AGG	22.9	7	52°37'40"	-131°25'24"	0
	Ramsay	RAM	1,623	14	52°33'30"	-141°23'07"	25
	Tar	TAR	6	6	52°39'37"	-131°25'14"	0
Laskeek Bay	Lost	LOS	5.3	3	52°48'12"	-131°29'17"	0
	Louise	LOU	>35,000	5	52°52'59"	-131°40'50"	11.5
	Low	LOW	9.6	5	52°54'32"	-131°32'10"	0
	Reef	REE	249	6	52°52'16"	-131°31'18"	8.3
	South Skedans	SSK	5.6	1	52°57'08"	-131°34'11"	12.5

Island areas from: (Catomeris, 2018; Stockton, 2004; Vila et al., 2004b). Deer population density estimated based on pellet counts and a Parks Canada survey of the deer population on Ramsay Island (R. Irvine, personal communication 2018).

2.1.2 Browsing History

The islands invaded by Sitka black-tailed deer vary in their browsing histories – the length of time since initial colonization and the intensity of the grazing pressure. On South Skedans Island, the earliest fraying scars on *Salix* spp. and *Alnus rubra* were dated to approximately 1985 and the frequency of fraying scars continued to increase until 1990 (Vila et al., 2004a). Based on this evidence, South Skedans and West Skedans were likely exposed to high browsing pressure for approximately 25 to 30 years prior to the sampling conducted for this current study. In contrast, browsing pressure has been high enough on Louise Island to reduce the stem regeneration of red huckleberry (*Vaccinium parvifolium*) as early as 1975, approximately 40 years prior to this study (Vila et al., 2004b). The dendrochronology of fraying scars on cedar, alder and willow suggests deer colonized Reef Island by 1952 (Vila et al., 2004a). The earliest detectable effect of deer browsing on the stem regeneration of red huckleberry on Reef Island and Ramsay Island occurred between 1940 and 1950 (Vila et al., 2004b), approximately 65 to 75 years prior to the sampling conducted on those islands for the current study. However, the age-class distribution of stems from salal (*Gaultheria shallon*) show that stem regeneration for this species began to decline at least 10 years earlier on Reef Island than on Ramsay Island (Vila et al., 2005) which suggests that Sitka black-tailed deer reached hyperabundant densities earlier in Laskeek Bay than in the areas of Gwaii Haanas sampled for this study.

Reef Island is unique among the deer-invaded islands because it was the site of multiple deer culls (Reimchen et al., 2008), which ultimately were unsuccessful in fully eradicating the resident deer population. Between 1997 and 2003, 85 deer were killed on Reef Island, with 5 deer estimated to have survived (Gaston et al., 2008). The abundance of understory vascular plants such as *Gaultheria shallon*, *Menziesia ferruginea* and *Vaccinium parvifolium* increased as a response to less browsing pressure (Gaston et al., 2008), but the deer population has since rebounded on Reef Island to approximately 23% of pre-cull levels based on density estimates in the current study (Table 2.1). Therefore, Reef Island was classified as a deer-invaded islands in subsequent data analysis in this thesis.

2.1.3 Anthropogenic Plant Communities

Haida Gwaii has been dominated by relatively stable temperate rainforest vegetation for at least 1800 years (Lacourse et al., 2007). The study site is located in the rainshadow of the Queen Charlotte Mountains in the Coastal Western Hemlock Wet Hypermaritime subzone (CWHwh1) of the Biogeoclimatic Ecosystem Classification (BEC) system (Banner et al., 2014). Unlike mainland CWH

subzones, Pacific silver fir (*Abies amabilis*) and Douglas-fir (*Pseudotsuga menziesii*) are conspicuously absent from these forests, dominated instead by *Tsuga heterophylla*, *Picea sitchensis* and *Thuja plicata*. Less frequently occurring tree species include yellow cedar (*Cupressus nootkatensis*), shore pine (*Pinus contorta*), red alder (*Alnus rubra*), mountain hemlock (*Tsuga mertensiana*), and Pacific yew (*Taxus brevifolia*) (Banner et al., 2014). In mature low elevation forests, the understory is dominated by shrubs such as red huckleberry (*Vaccinium parvifolium*), salal (*Gaultheria shallon*), oval-leaved blueberry (*Vaccinium ovalifolium*), false azalea (*Menziesia ferruginea*), twinberry (*Lonicera involucrata*), salmonberry (*Rubus spectabilis*), as well as ferns such as deer fern (*Blechnum spicant*), spiny wood fern (*Dryopteris expansa*) and sword fern (*Polystichum munitum*) (Golumbia, 2007; Pojar, 2008).

Despite late-successional forests of Haida Gwaii being described as "primary forests" (Daufresne and Martin, 1997) or "old growth forests" (Banner et al., 2014), a wide diversity of evidence suggests these forests are anthropogenic plant communities that have been extensively managed by the Haida people for thousands of years. Pollen and plant macrofossils extracted from lake sediments in the southern Haida Gwaii reveal a significant decline in the pollen of Cupressaceae beginning around 1000 BP, suggesting an intensification of the harvesting of *Thuja plicata* (Lacourse et al., 2007) which was and continues to be used by the Haida as a material for construction, transportation, clothing, art and other technology (Snyder, 1979). A map of Haida place names shows at least 3 historical village sites and other culturally significant locations on or nearby the islands of the study site (Bringhurst, 2011), suggesting that these regions were well-populated prior to contact with European Americans. In the current study, culturally modified trees with signs of bark stripping were identified on several islands in Laskeek Bay and Gwaii Haanas (Figure 2.1). Previous research identified potato (*Solanum tuberosum*) on Reef Island, suggesting that plant cultivation was common in the study area (Gaston et al., 2008). Indeed, pre-contact plant cultivation was ubiquitous among First Nations throughout the Pacific Northwest (Deur and Turner, 2005).

Figure 2.1: Culturally modified trees in the study site

2.1.4 Soils

A map of soil orders using the Canadian System of Soil Classification identifies the lowland soils of the study area as Podzols (Soil Landscapes of Canada Working Group, 2011). Parent materials of the mineral horizons have a diverse range of potential influences, including weathered regolith, glacial till, glacial outwash and marine deposits. Islands surveyed in the Laskeek Bay region share the Yakoun Formation as a common bedrock type, comprised of porphyritic andesite agglomerate, calcacerous scoriaceous lapilli tuff, volcanic sandstone, conglomerate, tuffaceous shale and coal (Brown, 1968). Islands surveyed in the Gwaii Haanas region share the Masset Formation as a common bedrock type, comprised of subaerial basalt flows, breccias, rhyolite ash flows, dacite, and dolerite (Brown, 1968). Parent material may also be comprised of glacial deposits layered over bedrock. Haida Gwaii was extensively glaciated during the Fraser glaciation, with lowlands covered by locally generated glaciers in equilibrium with the Cordilleran ice sheet in the Hecate lowlands (Brown, 1968). Therefore the parent material of the uppermost mineral horizons in both Laskeek Bay and Gwaii Haanas may consist of layers

of sandy glacio-fluvial outwash layered over glacial ablation and basal till (Brown, 1968). Marine deposits may have also influenced the soil parent materials of our study sites. During the Fraser glaciation and deglaciation periods, sea levels fluctuated widely due to glacial loading and rebound (Brown, 1968). The presence of marine fossils upland from the present shoreline suggests that at certain times, the sea level was at least 8 m higher than present levels (Brown, 1968). Paleoshorelines from 9,200 to 3,000 BP have been identified as high as 15 m above present sea level (Fedje and Christensen, 1999).

2.2 Experimental Design

2.2.1 Sampling Times and Locations

Field work occurred in August 2016 and July 2017 for Gwaii Haanas and Laskeek Bay, respectively. Penetrometer data was collected in 2017 for both regions. Soils were sampled in Gwaii Haanas during August 2016 and in Laskeek Bay during July 2017. The two regions were sampled and analyzed independently due to the potential differences in their soil parent material (Brown, 1968).

Previous research on the deer invasion was used as a preliminary method for identifying non-invaded islands to serve as appropriate negative controls, such as Lost Island or the Tar Islands (Vila et al., 2005, 2004b). However, an *a priori* decision was made to analyze data by classifying islands as deer-invaded or non-invaded based on observed signs of deer, including tracks, fewmishings (dung), fraying marks on trees (Edward of Norwich, 1406), and the presence or absence of an apparent browse line approximately 1.1 m above the ground, (Vila et al., 2003). Personal observations revealed that signs of deer were present on all plots on islands classified as deer-invaded. Similarly, deer signs were completely absent from the plots located on islands later classified as non-invaded. Of the 49 total plots used for this thesis, 21 plots were non-invaded and 28 plots were deer-invaded. Sample sizes in each treatment group are summarized in Table 2.2. Plots were randomly located on each island given the following constraints:

- Culturally sensitive places were avoided.
- Areas identified by Parks Canada or the Archipelago Management Board as conservation priorities were avoided.
- Areas with unusually low canopy cover (<25%) likely associated with stochastic processes or the historical resource management of the Haida were avoided.

- Areas that could be hazardous to work in, such as steep slopes or large blow downs, and areas that were difficult to access due to hazardous sea conditions were avoided.
- On deer-invaded islands, forested wetlands were avoided due to these ecosystem types being absent on non-invaded islands.

Table 2.2: Sample size (plots) of each treatment group.

	Gwaii Haanas	Laskeek Bay
Non-invaded	13	8
Deer-invaded	14	14

2.2.2 Sampling Method

Each plot was square-shaped with side lengths of 20 m for a total area of 400 m^2. Understory vascular plants were surveyed as described in Chapter 4. The soil profile was described to a depth of at least 50 cm from the surface of the uppermost organic horizon using a single soil pit in each plot. Where feasible, the depths of all organic soil horizons were noted, including the Oi horizons (slightly decomposed organic material), Oe horizons (organic material of intermediate decomposition) and Oa horizons (humic, highly decomposed organic material, Soil Survey Staff, 2014, 1999). To measure moisture content, a single sample was taken from the Oe horizon, equivalent to the F layer of the Canadian System of Soil Classification, respectively (Soil Classification Working Group, 1998). A pocket penetrometer was used to measure penetration resistance on the upper surface of the Oi horizon for all islands with the exception of Agglomerate Island which we were not allowed access to in 2017. Penetration resistance was measured using 50 technical replicates per plot. Soils were sampled for all other laboratory analyses using a 3 cm diameter probe to a depth of 30 cm in the organic horizons in 50 – 100 random locations in each plot. The Oi and Oa horizons were separated from the soil cores and only soil from the Oe horizon (F layer) was used for subsequent laboratory analysis. The Oe subsamples were combined into a single composite sample per plot. Soils from Gwaii Haanas were sampled in August of 2016 and were kept in cool conditions at approximately 4°C for 2 to 3 weeks. Soils from Laskeek Bay were sampled in July of 2017 and were kept in cool conditions at approximately 4°C for 3 to 4 weeks. Samples were sieved to 4 mm to further homogenize the soils and to remove longer root

fragments and wood. Samples were then stored at -20°C until subsequent use. Laboratory analyses on soils are described in the methods sections of Chapters 3, 5, 6 and 7.

CHAPTER 3 – Physical and Chemical Soil Properties of Deer-invaded and Non-invaded Islands

3.1 Introduction

3.1.1 Background

By altering the abiotic properties of the soil, such as pH, compaction or nutrient concentrations, aboveground herbivores may indirectly alter the abundance, diversity and composition of the soil fungal community (Tedersoo et al., 2014; Treseder, 2004). Perhaps the most direct way large aboveground herbivores affect soils is through their physical trampling of the ground which can lead to greater soil compaction. Most studies find the presence of herbivores to be associated with significantly greater soil compaction (Kumbasli et al., 2010; Sabo et al., 2017; Sharrow, 2007), which can be interpreted through measurements of soil bulk density, penetration resistance, total porosity or the depth of the upper soil horizons. For example, excluding white-tailed deer (*Odocoileus virginianus*) for as little as 2 years in temperate deciduous forests in Indiana resulted in significantly lower soil compaction compared with areas exposed to grazing (Shelton et al., 2014). Similarly, in forests dominated by sugar maples (*Acer saccharum*) in Wisconsin and Michigan, there was significantly greater penetration resistance and thinner E horizons in areas exposed to grazing by white-tailed deer compared with soils where the herbivores had been excluded for 10-20 years (Sabo et al., 2017). Based on the results of these previous studies, Sitka black-tailed deer likely increase the compaction of the soils on Haida Gwaii.

As a major driver of the diversity and structure of soil fungal communities (Tedersoo et al., 2014), pH is a crucial soil property to measure in the study of herbivore-soil interactions. Shifts in the composition of plant communities as a result of grazing by herbivores has the potential to both increase or decrease the pH of soil organic matter due to interspecific variation in the foliar pH of plants (Cornelissen et al., 2011, 2006). As an alternative mechanism, mammalian herbivores can more directly influence soil pH through nutrient inputs from their urine. Following the deposition of urea fertilizers or urine from sheep or cattle, soil pH may initially increase, but then tends to slowly decline during the nitrification process (Somda et al., 1997) as hydrogen ions are released through the subsequent oxidation of ammonium. Previous research has shown herbivores to have inconsistent effects on soil pH. Grazing by livestock such as sheep and cattle has been observed to increase soil pH in some grassland ecosystems (Wang et al., 2018), but in other grasslands studies, soils from grazed areas have lower pH compared with the soils where aboveground herbivores have been excluded (Hatton and

Smart, 1984). Other studies have found deer and other ungulates to have no significant effect on soil pH (Relva et al., 2014; Sabo et al., 2017). Based on these inconsistencies, it is difficult to predict how Sitka black-tailed deer would affect the pH of soils in Haida Gwaii.

Previous research has revealed the presence of aboveground herbivores to be associated with either increases or decreases in the concentrations of available nitrogen or phosphorus. In other cases, no significant differences in these soil nutrients are found. In a post-fire chronosequence comparing soils inside and outside of fenced plots excluding elk (*Cervus canadensis*), total nitrogen content was significantly lower in plots exposed to the herbivores (Stritar et al., 2010). Similarly, a study in the Scottish Highlands found lower nitrate and ammonium concentrations in plots exposed to grazing by red deer (*Cervus elaphus*) compared with plots inside herbivore exclosures (Harrison and Bardgett, 2004a). However, excluding invasive deer (*Cervus elaphus, Dama dama*) for 7 years in Patagonia found no significant difference in total nitrogen inside and outside of herbivore exclosures (Relva et al., 2014). Excluding deer for 4.5 years in a temperate hardwood forest in Pennsylvania did not result in significant differences in the concentrations of ammonium, nitrate or phosphate compared with soils outside of the herbivore exclosures (Burke et al., 2011). Likewise, a study comparing plots inside and outside of herbivore exclosures in the forests of Wisconsin and Michigan found no significant differences in extractable phosphorus, extractable potassium nor total nitrogen between soils inside and outside of the exclosures (Sabo et al., 2017). Excluding large herbivores for 24 years from a grassland in Uganda resulted in a significant increase in extractable phosphorus (Hatton and Smart, 1984). However, applications of dung from deer, sheep and cattle to a pasture in New Zealand resulted in higher phosphate and nitrate in the soil compared with untreated control plots (Williams and Haynes, 1995). Based on these studies, it is also difficult to predict the effects of Sitka black-tailed deer on soil nutrients, but they demonstrate the potential for deer to be associated with lower concentrations of ammonium and nitrate and potentially higher concentrations of phosphate.

The effects of Sitka black-tailed deer on the physiochemical soil properties of Haida Gwaii remain unclear. Consequently, it is imperative to assess the physical and chemical soil properties when evaluating potential links between aboveground herbivores and soil fungal communities. To improve our understanding of how aboveground herbivores affect the abiotic soil properties of temperate conifer forests, this chapter compares the physiochemical properties of soils from islands invaded or not invaded by Sitka black-tailed deer in the regions of Gwaii Haanas and Laskeek Bay. Soil properties that were analyzed included the depth of the organic horizons, penetration resistance, pH, available nitrogen

and available phosphorus. I expected the physical trampling of the soil by Sitka black-tailed deer to result in shallower organic horizons and higher penetration resistance on the soil surface. Based on the findings of previous research, I predicted that ammonium and nitrate concentrations would be lower in soils from deer-invaded islands while phosphate concentrations would be higher.

3.1.2 Objective and Hypotheses

The objective of the research presented in this chapter was to determine the physical and chemical properties of soils on islands invaded or not invaded by Sitka black-tailed deer, including the depth of the organic horizons, penetration resistance, pH, and the concentrations of ammonium, nitrate and phosphate, in Gwaii Haanas and Laskeek Bay. The following hypotheses were tested:

- H_0 The presence of Sitka black-tailed deer on the islands is not associated with differences in the physical or chemical properties of the soil.
- H_1 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the depth of the organic horizons.
- H_2 The presence of Sitka black-tailed deer on the islands is associated with an increase in soil penetration resistance.
- H_3 The presence of Sitka black-tailed deer on the islands is associated with a difference in soil pH.
- H_4 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the concentration of ammonium in the soil.
- H_5 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the concentration of nitrate in the soil.
- H_6 The presence of Sitka black-tailed deer on the islands is associated with an increase in the concentration of phosphate in the soil.

3.2 Methods

3.2.1 Laboratory Analysis

Field methods for determining penetration resistance, the depth of the organic horizons and for sampling soils for subsequent laboratory analyses are described in Chapter 2. Gravimetric moisture content was determined by measuring mass before and after oven-drying samples at 70°C for 48 hours. pH was measured using a 1:10 ratio by mass of air-dried soil and a solution of 0.01 M $CaCl_2$. Frozen soil samples were submitted to the BC Ministry of Environment & Climate Change Strategy Analytical

Laboratory for analysis of extractable ammonium and nitrate (Mulvaney, 1996), with moisture content measured to estimate concentrations of ammonium and nitrate on a dry mass basis. Extractable phosphate was determined using the Bray P-1 extraction method (Kuo, 1996) adapted for 96-well microplates (Corning, product no. C3904) as follows. After filtering through Whatman 42 filter paper (Sigma-Aldrich, product no. WHA1442125), 20 µL of extraction solution were added to each well along with 30 µL of 0.012 M molybdate solution, 15 µL of 0.06 M stannous chloride solution and 200 µL of deionized water, with 8 technical replicates per sample. Absorbance was read at a wavelength of 660 nm using a TECAN Spark 10M spectrophotometer and concentrations were determined using a standard calibration curve of monopotassium phosphate.

3.2.2 Statistical Analysis

All data were analyzed in the R 3.4.2 programming language (R Core Team, 2017) with the *car* and *ggplot2* packages in RStudio (RStudio Team, 2016). Because deer-invaded islands were safer to access during stormy weather, differences in soil moisture content between non-invaded and deer-invaded islands were likely confounded by variable amounts of daily precipitation during fieldwork. Therefore, the *treatment* factor (non-invaded vs deer-invaded) was not used in the analysis of soil moisture content. For all other physiochemical soil properties, both the *treatment* and *region* factors were included in the analyses. Normality was tested with the Shapiro-Wilk test (Shapiro and Wilk, 1965) and equality of variances was tested with Levene's test (Levene, 1960). Two-factor ANOVA was used to analyze data based on the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay). For data with non-normal or heteroscedastic distributions, transformations noted below were used to meet the assumptions of ANOVA or a Mann-Whitney U test was used (Mann and Whitney, 1947).

For the phosphate concentrations and penetration resistance, a square root transformation was used to correct for heteroscedasticity and non-normal distributions:

$$X' = X^{\wedge 1/2}$$

To control for false discoveries, the Benjamini-Hochberg procedure was used with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995). To control the family-wise error rate associated with multiple comparisons, a Bonferroni correction was applied (Dunn, 1961) by adjusting the alpha using the following equation, with $\alpha = 0.05$ and k = the number of comparisons:

$$\alpha' = \alpha/k$$

3.3 Results

3.3.1 Physical Properties

The cumulative depth of the organic soil horizons was significantly higher on non-invaded islands compared with deer-invaded islands ($F_{(1,45)} = 72.901$, $p < 0.001$) and there was a significant interaction between *treatment* and *region* ($F_{(1,45)} = 8.402$, $p = 0.006$). The organic soil horizons of soils from non-invaded islands had a combined depth with a mean of 40.5 cm ± 2.6 SE, while the organic horizons on deer-invaded islands had a combined depth with a mean of 19.0 cm ± 1.3 SE. In Gwaii Haanas, five out of thirteen plots on non-invaded islands had depths greater than 50 cm (Figure 3.1). There was no significant difference between the depth of the organic horizons between Gwaii Haanas and Laskeek Bay.

The penetration resistance of soils from deer-invaded islands (2.63 $kg \cdot cm^{-2}$ ± 0.07 SE) was more than two times higher than soils from non-invaded islands (0.74 $kg \cdot cm^{-2}$ ± 0.04 SE, $F_{(1,38)} = 486.44$, $p < 0.001$, Table 3.1, Figure 3.2). There was no significant difference in the penetration resistance of soils between Gwaii Haanas and Laskeek Bay and there was no significant interaction between the factors of *treatment* and *region*. Penetration resistance and the depth of the organic horizons were negatively correlated with each other (Pearson's correlation coefficient = -0.69, $R^2 = 0.48$, $p < 0.001$, Figure 3.3).

Soil moisture content was significantly higher in soils from Laskeek Bay (300.6% ± 11.4 SE) compared with Gwaii Haanas (156.8% ± 18.7 SE, $W = 63$, $p < 0.001$, Figure 3.4).

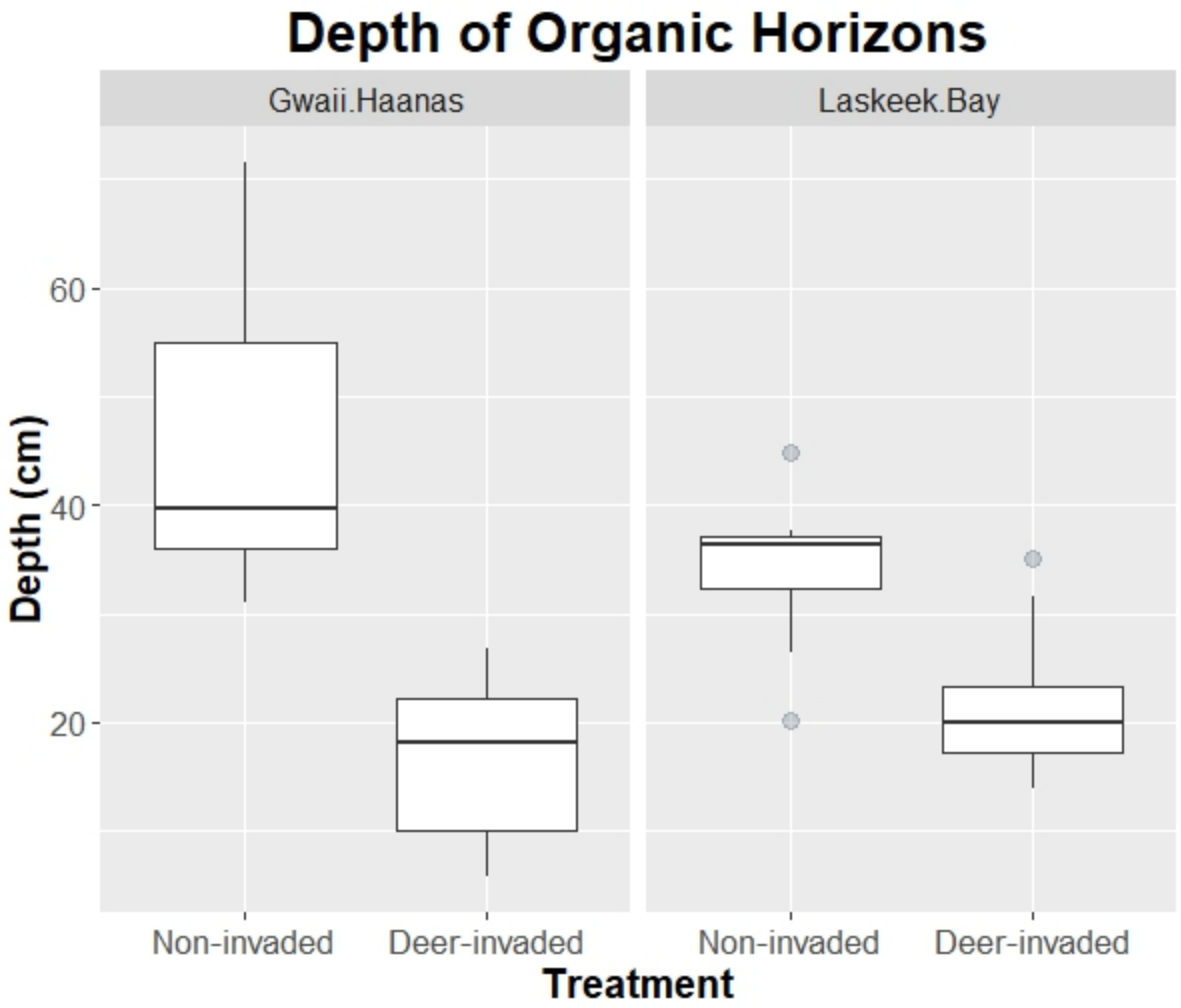

Figure 3.1: Depth of organic horizons of soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Table 3.1: Penetration resistance ($kg \cdot cm^{-2}$) in soils from non-invaded islands (n = 6, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE and omit data from Agglomerate Island.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	0.75 ± 0.06	0.73 ± 0.06
	Deer-invaded	2.75 ± 0.08	2.50 ± 0.10

Figure 3.2: Penetration resistance of the soil surface on non-invaded islands (n = 6, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

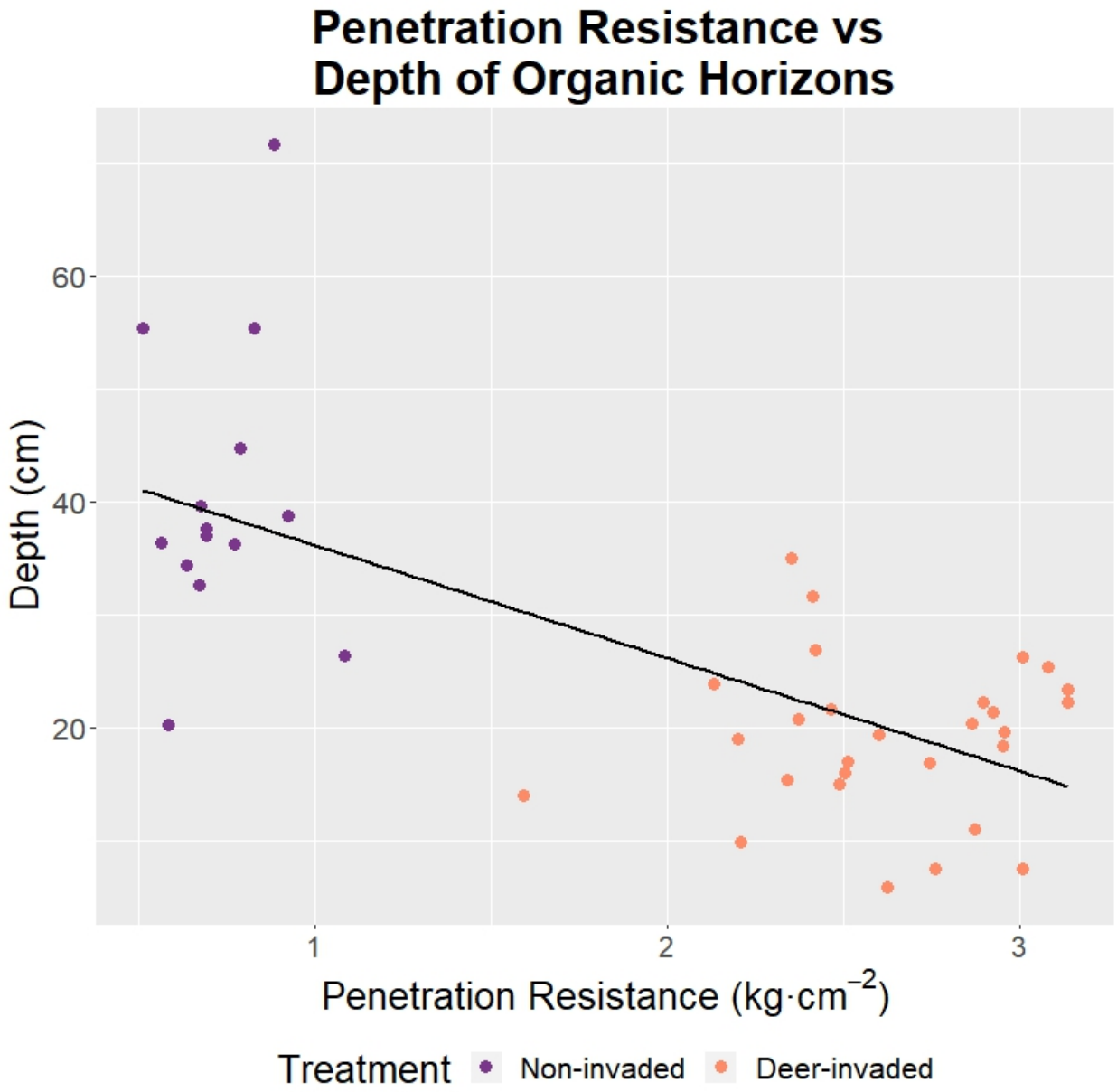

Figure 3.3: Correlation between the penetration resistance (kg·cm^{-2}) of the soil surface and the total depth of the organic horizons (cm) in soil from non-invaded islands (n = 6, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Data from Agglomerate Island are omitted. The line represents a linear regression between penetration resistance and depth.

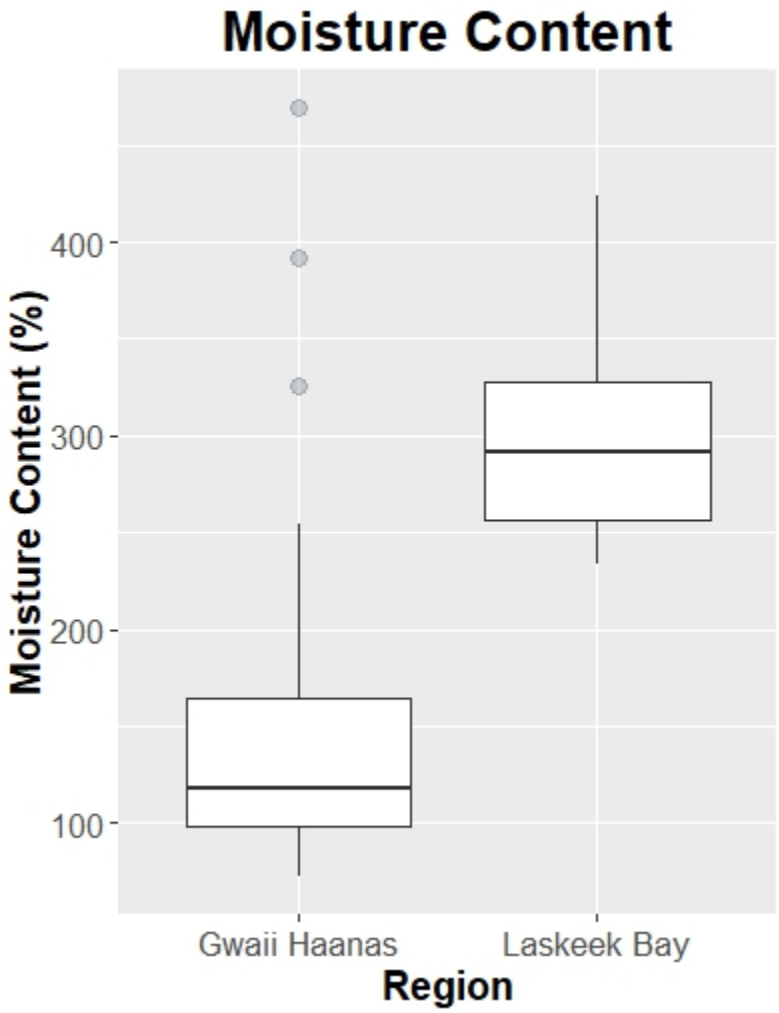

Figure 3.4: Moisture content of soils from islands of Gwaii Haanas (n = 27) and Laskeek Bay (n = 22). The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

3.3.2 Chemical Properties

The mean pH of soil from deer-invaded islands (3.68 ± 0.08) was significantly lower than the pH of soil from non-invaded islands (3.96 ± 0.07 SE, W = 440, p = 0.003). There was also a significant difference in the pH of soils in Gwaii Haanas (3.64 ± 0.06 SE) and Laskeek Bay (3.99 ± 0.09 SE, W = 134.5, p = 0.001, Table 3.2, Figure 3.5). As the assumptions of ANOVA could not be met, the interaction between the factors of *treatment* and *region* is not reported.

Table 3.2: pH of soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	3.85 ± 0.09	4.13 ± 0.09
	Deer-invaded	3.44 ± 0.05	3.91 ± 0.13

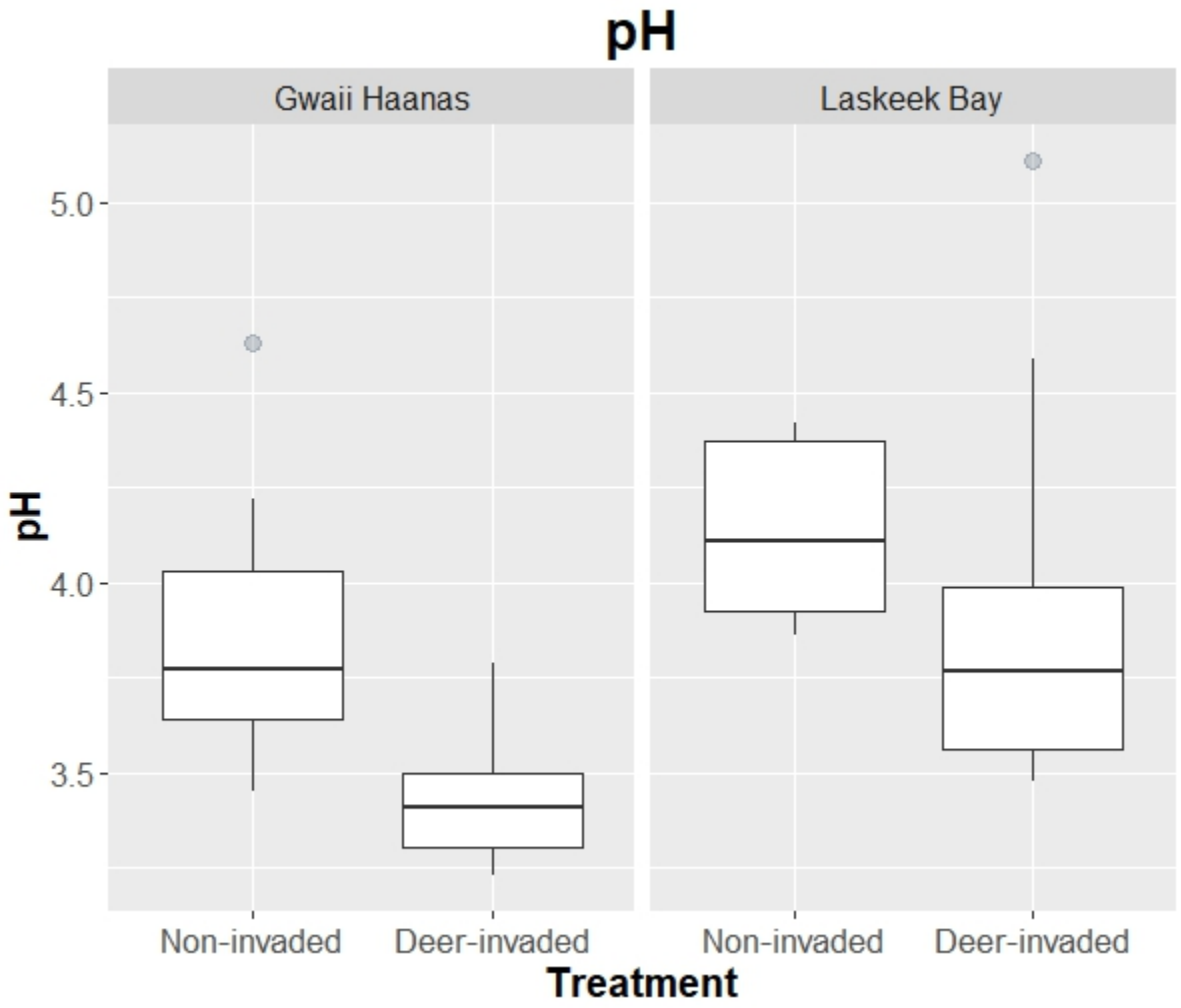

Figure 3.5: pH of soil from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Inorganic forms of nitrogen had similar concentrations in soils from non-invaded and deer-invaded islands. While the mean concentration of ammonium in soils from deer-invaded islands was 20% lower than in soils from non-invaded islands, the difference was not significant (W = 396, p-value = 0.053). The mean concentration of ammonium in soils from Laskeek Bay (53 $\mu g \cdot g^{-1}$ ± 6.6 SE) was significantly lower than in soils from Gwaii Haanas (75.8 $\mu g \cdot g^{-1}$ ± 2.8 SE, W = 491, $p < 0.001$, Figure 3.6). As the assumptions of ANOVA could not be met, the interaction between the factors of *treatment* and *region* is not reported.

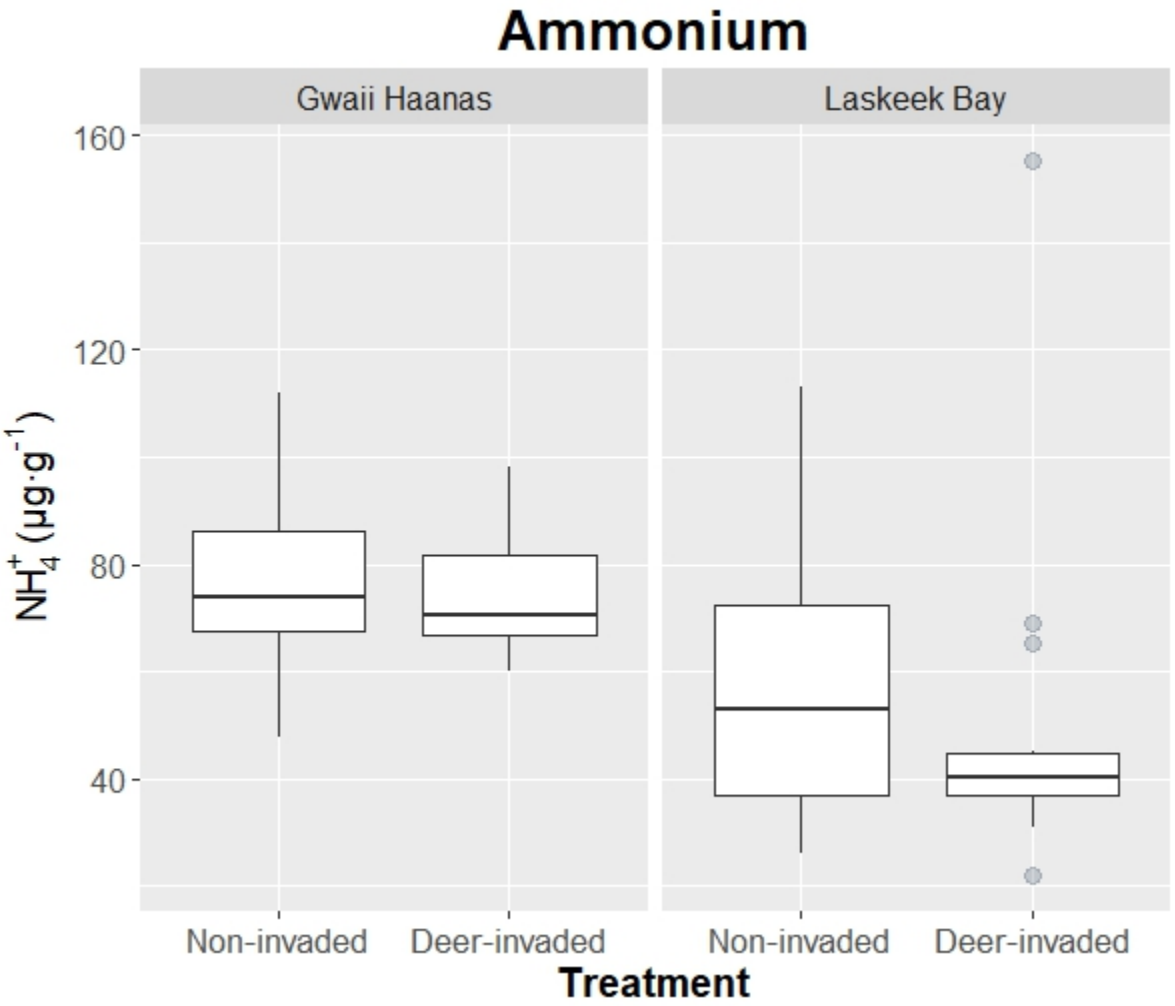

Figure 3.6: Ammonium concentrations in soil from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Likewise, there was no significant difference in the concentration of nitrate between soils from non-invaded and deer-invaded islands. However, mean concentration of nitrate in soils from Laskeek Bay (4.75 $\mu g \cdot g^{-1}$ ± 0.36 SE) was significantly higher than soils in Gwaii Haanas (3.26 $\mu g \cdot g^{-1}$ ± 0.26 SE, $F_{(1,45)}$ = 11.54, p = 0.001). There was no significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay).

The mean concentration of phosphate in soils from deer-invaded islands (10.12 $\mu g \cdot g^{-1}$ ± 0.8 SE) was significantly higher than in soils from non-invaded islands (7.54 $\mu g \cdot g^{-1}$ ± 0.59 SE, $F_{(1,45)}$ = 7.87, p = 0.007). There was no significant difference in the mean concentration of phosphate between soils from the regions of Gwaii Haanas and Laskeek Bay and there was no significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay, Table 3.3, Figure 3.7).

Table 3.3: Phosphate concentrations ($\mu g \cdot g^{-1}$) from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	6.65 ± 0.77	8.99 ± 0.65
	Deer-invaded	10.66 ± 1.40	9.58 ± 0.79

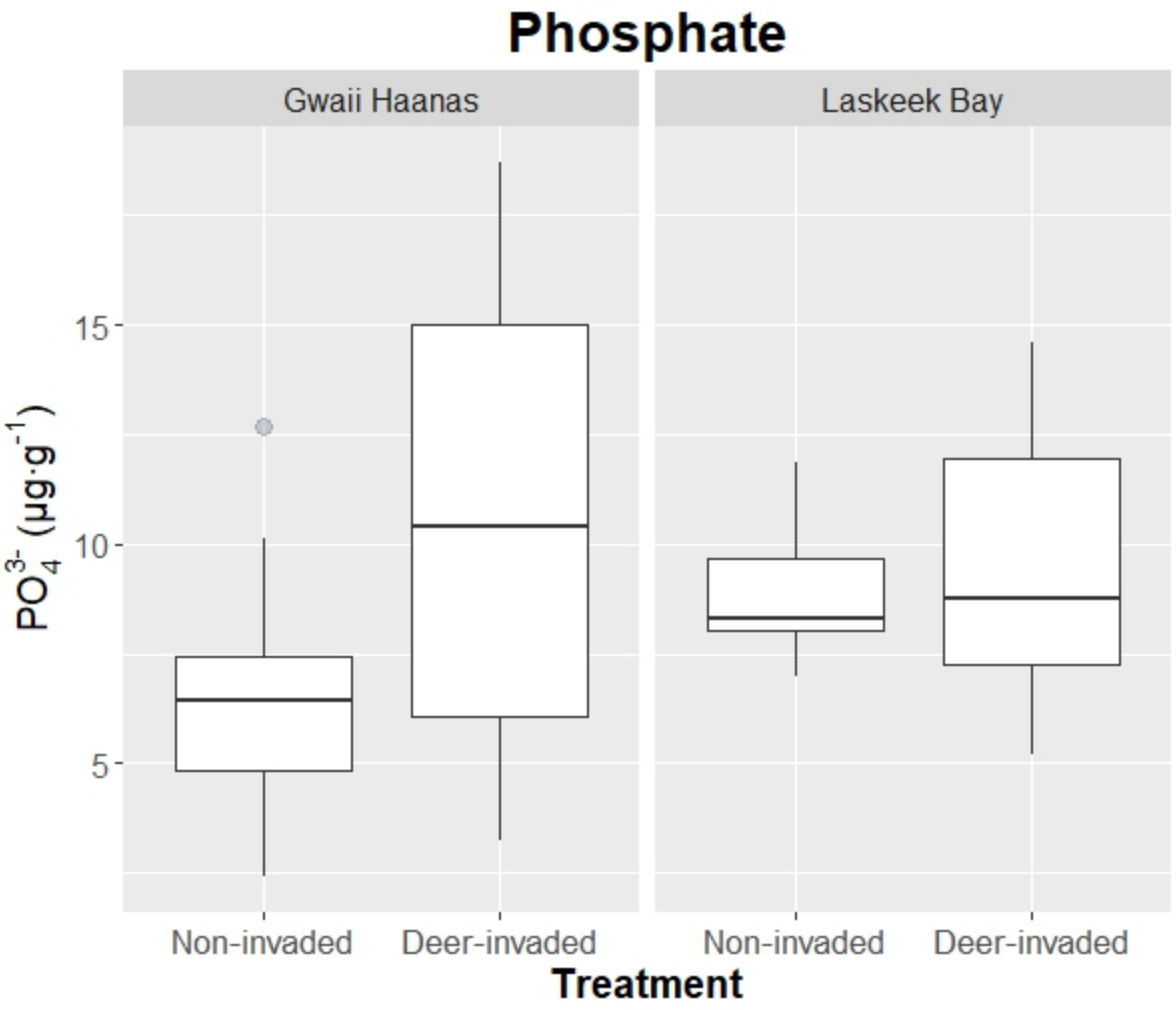

Figure 3.7: Phosphate concentrations in soil from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

3.4 Discussion

3.4.1 Physical Properties

Although the depth of the organic horizons was significantly correlated with soil surface penetration resistance, the linear regression between the two variables explained a relatively small portion of the variance, suggesting that other processes besides compaction are responsible for the difference in soil depth between non-invaded and deer-invaded islands. Shallower organic horizons on deer-invaded islands may be due in part to decreased root biomass as a result of aboveground herbivory by Sitka black-tailed deer. Severe defoliation can cause plants to reduce their resource allocation to roots (Eyles et al., 2009), resulting in less belowground plant biomass (Guo et al., 2012; Wang et al., 2017).

Soil compaction as measured through penetration resistance was higher on deer-invaded islands, confirming previous research which has found that large aboveground herbivores are often associated with higher soil compaction (Epelde et al., 2017; Kumbasli et al., 2010; Sabo et al., 2017; Sharrow, 2007). Soil compaction has a range of potential effects on soil biota. For example, soil compaction reduces pore space and belowground oxygen availability (Holden and Treseder, 2013) which can result in an increased abundance of anaerobic soil bacteria (Hartmann et al., 2014). Soil compaction can also decrease phosphorus uptake in plants (Nadian et al., 1998). A study on two forested sites in Switzerland found that soil compaction was associated with a decrease in the abundance of ectomycorrhizal fungi and an increase in the abundance of saprotrophic and pathogenic fungal taxa (Hartmann et al., 2014).

Soil moisture was significantly higher in the soils from Laskeek Bay compared with Gwaii Haanas, likely due differences in precipitation when those regions were sampled. Data from the nearby Sandspit weather station show that there were 45.9 mm of precipitation in July 2017 when the soils of Laskeek Bay were sampled, whereas there were only 18.7 mm of precipitation in August 2016 when the soils of Gwaii Haanas were sampled (Environment and Climate Change Canada, 2018).

3.4.2 Chemical Properties

Plant available forms of inorganic nitrogen (ammonium and nitrate) were similar between soils from non-invaded and deer-invaded islands. Similarly, analyses conducted by a colleague using a similar set of samples from Gwaii Haanas and Laskeek Bay revealed no significant differences in total nitrogen

or C:N ratios between soils from non-invaded and deer-invaded islands (Catomeris, 2018). Other studies have also found little relationship between aboveground herbivores and soil nitrogen concentrations. For example, after excluding reindeer for 10 years from sub-arctic meadows, one study found no significant differences in ammonium concentrations (Francini et al., 2014). Seasonal variation in nitrification rates associated with differences in soil moisture content may explain the differences in ammonium and nitrate concentrations between soils from Gwaii Haanas, sampled in August, and Laskeek Bay, sampled in July. A study on soils in a temperate deciduous forest in Pennsylvania found similar results, with significant differences in the concentrations of ammonium and nitrate in soils sampled in June and August of the same year (Burke et al., 2011). Nitrification rates tend to be positively correlated with soil moisture content up to certain thresholds (Dubey, 1968). Under warm conditions (20°C), nitrification rates reach a maximum when soil moisture is at field capacity (Maag and Vinther, 1996). Therefore, the higher soil moisture content in Laskeek Bay may have accelerated nitrification rates, resulting in less ammonium and higher nitrate than in the soils of Gwaii Haanas, sampled later in a drier time of year. Lower ammonium concentrations in the soils of deer-invaded islands in Laskeek Bay could be the result of greater volatilization of ammonia. For example, a study in Yellowstone National Park found that volatilization rates were higher in areas grazed by bison and elk compared with fenced areas (Douglas A Frank and Groffman, 1998). However, ammonium concentrations were similar between the deer-invaded and non-invaded islands of Gwaii Haanas, suggesting that differences in the rates of leaching or volatilization associated with the presence of Sitka black-tailed deer may vary between different times of the year.

Although pH was significantly lower in soils from deer-invaded islands compared with non-invaded islands, the difference was relatively small and is unlikely to strongly affect the abundance or diversity of the soil fungal community. For example, a study using liming treatments to create a pH gradient in the soils of a wheat field in the UK found the abundance of soil fungi to be unaffected by changes in pH, with fungal diversity only weakly related to the pH gradient (Rousk et al., 2010). Differences in pH between deer-invaded and non-invaded islands are more likely to affect the abundance, diversity and composition of the bacterial communities (Rousk et al., 2010).

However, higher phosphate concentrations on deer-invaded islands may have implications for the abundance of mycorrhizal fungi in those soils. Justus von Liebig's Law of the Minimum states that plant growth is limited not by the availability of total nutrients but by the scarcest resource (Gorban et al., 2011). Likewise, the plant investment hypothesis states that plants allocate resources to mycorrhizal

fungi as a response to limiting nutrients (Treseder, 2004). Experiments involving the anthropogenic deposition of nitrogen and phosphorus in fertilizers suggest that changes in nutrient availability can affect the diversity and abundance of mycorrhizal fungi (Bahr et al. 2013; Nouri et al. 2014; Treseder 2004; Zhang et al. 2011). A meta-analysis of mycorrhizal responses to nutrient additions found that mycorrhizal abundances decreased by of 32% under phosphorus fertilization (Treseder, 2004). For example, a study on arbuscular mycorrhizal fungi found that nitrogen starvation could negate the suppressive effects of phosphorus additions on mycorrhizal colonization in a *Petunia* species (Nouri et al., 2014). In the current study, phosphate was significantly higher in soils from deer-invaded islands, thus nitrogen availability may be an increasingly limiting resource for plants and microbial communities on deer-invaded islands.

3.5 Conclusion

The objective of the research presented in this chapter was to determine the physical and chemical properties of soils on islands invaded or not invaded by Sitka black-tailed deer, including the depth of the organic horizons, penetration resistance, pH, and the concentrations of ammonium, nitrate and phosphate. Soils from islands invaded by the Sitka black-tailed deer had significant differences in several physical and chemical properties compared with soils from non-invaded islands. The organic horizons were shallower and the penetration resistance was higher on deer-invaded islands, supporting the hypotheses that trampling by deer would increase the soil compaction, contributing to a decrease in the depth of the organic horizons. There were no significant differences in the concentrations of ammonium or nitrate between soils from deer-invaded and non-invaded islands, conflicting with the hypotheses that deer would be associated with increases in the concentrations of available forms of nitrogen. However, pH was significantly lower in soils from deer-invaded islands and phosphate was significantly higher in these soils, both supportive of the respective hypotheses that differences in pH would be associated with deer-invaded islands and that Sitka black-tailed deer would increase the concentrations of available phosphorus. Altogether, these results suggest that Sitka black-tailed deer are associated with distinct shifts in the physiochemical properties of the organic soil horizons of Haida Gwaii.

CHAPTER 4 – Plant Communities of Deer-Invaded and Non-Invaded Islands

4.1 Introduction

4.1.1 Background

Sitka black-tailed deer have well-documented effects on the plant communities of Haida Gwaii. A natural experiment comparing the plant communities in Laskeek Bay found the cover-abundance of understory plants on non-invaded islands to exceed 80%, whereas the cover-abundance of understory plants on deer-invaded islands was less than 10% (Stockton et al., 2005). Although the species richness of understory plants per plot was generally lower on deer-invaded islands, the effect of deer on plant species richness was greater in shoreline edge habitat than interior forest areas (Stockton et al., 2005). Long after invasive deer have initially colonized an island, their effects on vegetation can continue to intensify. Although Sitka black-tailed deer were likely present on Reef Island in Laskeek Bay since at least 1950 (Vila et al., 2004a), browsing pressure continued to increase after 1985, causing regenerating Sitka spruce (*Picea sitchensis*) to take increasingly longer amounts of time to grow beyond the browse line 1.1 m above the ground (Vila et al., 2002). Removing or reducing deer browsing can reverse the effects of deer on the diversity and abundance of understory plants. The culling of deer on Reef Island in Laskeek Bay between 1997 and 2003 resulted in a five-fold increase in the cover-abundance of understory shrubs in the interior forest plots (Chollet et al., 2016). The species richness of forbs, ferns and understory shrubs significantly increased following the deer culls (Chollet et al., 2016). Similarly, the cover-abundance of regenerating western redcedar (*Thuja plicata*) seedlings was higher in herbivore exclosures than in areas exposed to browsing by Sitka black-tailed deer (Martin and Baltzinger, 2002; Stroh et al., 2008). Browsing by invasive deer has also shifted the successional trajectory of the forests on Haida Gwaii because the deer preferentially consume some plant species over others. Research on the age-class distributions of red huckleberry (*Vaccinium parvifolium*) and salal (*Gaultheria shallon*) on islands invaded by Sitka black-tailed deer reveals that the huckleberry populations on deer-invaded islands began to decline approximately 30 to 40 years prior a decline in salal, suggesting that huckleberry is a more palatable species to the deer (Vila et al., 2005, 2004b). Areas of Haida Gwaii exposed to hunters had reduced browsing pressure and higher regeneration of *Thuja plicata*, but hunting had no effect on the abundance of regenerating *Tsuga heterophylla* and *Picea sitchensis* (Martin and Baltzinger, 2002). The same study found evidence that browsing pressure was higher in western

redcedar than in western hemlock or Sitka spruce, suggesting that deer preferentially eat *Thuja plicata* over *Tsuga heterophylla* (Martin and Baltzinger, 2002).

Although the effects of Sitka black-tailed deer on the plant communities of Haida Gwaii have been extensively researched, it was imperative to survey understory plants in the current study because of the role of plants as mediators between aboveground herbivores and belowground organisms associated with plant roots (Van Der Putten et al., 2001). Furthermore, the diversity and abundance of plant communities is a major driver of soil fungal communities (Reininger et al., 2015). Thus, changes to the plant community through herbivory by Sitka black-tailed deer may indirectly affect soil fungi that depend on the root and leaf litter, root exudates or abiotic conditions facilitated by those plants (Bardgett and Wardle, 2003; Kardol and De Long, 2018). To improve our understanding of the role of plants as potential mediators between the aboveground effects of Sitka black-tailed deer and the belowground fungal community, this chapter describes a survey of the understory plants on deer-invaded and non-invaded islands in Laskeek Bay and Gwaii Haanas. Based on the extensive research previously conducted on the effects of herbivores on plant communities, both globally and on Haida Gwaii, the abundance and species richness of understory plants was predicted to be lower on deer-invaded islands. However, by reducing the abundance of the most dominant plant taxa, Pielou's species evenness, a measure of how total abundance is distributed among the different species (Pielou, 1975), may be higher on deer-invaded islands. The Shannon index, a measure of ecological diversity, is influenced more greatly by species evenness than by species richness, and therefore is likely to be coupled with differences in species evenness on deer-invaded islands, especially if differences in species richness are minor (Shannon, 1948; Strong, 2016). Lastly, by asymmetrically decreasing or increasing the relative abundance of different plant species, there is likely to be a difference in the composition of the plant communities on deer-invaded islands compared with non-invaded islands.

4.1.2 Objective and Hypotheses

The objective of the research presented in this chapter was to determine the abundance, diversity and composition of the understory plant community on islands invaded or not invaded by Sitka black-tailed deer in Gwaii Haanas and Laskeek Bay. The following hypotheses were tested:

H_0 The presence of Sitka black-tailed deer on the islands has no effect on the abundance, diversity or structure of the plant community.

H_1 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the abundance of understory plants.

H_2 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the species richness of the plant community.

H_3 The presence of Sitka black-tailed deer on the islands is associated with an increase in the species evenness of the plant community.

H_4 The presence of Sitka black-tailed deer on the islands is associated with an increase in the Shannon diversity of the plant community.

H_5 The presence of Sitka black-tailed deer on the islands is associated with a difference in the composition of the plant community.

4.2 Methods

4.2.1 Vegetation Survey

With the exception of tree species noted below (Table 4.1), all other vascular plants in the lower 3 m forest stratum were surveyed in each plot, hereafter referred to as understory plants. Species were identified according to Pojar and MacKinnon (1994) and the percent cover-abundance of each species was estimated using a modified version of the Braun-Blanquet cover-abundance scale (Braun-Blanquet, 1932; Mueller-Dombois and Ellenberg, 1974) with finer cover-abundance divisions (Table 4.2). The midpoints of each cover-class were used in subsequent analysis because the standard error of mean cover-abundance tends to be normally distributed when using visual methods of cover estimation, with larger error associated with intermediate cover-abundance and lower error associated with extremely low or high cover-abundance (Hatton et al., 1986).

Table 4.1: Tree species omitted from plant survey

Scientific Name	Common Name
Alnus rubra	red alder
Malus fusca	Pacific crabapple
Picea sitchensis	Sitka spruce
Thuja plicata	western redcedar
Tsuga heterophylla	western hemlock

Table 4.2: Modified Braun-Blanquet cover-abundance scale

Cover Class (%)	Midpoint (%)	Area (m^2)	Midpoint (m^2)
0 - 0.25	0.125	0 - 1	0.5
0.25 - 0.5	0.375	1 - 2	1.5
0.5 - 1	0.75	2 - 4	3
1 - 5	3	4 - 20	12
5 - 15	10	20 - 60	40
15 - 25	20	60 - 100	80
25 - 50	37.5	100 - 200	150
50 - 75	62.5	200 - 300	250
75 - 95	85	300 - 380	340
95 - 100	97.5	380 - 400	390

4.2.2 Statistical Analysis

All data were analyzed in the R 3.4.2 programming language (R Core Team, 2017) with the *vegan*, *car*, *labdsv*, and *ggplot2* packages in RStudio (RStudio Team, 2016). Species richness was calculated as α-diversity at the plot level (Whittaker, 1972). The relative abundances of understory plant species were used for determining the diversity metrics of Pielou's species evenness (Pielou, 1975) and the Shannon diversity index (Shannon, 1948). Normality was tested with the Shapiro-Wilk test (Shapiro and Wilk, 1965) and equality of variances was tested with Levene's test (Levene, 1960). Three-factor ANOVA was used to analyze data based on the factors of *treatment* (non-invaded vs deer-invaded), *region* (Gwaii Haanas vs Laskeek Bay) and *island* as a nested factor within *region* (Table 2.1, Chapter 2). For the cover-abundance data, a logarithmic transformation was used to correct for heteroscedasticity and non-parametric distributions:

$$X' = \log_{10}(X)$$

To test for changes in community composition, a non-parametric permutational multivariate ANOVA was applied using the Bray-Curtis dissimilarities (Bray and Curtis, 1957) of the relative of understory plant species. Data were visualized using principal coordinates analysis (PCoA) of the same Bray-Curtis dissimilarities. In applying a canonical analysis of principal coordinates (CAP) (Anderson and

Willis, 2003), a Hellinger transformation (Legendre and Gallagher, 2001) was used on the absolute abundances to avoid an apparent arch effect in the PCoA (Morton et al., 2017). The PCoA and CAP analyses were performed with the *capscale* function using the *vegan* package and plotted using the *ggplot2* package (https://github.com/levybooth/multivariate_plots) in in the R 3.4.2 programming language (R Core Team, 2017).

Indicator species values were determined with the method described by Dufrêne & Legendre (1997) with $\alpha = 0.01$ according to the following equation, where *Frequency* is the percentage of samples in a given treatment group in which a given species is present, and where *Fidelity* is the percentage of the total reads of a given species that are present in the treatment group.

$$Indicator\ Value = Frequency * Fidelity$$

To control for false discoveries, the Benjamini-Hochberg procedure was used with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995).

4.3 Results

4.3.1 Plant Abundance

The presence of Sitka black-tailed deer was associated with a significant decrease in the cover-abundance of understory plants ($F_{(1,40)} = 298.60$, $p < 0.001$), with a mean cover of 83% ± 8.4 SE and 10% ± 4.3 SE on non-invaded and deer-invaded islands, respectively (Table 4.3, Figure 4.1). Cover abundance of understory plants was also significantly higher in the region of Laskeek Bay compared with Gwaii Haanas ($F_{(1,40)} = 17.05$, $p < 0.001$), with a mean of 50.6% ± 11.4 SE in Laskeek Bay and 33.9% ± 7.9 SE in Gwaii Haanas. There was no significant interaction between the *treatment* and *region* factors but there were significant differences in cover-abundance between different islands ($F_{(5,40)} = 15.57$, $p < 0.001$, Appendix A § Tables A.1-5). Understory plants on Agglomerate Island had unusually low mean cover-abundance (46.4% ± 11.4 SE) compared with the mean cover abundances of other non-invaded islands, which were all above 90%. West Skedans Island and South Skedans Island, both deer-invaded, had means of 84.3% ± 14.7% and 43.5% (SE not applicable), respectively. In contrast, the mean cover-abundances of understory plants on the other deer-invaded islands were 4.6% ± 1.6 SE (Louise Island), 2.2% ± 0.63 SE (Reef Island) and 2.6% ± 0.43 SE (Ramsay Island).

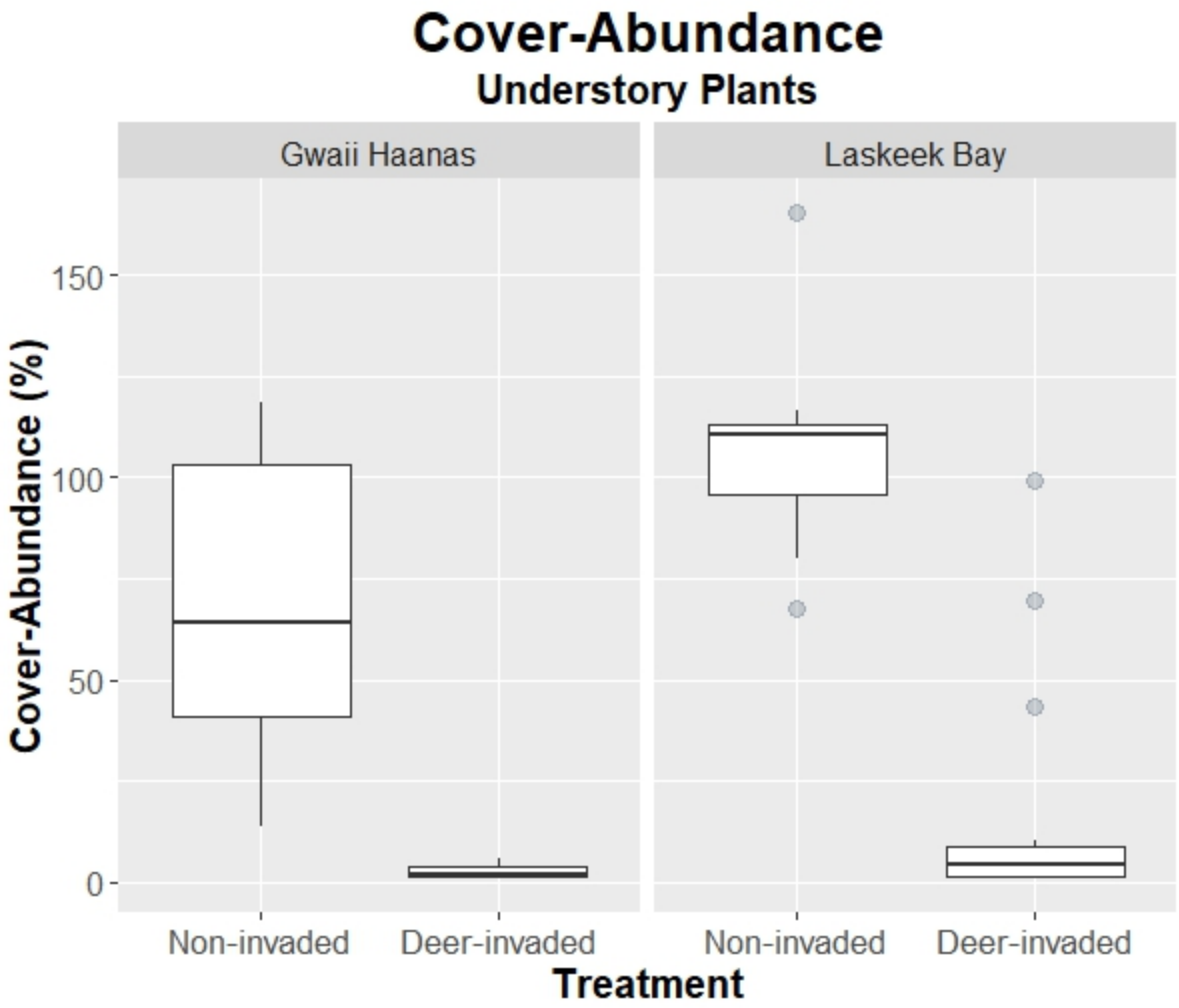

Figure 4.1: Cover-abundance of the understory plants on non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Table 4.3: Cover-abundance of understory plants on non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) from the Gwaii Haanas and Laskeek Bay regions, respectively. Values are mean percentages ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	67.5 ± 10.1	108 ± 10.2
	Deer-invaded	2.6 ± 0.4	17.8 ± 8.2

4.3.2 Diversity and Community Structure

While islands invaded by Sitka black-tailed deer were associated with a mean increase of 1.8 plant species per plot, the difference in the species richness of plants on deer-invaded and non-invaded islands was not significant ($F_{(1,40)} = 3.10$, $p = 0.086$, Table 4.4). Likewise, there was no significant difference in the species richness of plants between the regions of Gwaii Haanas and Laskeek Bay, and there was no significant difference in the species richness between individual islands. There was also no significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay).

Table 4.4: Species richness of understory plants on non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) from the Gwaii Haanas and Laskeek Bay regions, respectively. Values are mean species per plot ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	8.5 ± 0.8	9.2 ± 1.2
	Deer-invaded	11.6 ± 1.3	9.6 ± 0.8

There was a significant increase in Pielou's species evenness of the understory plant communities on deer-invaded islands compared with non-invaded islands. ($F_{(1,40)} = 62.305$, $p < 0.001$, Figure 4.3). Deer-invaded islands had a mean species evenness of 74.6% ± 5.0 SE while non-invaded islands had a species evenness of 35.2% ± 3.9 SE. There was no significant difference in the species evenness between the regions of Gwaii Haanas and Laskeek Bay, however there was a significant interaction between the factors of *treatment* and *region* ($F_{(1,40)} = 8.32$, $p = 0.006$). The species evenness of Ramsay Island (84.3% ± 11.4 SE) was more than two-times greater than mean species evenness of the non-invaded islands of the Gwaii Haanas region (31.4% ± 4.2 SE), whereas in Laskeek Bay, the mean species evenness on deer-invaded islands was only 52% greater the non-invaded islands (Table 4.5). This difference in effect size between Gwaii Haanas and Laskeek Bay was driven largely by outliers. There were significant differences in the species evenness of individual islands ($F_{(5,40)} = 7.09$, $p < 0.001$, Appendix A § Tables A.1-2). West Skedans Island and South Skedans Island had means of 14.3% ± 8.8 SE and 24.4% (SE not applicable), respectively, which were lower than the mean species evenness of non-invaded islands (35.2% ± 3.9 SE).

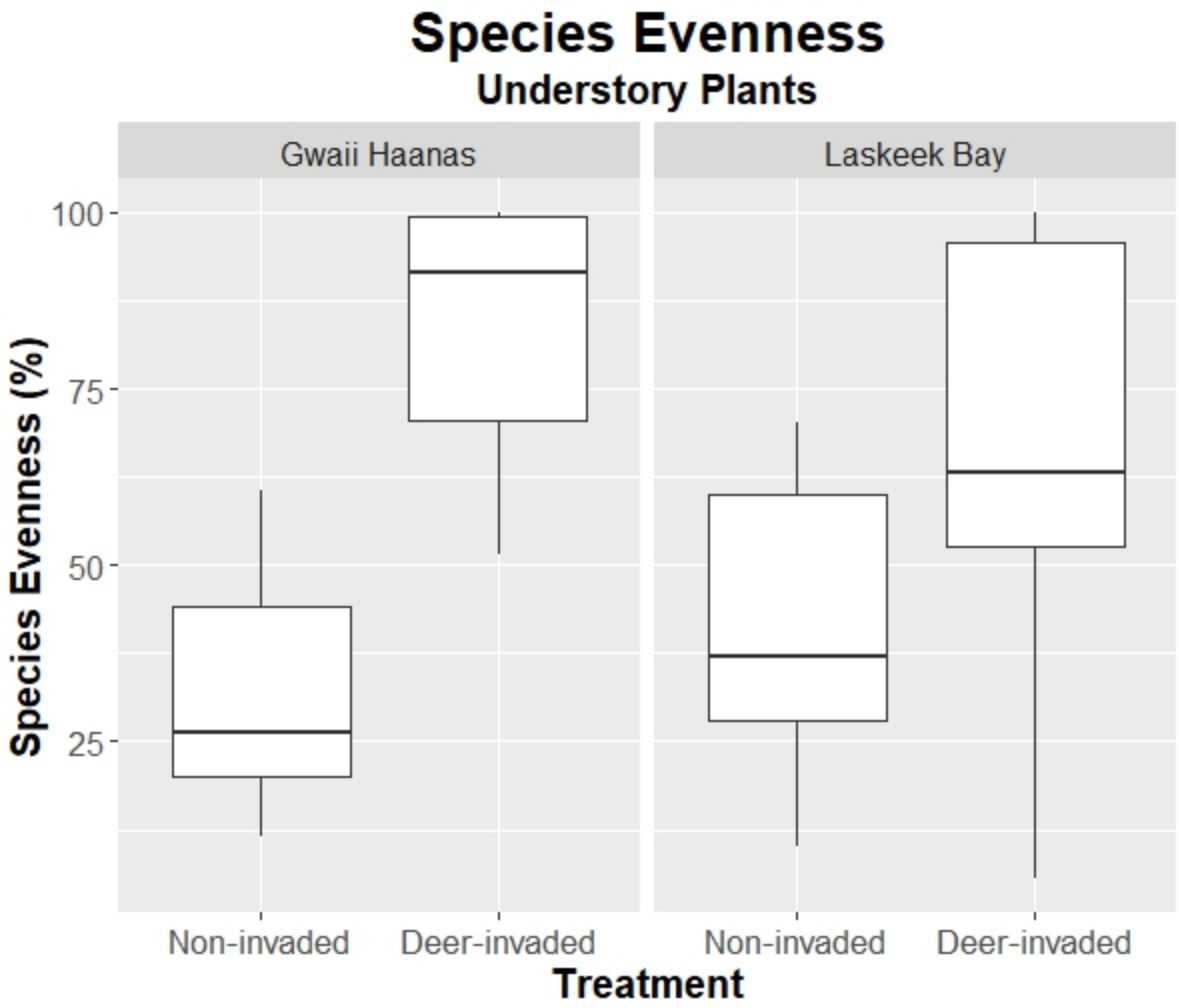

Figure 4.3: Species evenness of the understory plants on non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Table 4.5: Species evenness of understory plants on non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) from the Gwaii Haanas and Laskeek Bay regions, respectively. Values are mean percentages ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	31.4 ± 4.2	41.4 ± 7.6
	Deer-invaded	84.3 ± 4.7	64.9 ± 8.3

The understory plant communities on deer-invaded islands had a significantly higher Shannon diversity index than the understory plants on non-invaded islands ($F_{(1,40)} = 53.716$, $p < 0.001$). The mean Shannon diversity index of non-invaded and deer-invaded islands was 0.76 ± 0.10 SE and 1.72 ± 0.13 SE, respectively. There was no significant difference in the mean Shannon diversity index between the regions of Gwaii Haanas and Laskeek Bay. However, there was a significant interaction between the *treatment* and *region* factors ($F_{(1,40)} = 10.19$, $p = 0.003$). The differences in Shannon diversity index between deer-invaded and non-invaded islands was greater in Gwaii Haanas than in Laskeek Bay (Table 4.6, Figure 4.4). This interaction was largely driven by outliers among the individual islands ($F_{(5,40)} = 5.277$, $p < 0.001$, Appendix A § Tables A.1-2). West Skedans and South Skedans, both located in Laskeek bay, had means of 0.27 ± 0.18 SE and 0.64 (SE not applicable), respectively, similar to the Shannon diversity index of most non-invaded islands: 0.65 ± 0.12 SE (Tar Island), 0.65 ± 0.15 SE (Agglomerate Island) and 0.66 ± 0.21 SE (Low Island). The understory plants of Lost Island, also from Laskeek Bay, had a relatively high mean Shannon diversity index of 1.42 ± 0.27 SE compared with the other non-invaded islands and was more similar to the Shannon diversity index of deer-invaded islands.

Table 4.6: Shannon diversity index of understory plants on non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) from the Gwaii Haanas and Laskeek Bay regions, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	0.65 ± 0.09	0.95 ± 0.21
	Deer-invaded	2.00 ± 0.14	1.44 ± 0.19

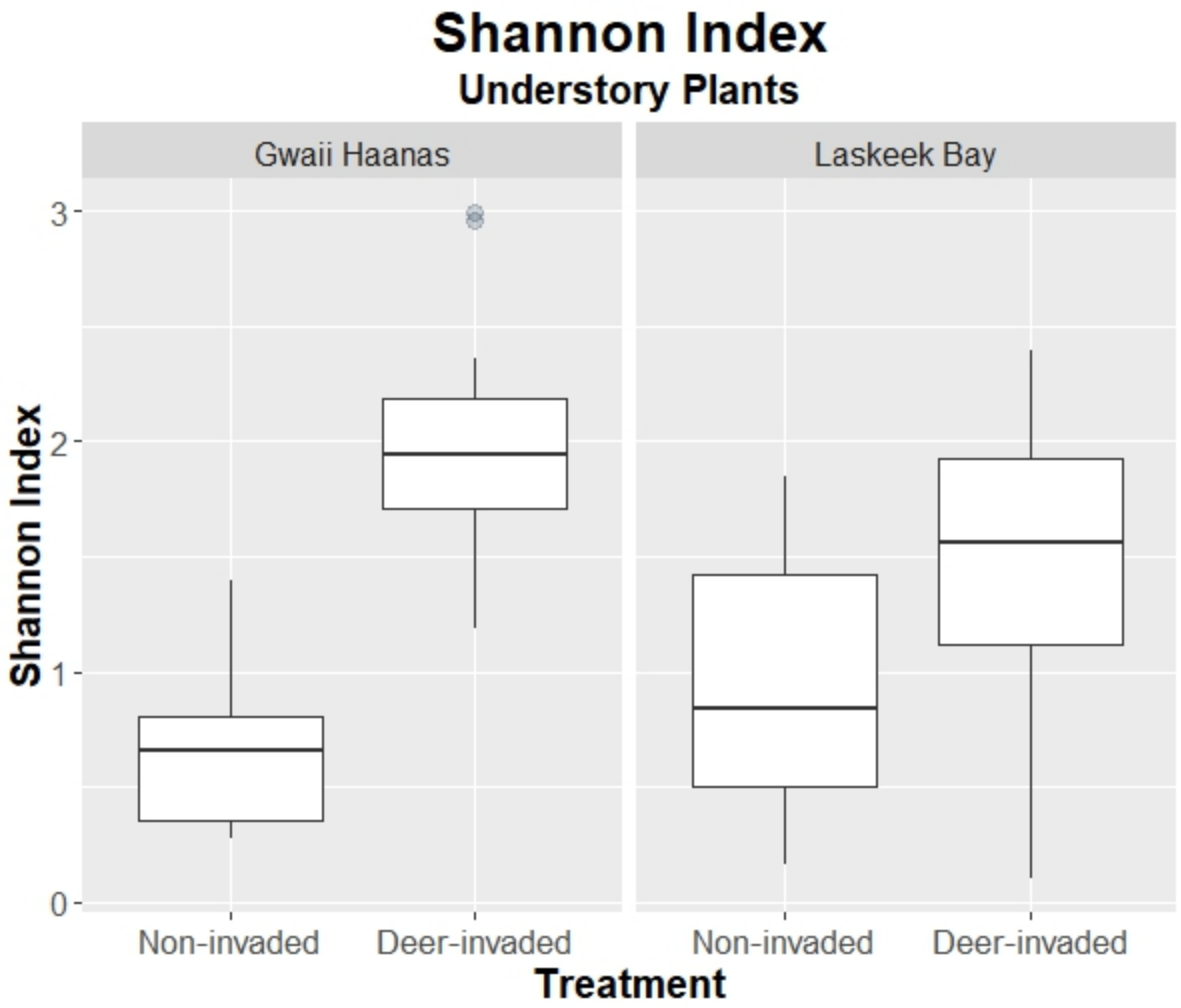

Figure 4.4: Shannon diversity index of the understory plants on non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Analysis of community composition using a permutational multivariate ANOVA revealed a significant difference in the understory plant communities of islands invaded by Sitka black-tailed deer and those of non-invaded islands ($F_{(1,40)} = 18.8763$, $R^2 = 0.25$, $p < 0.001$). There was also a significant difference between the plant communities of Gwaii Haanas and Laskeek Bay ($F_{(1,40)} = 3.1370$, $R^2 = 0.04$, $p = 0.026$). There was a significant interaction between the *treatment* and *region* factors ($F_{(1,40)} = 2.4234$, $R^2 = 0.03$, $p = 0.038$), however, analysis of the results with the Benjamini-Hochberg procedure suggests this may be a false discovery. There was also a significant difference in the understory plant community between individual islands ($F_{(5,40)} = 2.09$, $R^2 = 0.14$, $p = 0.012$). Altogether, 47% of the variance in the

plant community could be explained by differences between deer-invaded and non-invaded islands, differences in the plant communities of Gwaii Haanas and Laskeek Bay, and differences in the plant communities of individual islands. Principal coordinates analysis (PCoA) of the plant community using Bray-Curtis dissimilarities of the Hellinger-transformed cover-abundances revealed distinct separation of the plots from deer-invaded and non-invaded islands along the primary axis, with the exception of the plots from West Skedans Island and South Skedans Island, outliers with plant communities more similar to non-invaded islands (Appendix A § Figure 1).

In a canonical analysis of principal coordinates (CAP), a model constrained with the factors of *treatment*, *region*, and *island*, both axes 1 and 2 were found to be significant after the Bonferroni correction ($F_{(1,40)} = 31.64$, $P = 0.001$; $F_{(1,40)} = 5.0459$, $p = 0.024$, respectively). Plots from deer-invaded and non-invaded islands generally separated along the primary axis which explained 36.8% of the variance. Three outliers among the deer-invaded plots, representing West Skedans Island and South Skedans Island, had less dissimilarity with the non-invaded plots than the other deer-invaded plots. There was greater within-group variance among plots from non-invaded islands than deer-invaded islands along the secondary axis, representing 6.2% of the overall variation in the plant community (Figure 4.6).

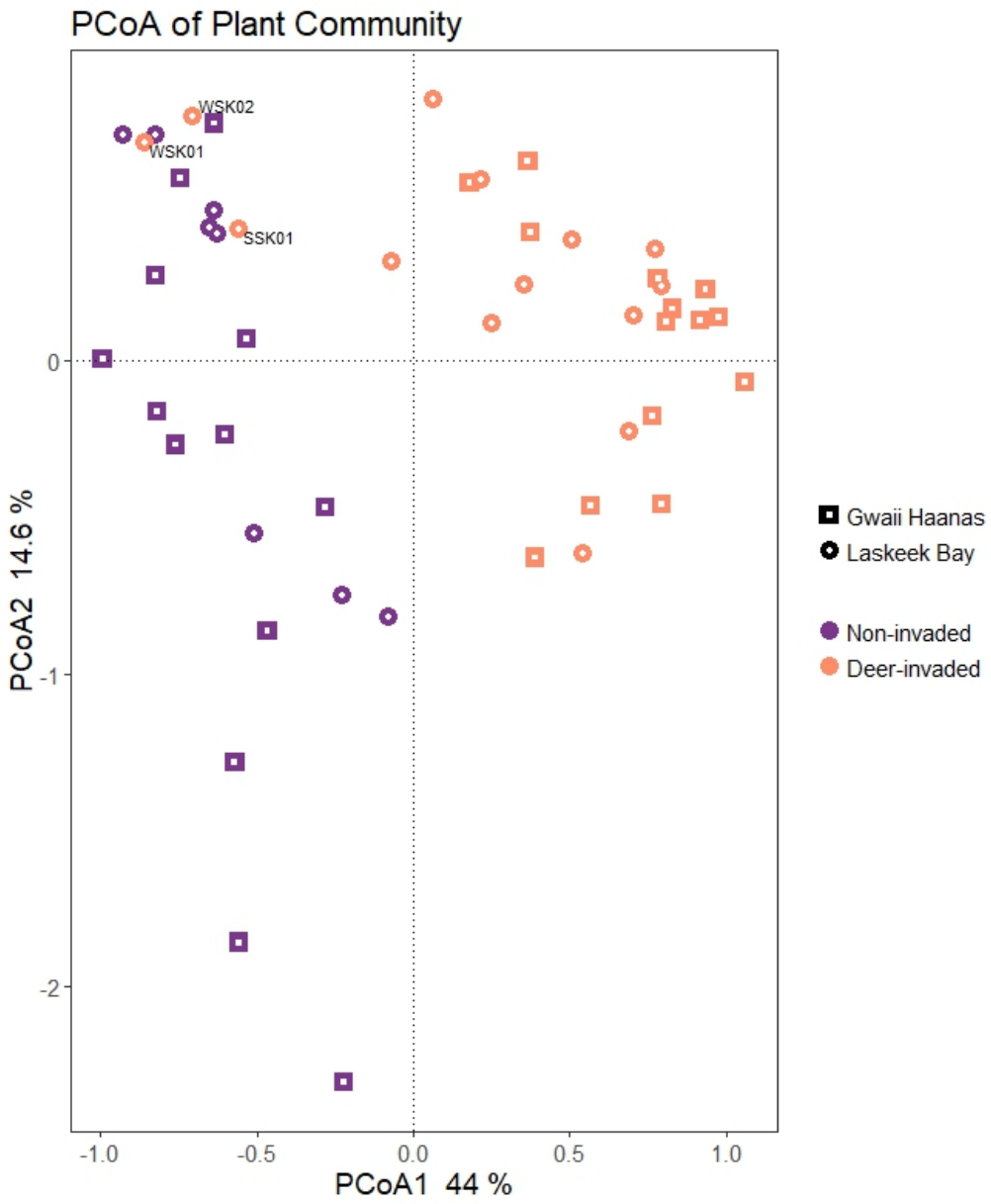

Figure 4.5: Principal coordinates analysis (PCoA) of the plant community using Bray-Curtis dissimilarities of Hellinger-transformed cover-abundances, comparing plots from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively.

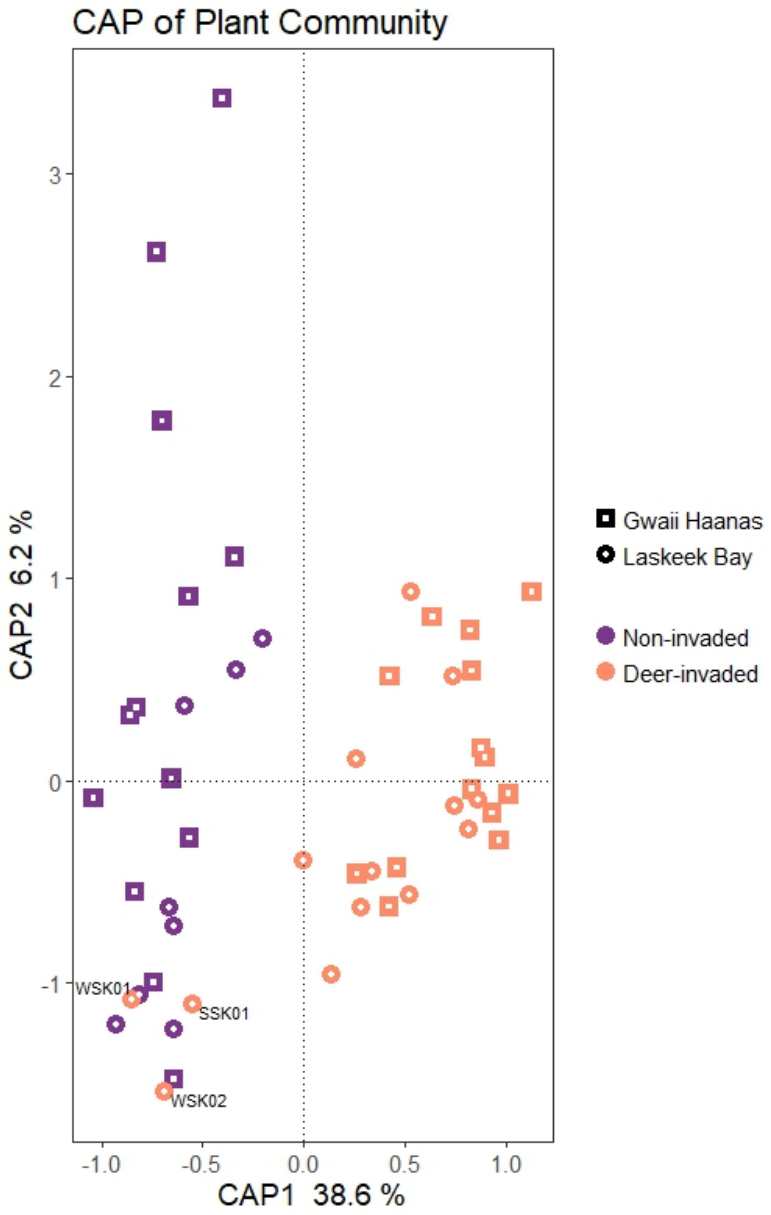

Figure 4.6: Canonical analysis of principal coordinates (CAP) of the plant community using Bray-Curtis dissimilarities of Hellinger-transformed cover-abundances, comparing plots from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively, constrained using the factors of *treatment* (non-invaded vs deer-invaded), *region* (Gwaii Haanas vs Laskeek Bay) and *island* (Agglomerate, Lost, Louise, Low, Ramsay, Reef, Tar).

4.3.3 Indicator Species

Indicator analysis revealed four understory plant species as significant indicators of non-invaded islands ($P < 0.05$): sword fern (*Polystichum munitum*), salal (*Gaultheria shallon*), twinberry (*Lonicera involucrata*) and bracken fern (*Pteridium aquilinum*). Ten understory plant species were significant

indicators of invaded islands ($P < 0.05$, Table 4.7), most of which are ferns, grasses and herbaceous plants. Two shrubs species were significant indicators of deer-invaded islands: *Menziesia ferruginea* and *Vaccinium parvifolium*.

Table 4.7: Indicator species of the understory plant communities on non-invaded islands (n = 21) and deer-invaded islands (n = 28). Values are indicator species values for the respective *treatment* factor.

Treatment	Species	Indicator Value
Non-invaded	*Polystichum munitum*	0.88
	Gaultheria shallon	0.71
	Lonicera involucrata	0.67
	Pteridium aquilinum	0.35
Deer-invaded	*Menziesia ferruginea*	0.92
	Vaccinium parvifolium	0.82
	Moneses uniflora	0.80
	Blechnum spicant	0.72
	Neottia banksiana	0.69
	Neottia cordata	0.62
	Driopteris expansa	0.41
	Luzulla parviflora	0.30
	Stellaria crispa	0.27
	Bromus sitchensis	0.23

4.4 Discussion

4.4.1 Plant Abundance, Diversity and Community Structure

The overall abundance of understory plants was lower on deer-invaded islands compared with non-invaded islands, consistent with results in previous studies on Haida Gwaii that have shown Sitka black-tailed deer to be associated with a large decrease in the abundance of red huckleberry (*Vaccinium parvifolium*), salal (*Gaultheria shallon*) and other shrubs, ferns and herbs (Chollet et al., 2016; Stockton et al., 2005; Vila et al., 2005, 2004b). However, in contrast to previous research, plant species richness was not found to be significantly different between non-invaded and deer-invaded islands. Previous research has found the decrease in plant species richness (plot level) on deer-invaded islands to be greater in forest edge habitat than in interior forest habitat (Stockton et al., 2005). In this study, plots were located in the interior forest areas where the effects of deer on species richness are less pronounced.

Plots on Ramsay Island heavily skewed the results on plant species richness. On deer-invaded islands in Laskeek Bay, the species richness of understory plants was similar to that on non-invaded islands. The species richness of plants on Ramsay Island may have been higher due to several plots being located within riparian corridors, whereas freshwater riparian areas were absent on the non-invaded islands owing to their small size. Riparian corridors have variable disturbance regimes and complex environmental gradients which often results in them having unique plant communities (Goebel et al., 2003) with high biodiversity (Naiman et al., 1993). Plant species richness can also be higher along stream banks than in upland forest areas (Rawat and Chandra, 2014). Therefore, the higher plant species diversity found on Ramsay Island may not be causally linked to the invasion of Sitka black-tailed deer on the island.

The Shannon diversity index of the plant communities was higher on deer-invaded islands. However, the Shannon diversity index is determined by two key attributes, the species richness and species evenness (Strong, 2016). Because there was no significant difference in the mean species richness of non-invaded and deer-invaded islands, the increase in Shannon diversity associated with the understory plants of deer-invaded islands was driven largely by differences in species evenness. Higher within-plot species evenness suggests that the relative cover-abundance of understory plants is distributed more equitably among species, in contrast to the plant communities of non-invaded islands which are more likely to be dominated by a minority of the plant species.

In a study on Reef Island following several deer culls, the abundance and species richness of native understory plants increased as a response to decreased browsing pressure (Chollet et al., 2016). However, results from this current study suggest that the abundance and species richness of understory plants on Reef island are now comparable to other deer-invaded islands such as Louise and Ramsay. Indeed, the density estimates of resident deer populations, based on pellet counts suggest that, at the time of this study, the deer population on Reef had increased to approximately 70% of the population density on Louise Island (Table 2.1, Chapter 2). Without ongoing measures to control the deer population, the gains made through deer culls are likely to be temporary.

Canonical analysis of principal coordinates revealed that while the greatest amount of variance in the plant community can be explained by the invasion of Sitka black-tailed deer on certain islands, there is a significant amount of variation within the plant communities of deer-invaded and non-invaded islands explained by differences between regions and individual islands.

4.4.2 Indicator Species

Red huckleberry (*Vaccinium parvifolium*) was an unexpected indicator species for deer-invaded islands. Previous research has shown that Sitka black-tailed deer preferentially browse red huckleberry compared with other species such as *Gaultheria shallon* (Vila et al., 2005, 2004b), *Tsuga heterophylla* and *Picea sitchensis* (Martin and Baltzinger, 2002). However, as a long-lived shrub, red huckleberry can survive for long periods of time under intensive browsing if its stems are able to grow above the browse line (Vila et al., 2004b). By reducing the abundance of plant species that are otherwise highly dominant on non-invaded islands, Sitka black-tailed deer indirectly increase the relative abundance of tolerant species such as red huckleberry. Furthermore, the stems of red huckleberry found on deer-invaded islands tend to be significantly taller and older than the stems found on non-invaded islands because deer consume younger stems before they can grow above the browse line (Vila et al., 2004b). Therefore, the status of red huckleberry and other plants as indicator species of deer-invaded islands should not be interpreted as evidence that Sitka black-tailed deer necessarily benefit these plant species or increase their abundance. Rather, indicator species may be useful for the monitoring of the ecological integrity of these islands or as an assessment tool for habitat restoration projects (Siddig et al., 2016).

4.4.3 Outliers

Despite signs of deer presence on West Skedans and South Skedans, the plant communities of these two islands were distinct outliers among the deer-invaded islands and shared many similarities with non-invaded islands. The cover-abundance of understory plants on West Skedans Island was more than eight-times greater than the mean cover-abundance of all deer-invaded islands, while the cover-abundance of understory plants on South Skedans was four-times greater. The species evenness and Shannon diversity index of these two islands was more similar to non-invaded islands, and the composition of the plant communities on West Skedans and South Skedans were more similar to non-invaded islands than they are with the other deer-invaded islands. Overall, this evidence suggests that despite browsing pressure from Sitka black-tailed deer, the plant communities of these two islands are relatively intact. The small size of West Skedans (8.2 ha) and South Skedans (5.6 ha) may prevent a large resident population of deer from sustaining itself there (Table 2.1, Chapter 2). The intermediary effects on these islands may also be due Sitka black-tailed being rare or absent prior to the year 1980 (Gaston

et al., 2006), whereas other deer-invaded islands such as Reef Island and Ramsay Island may have had deer present since the year 1950 (Vila et al., 2005, 2004a, 2004b).

4.5 Conclusion

The objective of the research presented in this chapter was to determine the abundance, diversity and composition of the understory plant community on islands invaded or not invaded by Sitka black-tailed deer. There was significantly lower plant cover-abundance on deer-invaded islands than on non-invaded islands, similar to findings in previous studies and supportive of the hypothesis that deer reduce the cover-abundance of understory plants. However, the species richness of the plant community was associated with the presence or absence of Sitka black-tailed deer, contrary to the initial hypothesis that deer-invaded islands would have lower plant species richness. The species evenness and Shannon diversity index of the plant communities were higher on deer-invaded islands, demonstrating how the Shannon diversity index is disproportionately affected by differences in species evenness compared with species richness. The invasion of islands on Haida Gwaii by Sitka black-tailed deer is associated with a shift in the composition of understory plant communities and a reduction in the variation within the plant community compared with non-invaded islands. Finally, this research identified several plant species that can be used as indicators for the ecological monitoring of plant communities affected by Sitka black-tailed deer on Haida Gwaii, or for the prioritization of species for habitat restoration and conservation purposes.

CHAPTER 5 – Fungal Communities of Deer-Invaded and Non-Invaded Islands

5.1 Introduction

5.1.1 Background

Soil fungi are integral to many ecological processes such as the decomposition of organic matter (Baldrian, 2009), the uptake of nutrients by plants (Jeffries et al., 2003) and the pathogenesis of plants and animals (Tedersoo et al., 2014). Saprotrophic fungi derive their carbon through the decomposition of leaf and root litter in the soil and are capable of breaking down complex forms of organic matter through the production of hydrolytic and oxidative enzymes (Baldrian, 2009). Soil fungi that colonize the tissues of living organisms exist on a continuum between pathotrophy and symbiotrophy (Johnson et al., 1997), but they can generally be grouped into two functional types: pathogens and mutualistic endophytes such as mycorrhizal fungi. Through the decay of living plant tissue or the control of nutrient uptake, pathogenic fungi have negative effects on their hosts and play an important roles in the structuring of plant communities (Klironomos, 2002). In contrast, mycorrhizal fungi form mutualisms with plants in which they exchange nutrients and water for carbohydrates photosynthesized by their plant hosts (Tedersoo et al., 2014; Vance, 2001).

The fungal communities on Haida Gwaii remain poorly characterized but are likely comprised of several different functional groups. A survey of aboveground fruiting bodies identified a total of 605 species of fungi on Haida Gwaii, most of which were identified as saprotrophic or ectomycorrhizal species (Kroeger et al., 2012). While late-successional temperate conifer forests tend to be dominated by ectomycorrhizal fungi (Izzo et al., 2005; Molina and Trappe, 1982; Weber et al., 2005a), the old growth forests of Haida Gwaii likely support a variety of mycorrhizal functional groups in addition to saprotrophic and pathogenic taxa. Western redcedar (*Thuja plicata*), one of the dominant tree species on Haida Gwaii, forms symbiotic relationships with arbuscular mycorrhizal (AM) fungi (Weber et al., 2005b). Entirely restricted to Glomeromycota, AM fungi are generally considered to be obligate mutualists, completely dependent on their plant host as a source of carbon (Bago and Bécard, 2002). Understory plant species such as grasses and forbs also likely form mutualisms with arbuscular mycorrhizal fungi which have associations with over two thirds of terrestrial plant species (Hodge, 2000; Read et al., 1976). In contrast, the other two most dominant tree species on Haida Gwaii, western hemlock (*Tsuga heterophylla*) and Sitka spruce (*Picea sitchensis*), form symbiotic relationships with

ectomycorrhizal (EcM) fungi (Flynn et al., 1998; Kropp and Trappe, 1982). As facultative mutualists, ectomycorrhizal fungi are capable of producing extracellular enzymes (Bödeker et al., 2014) and thus retain the capacity to decompose organic matter, although to a lesser extent than saprotrophic fungi (Baldrian, 2009). Ericoid mycorrhizal (ErM) fungi represent a third major functional group of symbiotic fungi likely to occur on Haida Gwaii, colonizing the roots of salal (*Gaultheria shallon*) and other ericaceous plant species (Straker, 1996; Xiao and Berch, 1995). As with EcM fungi, ericoid mycorrhizal fungi are thought to be facultative mutualists capable of subsisting as saprotrophic organisms to varying degrees (van der Heijden et al., 2015). Although basidiomycetous ErM fungi exist (Allen et al., 2003), ericoid mycorrhizal fungi are especially represented by Helotiales (Ascomycota) which also contains many recognized species of dark septate endophytes (Zijlstra et al., 2005). Ericaceous plant species such as salal may also serve as alternative hosts for the same fungi that form ectomycorrhizas on trees in the pine family (Lukešová et al., 2015). Orchid species on Haida Gwaii such as *Neottia cordata* and *Neottia banksiana* (Cheney et al., 2007) likely form relationships with their own distinct functional group of root endophytes in Serendipitaceae (Weiß et al., 2016).

Aboveground herbivores can indirectly affect soil fungi by altering the physical and chemical properties of soils, causing shifts in the soil microbial communities towards species better adapted to those conditions. For example, through changes in the size and distribution of pore spaces, soil compaction can reduce the growth of soil fungi (Glenn et al., 1987). A study on two forested sites in Switzerland found soil compaction to be associated with a decrease in the relative abundance of ectomycorrhizal fungi, whereas saprotrophic and pathogenic fungi were associated with higher soil compaction (Hartmann et al., 2014). Furthermore, the reduction in porosity associated with soil compaction limits air and water conductivity in the soil, which in turn encourages the proliferation of anaerobic bacteria, which are more competitive under those conditions (Hartmann et al., 2014). On the other hand, by decreasing the diversity of organic substrates entering the soil, aboveground herbivores may reduce the ecological niche space available for saprotrophic fungi (Maltz et al., 2017), which may counteract the effects of soil compaction on the abundance of saprotrophic fungi. A global survey of soil fungi found soil pH to be the strongest predictor of the species richness of ectomycorrhizal fungi in ecosystems dominated by ectomycorrhizal plant species (Tedersoo et al., 2014). Similarly, a global meta-analysis on belowground microbial communities found pH and C:N ratios to be strong predictors of the relative abundance of fungi and bacteria (Fierer et al., 2009). In a long-term study of a pH gradient in a UK wheat field, with pH ranging from 4.0 to 8.3, pH was strongly correlated with shifts in the diversity and composition of bacterial communities, whereas the abundance of soil fungi was not correlated with

pH and fungal diversity was only weakly linked, likely due to soil fungi tolerating a wider range of pH than bacteria (Rousk et al., 2010). Shifts in the chemical properties of the soil can also modify the interactions between plants and mycorrhizal fungi. Fertilizing soil with mineral forms of nitrogen tends to decrease the overall abundance and diversity of soil fungi and alter the composition of fungal communities (Allison et al., 2007; Bittman et al., 2005). Likewise, increases in available phosphorus and nitrogen tend to be associated with decreases in the abundance of mycorrhizal fungi (Treseder, 2004), possibly due to reductions in the carbon allocated by plants to their root systems when they are less limited by nutrient availability (Haynes and Gower, 1995). By increasing soil compaction and phosphorus availability, Sitka black-tailed deer may drive an overall decrease in the abundance and diversity of soil fungi, with saprotrophic fungi likely to increase in abundance relative to mycorrhizal fungi. However, decreases in available nitrogen associated with the herbivores could have the opposite effect on fungal diversity or the abundance of mycorrhizal fungi.

Aboveground herbivores can also influence the abundance, diversity and composition of soil fungi through their direct effects on plant communities. Plants exist at the nexus of complex multitrophic interactions, linking herbivores in the aboveground subsystem with the pathogens, mutualists and herbivores associated with the rhizospheres of plants in the belowground subsystem (Van Der Putten et al., 2001). Shifts in plant community composition can alter soil microbial communities through a variety of mechanisms, including: i) differences in the quantity and chemical composition of leaf and root litter between different plant species, ii) interactions between living plant roots and the biotrophic or saprotrophic soil organisms in the rhizosphere , and iii) changes to soil microclimates and other abiotic properties (Kardol and De Long, 2018). A study on subtropical forests in southeastern China found significant differences in the fungal community composition between different stand ages and a significant correlation between plant community composition and different taxonomic and functional groups of fungi (Wu et al., 2013). Likewise, a study using 454 pyrosequencing to characterize soil fungal communities found decreases in the abundance of native plants to be associated with significant differences in the community composition of soil fungi (Reininger et al., 2015). Because plants species vary in the species of mycorrhizal fungi which they support, changes in the composition of plant communities are associated with shifts in the composition of mycorrhizal fungal communities (Hausmann and Hawkes, 2009). As arbuscular mycorrhizal fungi are obligate biotrophs, they may be particularly vulnerable to the loss of their plant hosts (Bago and Bécard, 2002). Likewise, because the understories of Haida Gwaii are dominated by ericaceous shrubs such as salal, the abundance of ErM fungi may also decline as a result of the consumption of their plant hosts by deer.

Through long-term shifts in the functional composition of plant communities, aboveground herbivores can also affect the abundance and diversity of soil fungi by altering the diversity and composition of leaf litter and root exudates entering the soil (Bardgett and Wardle, 2003), which may explain why plant diversity is often positively correlated with the abundance and diversity of soil fungi (Maltz et al., 2017; Zak et al., 2003). Plant species vary in the quality of their leaf and root litter, as well as the composition of their root exudates. Although most tree species exude glucose into their rhizospheres, there is considerable variation between the other types of sugars and amino acids that plant species deposit into the soil from their roots (Grayston et al., 1997). Thus, greater diversity of root exudates in soil is also associated with greater fungal biomass (Broeckling et al., 2008). Likewise, decreases in the diversity of leaf litter results in an associated decline in the diversity of soil fungi (Maltz et al., 2017). In summary, by decreasing the diversity and shifting the composition of plant communities, Sitka black-tailed deer are likely to drive differences in the composition of fungal communities and declines in the overall abundance and diversity of soil fungi. Functional groups such as AM fungi and ErM fungi that form mutualisms with understory plants consumed by deer are especially likely to decline in abundance on deer-invaded islands.

While the effects of Sitka black-tailed deer on the aboveground plants and animals of Haida Gwaii has been well documented (Chollet et al., 2016, 2014; Stockton et al., 2005), less in known about their potential effects on the soil fungal community. This chapter presents research comparing the biomass, diversity and structure of the soil fungal communities in soils from non-invaded and deer-invaded islands in the regions of Gwaii Haanas and Laskeek Bay. Molecular techniques were used to identify fungal taxa based on their DNA sequences and ergosterol, a membrane lipid found in soil fungi, was used as a biomarker for fungal biomass. I hypothesized that there would be significant differences in the composition of the soil fungal communities between non-invaded and deer-invaded islands, largely driven by differences in the diversity and abundance of the plant community, or differences in the physical and chemical properties of the soil. Based on previous studies of the plant communities of Haida Gwaii, I expected that plant diversity would be lower on deer-invaded islands which would result in an associated decline in the species richness of soil fungi. However, I expected Sitka black-tailed deer would be associated with an increase in the species evenness of soil fungi as a result of the homogenization of understory vascular plants. The Shannon diversity index, although also influenced by species richness, is more strongly affected by differences in species evenness (Strong, 2016), and therefore may be positively correlated with increases in species evenness irrespective of any differences in the species richness of the fungal community. I predicted that arbuscular and ericoid mycorrhizal

fungi associated would be lower in abundance on deer-invaded islands as a result of their host plants being consumed by Sitka black-tailed deer. As mature conifer trees on Haida Gwaii are too tall for their foliage to be browsed by ungulates, Sitka black-tailed deer likely have negligible effects on the abundance of ectomycorrhizal fungi associated with overstory trees such as western hemlock and Sitka spruce. As a result of the overall decline of AM and ErM fungi, as well as decreases in overall plant diversity, I expected that total fungal biomass would be lower in the soils of deer-invaded islands. Likewise, I expected taxonomic groups associated with arbuscular mycorrhizal fungi (Glomeromycota) and ericoid mycorrhizal fungi (Helotiales) to be more associated with non-invaded islands.

5.1.2 Objective and Hypotheses

The objective of the research presented in this chapter was to determine the biomass, diversity and composition of the soil fungal communities on islands invaded or not invaded by Sitka black-tailed deer in Gwaii Haanas and Laskeek Bay. The following hypotheses were tested:

- H_0 The presence of Sitka black-tailed deer on islands has no effect on the biomass, diversity or structure of the soil fungal community.
- H_1 The presence of Sitka black-tailed deer on islands is associated with a decrease in the biomass of soil fungi.
- H_2 The presence of Sitka black-tailed deer on islands is associated with a decrease in the species richness of the soil fungal community.
- H_3 The presence of Sitka black-tailed deer on islands is associated with an increase in the species evenness of the soil fungal community.
- H_4 The presence of Sitka black-tailed deer on islands is associated with an increase in the Shannon diversity of the soil fungal community.
- H_5 The presence of Sitka black-tailed deer on islands is associated with a difference in the composition of the soil fungal community, with fewer arbuscular mycorrhizal fungi and ericoid mycorrhizal fungi.

5.2 Methods

5.2.1 Fungal Biomass and Next Generation Sequencing

Ergosterol was used as an indicator of fungal biomass (Beni et al., 2014; Montgomery et al., 2000). Frozen soil samples were submitted to the Ministry of Environment & Climate Change Strategy

Analytical Laboratory for ergosterol analysis via ethanol extraction and high-performance liquid chromatography (Beni et al., 2014)

Next generation sequencing was used to determine the composition of the fungal community according to the following methods. Universal DNA was extracted from 0.15 g freeze-dried soil which had been ground to fine powder with a mortar and pestle. DNA was extracted using the Qiagen DNeasy PowerSoil Kit (product no. 12888-100, formerly sold by MO BIO as the PowerSoil DNA Isolation Kit). DNA was also extracted from a blank sample to account for potential contamination in the laboratory. DNA extracts were kept at -20°C until further use.

For amplifying and barcoding DNA, a two-stage PCR approach was used. The second internal transcribed spacer region (ITS2) of the fungal rDNA was amplified using the gITS7 forward primer (Ihrmark et al., 2012) and the ITS4 reverse primer (White et al., 1990) with Illumina overhang adapter sequences appended to each primer. The first PCR was conducted in a Bio-Rad MJ Mini thermal cycler in 50 µL reactions: 100 ng template, 2.5 µL forward primer (10 µM), 1.5 µL reverse primer (10 µM), 25 µL KAPA HiFi HotStart ReadyMix and DNAase-free water to a final volume of 50 µL. To minimize non-target amplification, a touchdown protocol was optimized for these specific samples: 4 minutes at 98°C; 6 cycles of 20 seconds at 98°C, 15 seconds at 63°C to 59°C, 40 seconds at 72°C; 21 cycles of 20 seconds at 98°C, 15 seconds at 58°C, 40 seconds at 72°C; and 1 minute at 72°C.

PCR products were cleaned with Agencourt AMPure XP beads (product no. A63880) and DNA was quantified with a Qubit dsDNA HS Assay Kit (product no. Q32851). PCR products were sent to the University of British Columbia Sequencing and Bioinformatics Consortium where a second stage of PCR was conducted to attach indexes and sequencing adapters to the amplicons. After further magnetic bead cleanup and fluorometric quantification, PCR products were sequenced on an Illumina MiSeq System.

To identify taxa, reads were processed through the PIPITS automated bioinformatics pipeline (Gweon et al., 2015) trained on the UNITE database, retrained on 01-12-2017 (Kõljalg et al., 2005). Operational taxonomic units (OTU) were clustered using a threshold of 97% sequence similarity and taxa were identified to the lowest possible phylogenetic level. Taxa were assigned to functional groups using the FUNGuild database (Nguyen et al., 2016). Twenty-two OTUs that were not assigned to the fungal kingdom were removed from the dataset. Reads corresponding with the blank sample were subtracted from the OTUs of all other samples in the dataset prior to subsequent analysis.

5.2.2 Statistical Analysis

All data were analyzed in the R programming language (version 3.4.2 , R Core Team, 2017) with the *vegan*, *car*, *labdsv*, *DESeq2*, and *ggplot2* packages using RStudio (RStudio Team, 2016). Statistical significance was interpreted using an α of 0.05. Species richness was calculated as α-diversity at the plot level (Whittaker, 1972). The relative abundances of OTUs were used for determining the diversity metrics of Pielou's species evenness (Pielou, 1975) and Shannon's diversity index (Shannon, 1948). Normality was tested with the Shapiro-Wilk test (Shapiro and Wilk, 1965) and equality of variances was tested with Levene's test (Levene, 1960) using the *car* package. Three-factor ANOVA was used to analyze data based on the factors of *treatment* (non-invaded vs deer-invaded), *region* (Gwaii Haanas vs Laskeek Bay) and *island* as a nested factor within *region* (Table 2.1, Chapter 2).

To test for differences in community composition, a permutational multivariate ANOVA was applied to the Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957) of rarefied reads using the *adonis* function in the *vegan* package. Data were visualized using principal coordinates analysis (PCoA) of the Bray-Curtis dissimilarities and then constrained to the model using canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003). Distance-based redundancy analysis (db-RDA) was used to identify potential explanatory variables (Legendre and Anderson, 1999) from the set of edaphic properties, plant cover-abundance, plant diversity metrics and indicator plant species abundances described in Chapters 3 and 4. Variables were added to the model using a forward-selection process and their significance was tested using a permutational ANOVA. The PCoA, CAP and db-RDA analyses were performed with the *capscale* function in the *vegan* package and plotted using the *ggplot2* package using code written by David Levy-Booth (https://github.com/levybooth/multivariate_plots).

Indicator species values were determined using the method described by Dufrêne and Legendre (1997) with α = 0.01 according to the following equation, where *Frequency* is the percentage of samples in a given treatment group in which a given species is present, and where *Fidelity* is the percentage of the total reads of a given species that are present in the treatment group.

$$Indicator\ Value = Frequency * Fidelity$$

To control for false discoveries, the Benjamini-Hochberg procedure was used with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995). To control the family-wise error rate associated

with multiple comparisons of the ergosterol data, a Bonferroni correction was applied (Dunn, 1961) by adjusting the alpha using the following equation, with $\alpha = 0.05$ and k = the number of comparisons:

$$\alpha' = \alpha/k$$

5.3 Results

5.3.1 Fungal Biomass

There was no significant difference in the concentration of ergosterol between the soils of non-invaded and deer-invaded islands (W = 326, p = 0.528). Ergosterol concentrations tended to be lower in soils from Gwaii Haanas (33.6 $\mu g \cdot g^{-1}$ ± 2.0 SE) compared with Laskeek Bay (47.8 $\mu g \cdot g^{-1}$ ± 5.4 SE). However, when using a Bonferroni-adjusted alpha (0.025), there was no significant difference in the concentrations of ergosterol between the two regions (W = 194, p = 0.039, Table 5.1, Figure 5.1). Furthermore, analysis using the Benjamini-Hochberg procedure suggests that differences in ergosterol concentrations between the two regions may be a false discovery. As the assumptions of ANOVA could not be met for the ergosterol data, the interaction between the factors of *treatment* and *region* is not reported (Appendix B § Table B.1).

Table 5.1: Ergosterol concentrations ($\mu g \cdot g^{-1}$) in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	33.4 ± 1.7	56.9 ± 8.3
	Deer-invaded	33.9 ± 3.6	42.5 ± 6.9

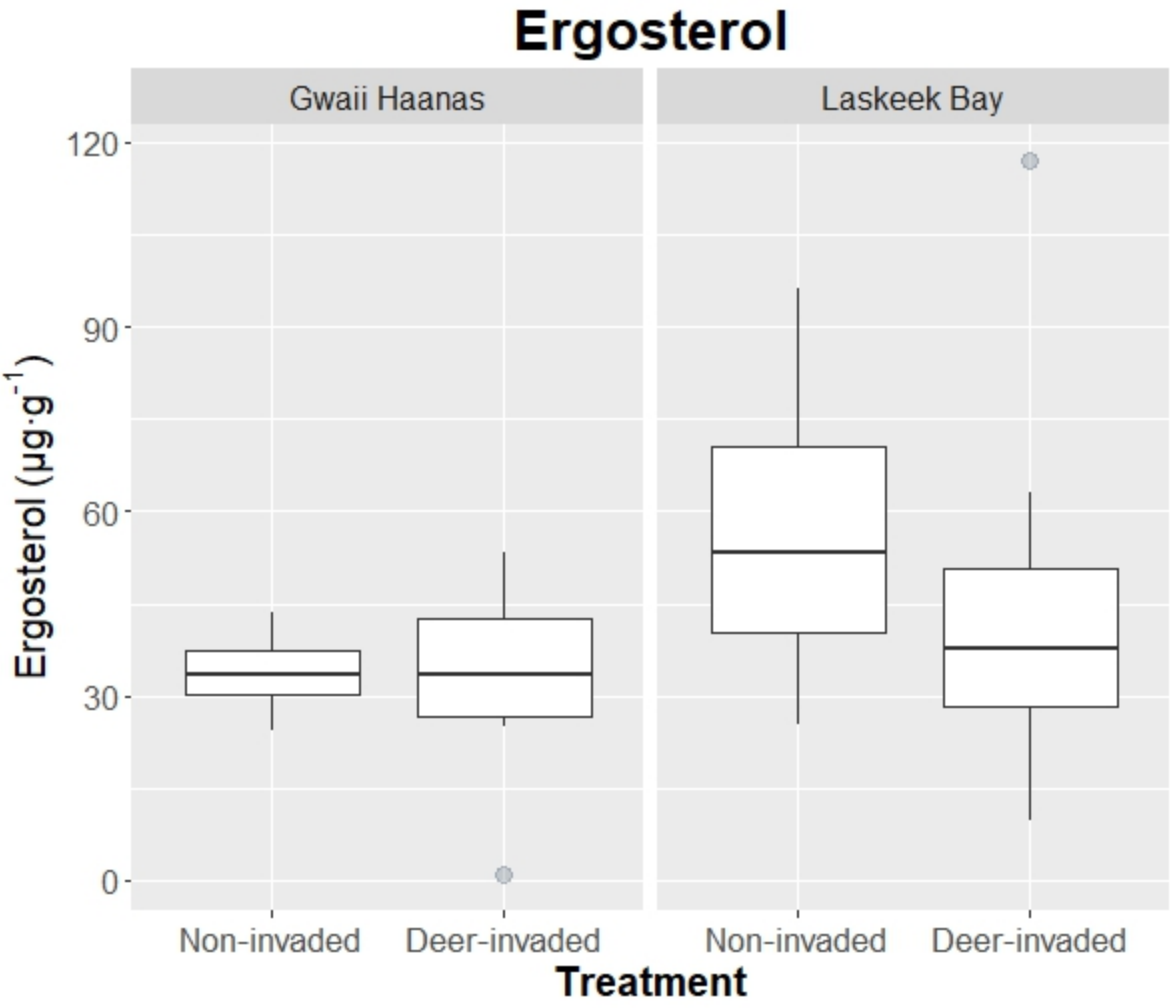

Figure 5.1: Ergosterol concentrations in soil from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

5.3.2 Diversity and Structure of the Fungal Community

There was no significant difference in the mean OTU richness of the fungal communities between soils from non-invaded and deer-invaded islands (p = 0.122), nor in the mean OTU richness of the fungal communities between soils from Gwaii Haanas and Laskeek Bay (p = 0.135, Table 5.2). There was also no significant difference in the mean OTU richness of individual islands (p = 0.105, Appendix B § Table B.1). Lastly, there was no significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay, p = 0.086, Figure 5.2)

Table 5.2: OTU richness of the fungal community in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. Values are mean OTUs per plot ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	1382.8 ± 45.1	1579.4 ± 41.9
	Deer-invaded	1544.3 ± 60.3	1534.6 ± 65.4

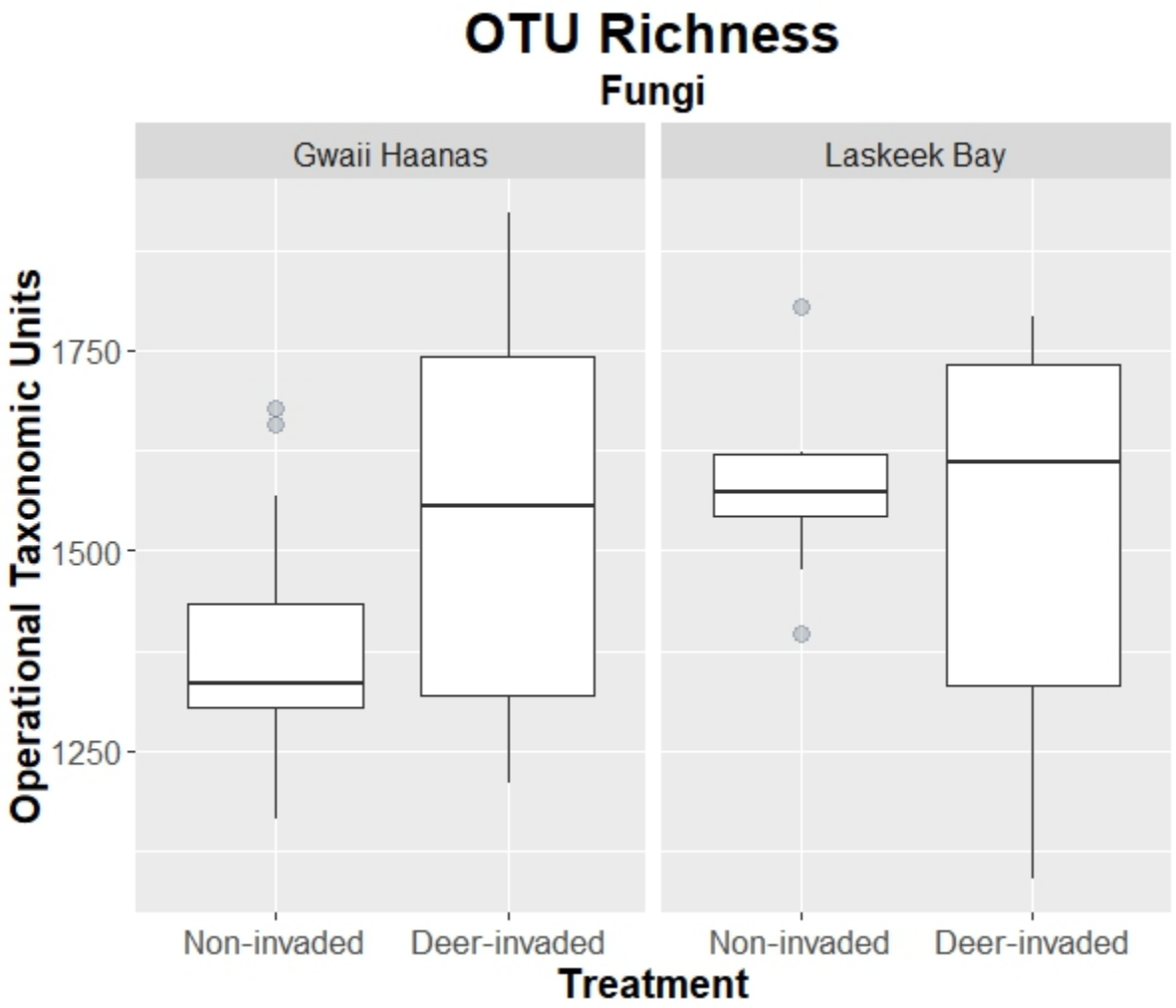

Figure 5.2: OTU richness of the fungal community in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

The mean OTU evenness of the fungal community was significantly higher in soils from deer-invaded islands compared with non-invaded islands ($F_{(1,40)} = 6.98$, $p = 0.012$, Table 5.3), but not between soils from Gwaii Haanas and Laskeek Bay. However, there was a significant interaction ($F_{(1,40)} = 13.25$, $p < 0.001$) between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay). In Gwaii Haanas, soils from deer-invaded islands had a higher OTU evenness compared with non-invaded islands, whereas in Laskeek bay, soils from deer-invaded islands had a lower OTU evenness compared with non-invaded islands (Figure 5.3). There was also a significant difference in the OTU evenness between individual islands ($F_{(5,40)} = 5.84$, $p < 0.001$, Appendix B § Table B.1). The mean OTU evenness of the fungal community in soils from Agglomerate Island (non-invaded) was unusually low (63.1% ± 1.0 SE) in contrast to that of other non-invaded islands (66.3% ± 0.7 SE) whereas the mean OTU evenness of the fungal community in soils from West Skedans Islands (deer-invaded) was unusually high (71.3% ± 0.68 SE) compared with the mean OTU evenness of other deer-invaded islands (68.0% ± 0.5 SE).

Table 5.3: OTU evenness of the fungal community in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. Values are mean percentages ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	65.0 ± 0.9	68.4 ± 0.9
	Deer-invaded	68.6 ± 0.5	67.4 ± 0.8

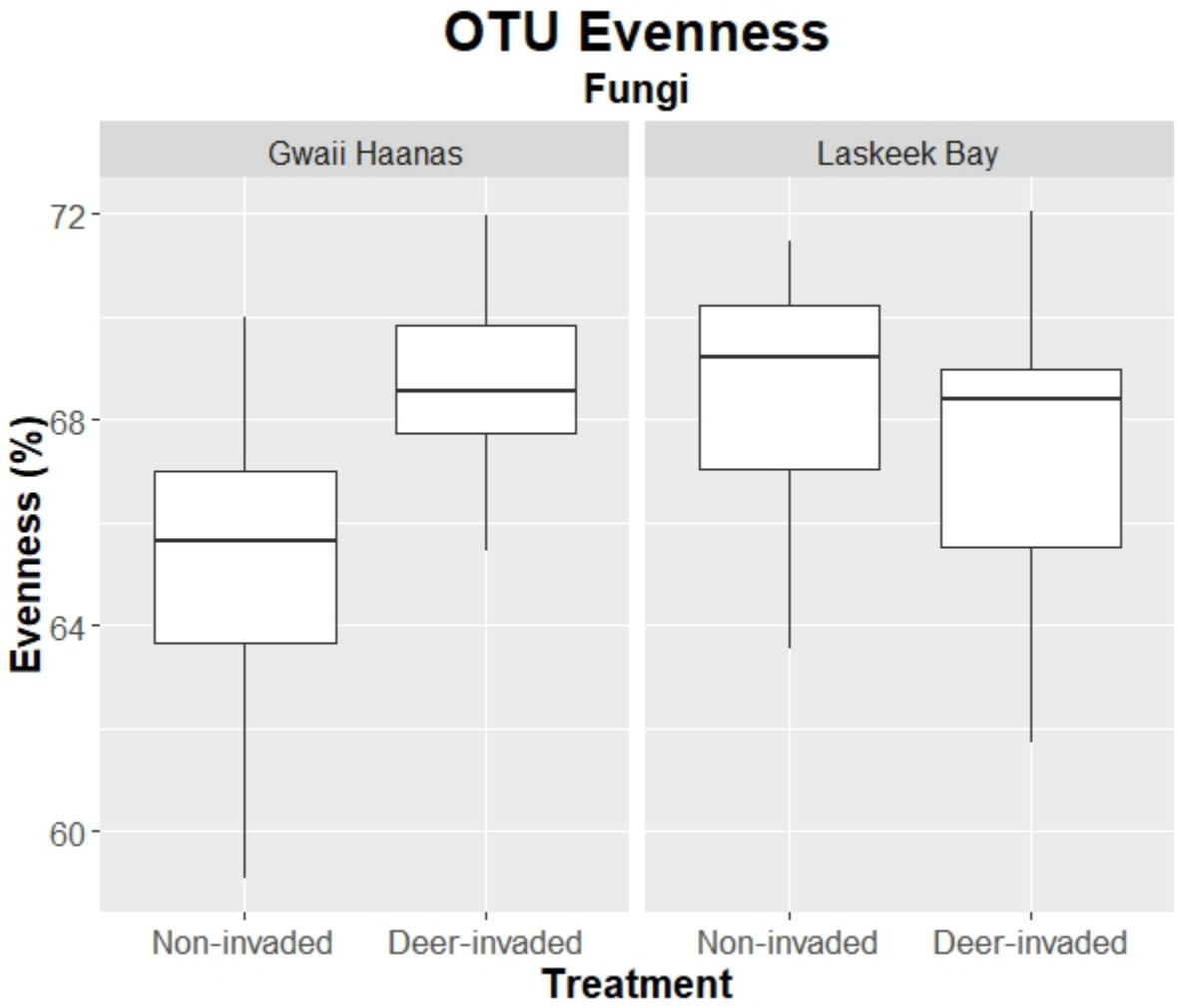

Figure 5.3: OTU evenness of the fungal community in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

The Shannon diversity index of the fungal community was significantly higher ($F_{(1,40)} = 5.65$, $p = 0.022$) in soils from deer-invaded islands than in soils from non-invaded islands. There was no significant difference in the Shannon diversity index between soils from Gwaii Haanas and Laskeek Bay; however, there was a significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas), with the Shannon diversity index of the fungal community tending to be higher in soils from Ramsay Island (deer-invaded) in Gwaii Haanas, but lower in soils from deer-invaded islands in Laskeek Bay (Table 5.4, Figure 5.4). The Shannon diversity index was unusually low in the non-invaded islands of Gwaii Haanas and there were significant differences in the Shannon diversity index between

individual islands ($F_{(5,40)}$ = 4.82, p = 0.002). The fungal community in soils from Agglomerate Island (non-invaded) in Gwaii Haanas had a mean Shannon diversity index of 4.53 ± 0.09 SE, whereas all other islands had a mean Shannon diversity index greater than 4.70 (Appendix B § Table B.1).

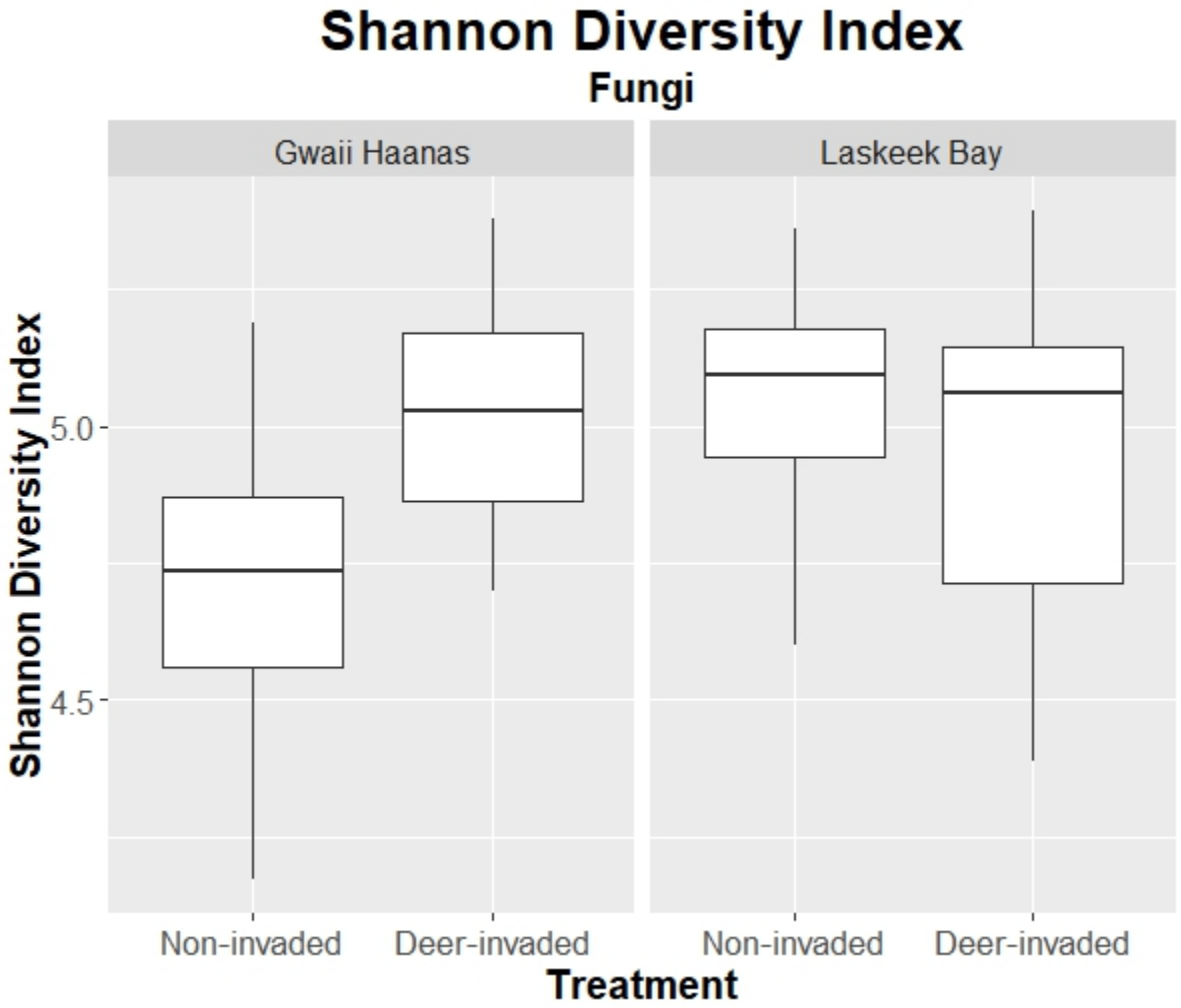

Figure 5.4: Shannon diversity index of the fungal community in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Table 5.4: Shannon diversity index of the fungal communities in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	4.70 ± 0.08	5.04 ± 0.08
	Deer-invaded	5.03 ± 0.05	4.94 ± 0.09

Permutational analysis of variance (PERMANOVA) of the fungal communities revealed there were significant differences in community composition in soils from non-invaded and deer invaded islands ($R^2 = 0.11$, $F_{pseudo} = 6.55$, $p = 0.001$), in soils from Gwaii Haanas and Laskeek Bay ($R^2 = 0.05$, $F_{pseudo} = 3.37$, $p = 0.001$), and in soils from individual islands ($R^2 = 0.16$, $F_{pseudo} = 2.06$, $p = 0.001$). There was also a significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay, $R^2 = 0.04$, $F_{(1,40)} = 2.27$, $p = 0.001$). Altogether, 36% of the variation in the fungal community could be explained by the presence of Sitka black-tailed deer on islands or by inherent differences between regions and individual islands (Appendix B § Table B.2).

Principal coordinates analysis (Figure 5.5, Appendix B § Figure B.1) revealed distinct separation of the fungal communities in soils from non-invaded and deer-invaded islands and regional differences in the fungal communities in soils from non-invaded islands. The fungal communities in soils from deer-invaded islands, however, were relatively similar between the two regions of Gwaii Haanas and Laskeek Bay. A model using the canonical analysis of principal coordinates (CAP) constrained the data with the factors of *treatment* (non-invaded vs deer-invaded), *region* (Gwaii Haanas vs Laskeek Bay), and *island* (Figure 5.6). Axes 1 and 2 were significant ($p < 0.001$) with the primary axis representing 16.1% of the variance and the secondary axis representing 6%.

Distance-based redundancy analysis (db-RDA) of the fungal community revealed four significant explanatory variables: total plant cover-abundance, soil pH, the depth of the organic horizons and the concentrations of ammonium in the soil (Appendix B § Table B.2). Other soil properties, such as moisture content, phosphate concentrations and nitrate concentrations were not significant variables and were not included in the model. Similarly, the species richness, species evenness, Shannon diversity index and the cover-abundances of the individual indicator species of the plant community (Chapter 4) were not significant variables and were not included in the model (Figure 5.7).

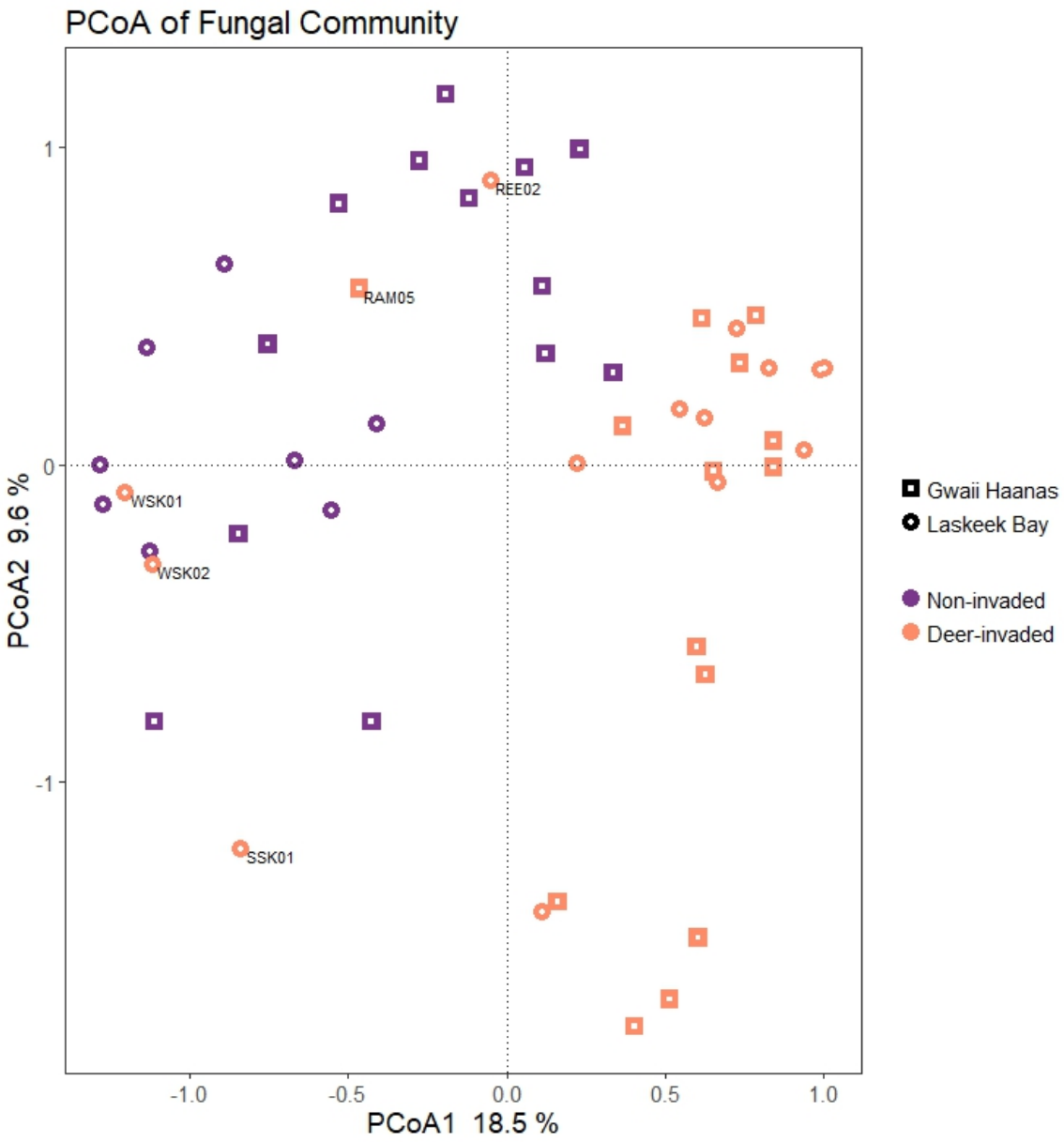

Figure 5.5: Principal coordinates analysis (PCoA) of the fungal community using Bray-Curtis dissimilarities of rarefied reads for soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively.

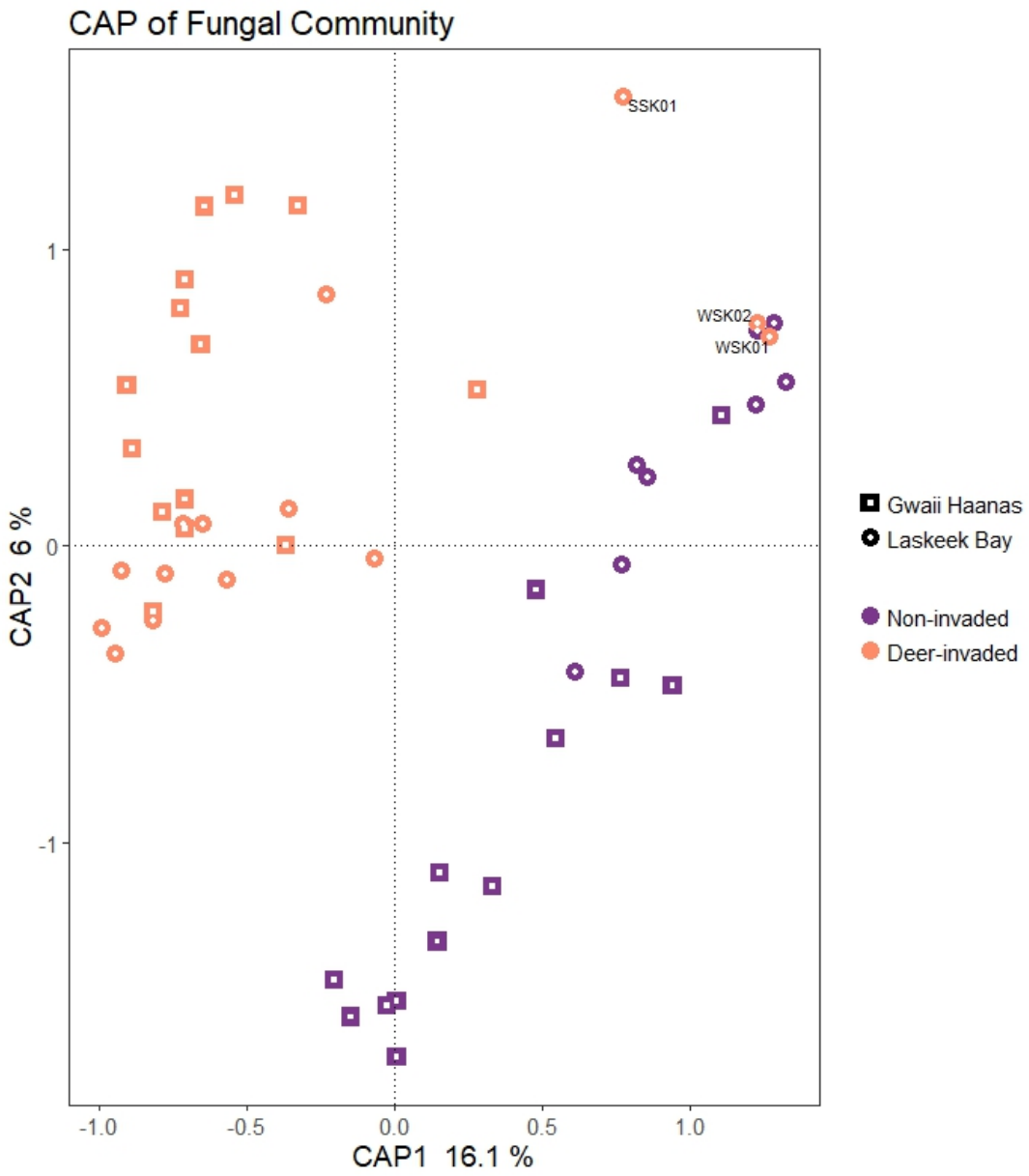

Figure 5.6: Canonical analysis of principal coordinates (CAP) of the fungal community using Bray-Curtis dissimilarities of rarefied reads for soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively, constrained using the factors of *treatment* (non-invaded vs deer-invaded), *region* (Gwaii Haanas vs Laskeek Bay) and *island* (Agglomerate, Lost, Louise, Low, Ramsay, Reef, South Skedans, Tar, West Skedans).

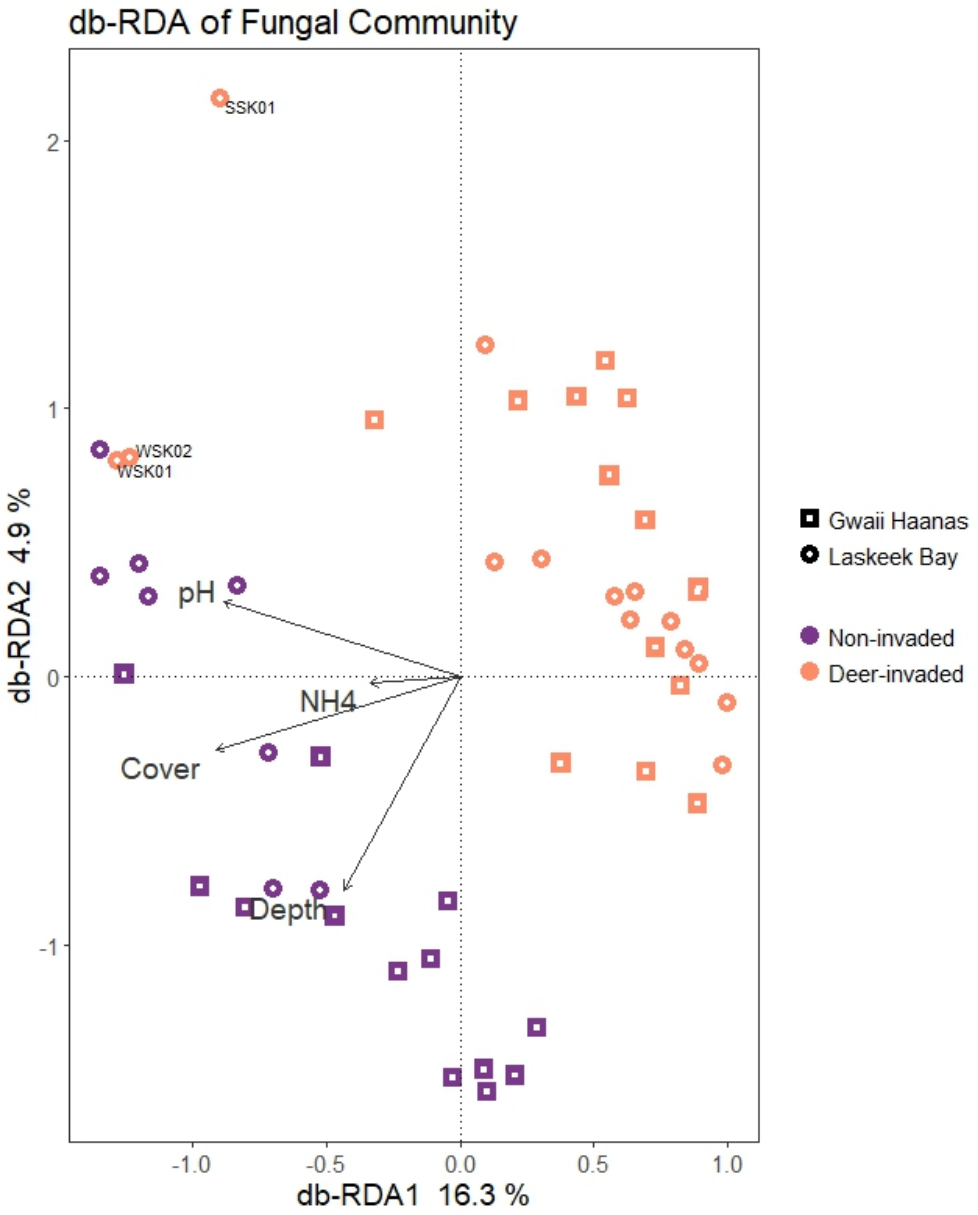

Figure 5.7: Distance-based redundancy analysis (db-RDA) of the fungal in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the regions of Gwaii Haanas and Laskeek Bay, respectively, constrained using the cover-abundances of understory plants (*cover*), soil pH, the depth of the organic horizon (*depth*) and the concentrations of ammonium (*NH4*) in the soil.

5.3.3 Functional Groups

Of the 11,944 total operational taxonomic units (OTU), 4,748 OTUs were identified to one or more mutually inclusive functional groups, including 2,558 species of saprotrophic fungi, 1,073 species of pathogenic fungi and 3,109 species of symbiotrophic fungi. Of the identifiable symbiotrophic fungi,

there were 63 species of arbuscular mycorrhizal fungi, 2,238 species of ectomycorrhizal fungi and 262 species of ericoid mycorrhizal fungi. All 262 OTUs of ericoid mycorrhizal fungi were identified as dark septate endophytes in the FUNGuild database.

There was no significant difference in the number of reads identified with arbuscular mycorrhizal fungi in soils from non-invaded and deer-invaded islands. OTUs identified as arbuscular mycorrhizal fungi represented fewer than 0.02% of the total identifiable reads and were completely absent in 25% of the samples. There were no significant differences in the number of reads identified as arbuscular mycorrhizal fungi between soils in Gwaii Haanas and Laskeek bay, nor between the soils of individual islands.

Similarly, there were no significant differences in the relative abundance of reads for pathotrophic or saprotrophic fungi between the soils of non-invaded and deer-invaded islands. The relative abundance of pathotrophic fungi was significantly higher in soils from Laskeek Bay (19.3%) than in soils from Gwaii Haanas (14.8%, $F_{(1,40)} = 7.55$, $p = 0.009$). Differences in the relative abundance of pathotrophic fungi were similar between individual islands. Saprotrophic fungi had significantly more reads in the soils from Laskeek Bay than Gwaii Haanas ($F_{(1,40)} = 11.39$, $p = 0.002$), but the differences were largely driven by unusually high abundances of saprotrophic fungi on the islands of South Skedans, West Skedans and Lost, all in Laskeek Bay. There was a significant difference in the number of reads identified as saprotrophic fungi between the soils of individual islands ($F_{(5,40)} = 5.31$, $p < 0.001$, Table 5.7).

OTUs identified as ectomycorrhizal fungi were highly abundant and represented 70% of the total identifiable reads. There was no significant difference in the mean number of reads identified as ectomycorrhizal fungi between soils from non-invaded and deer-invaded islands. Soils from Laskeek Bay had significantly fewer reads of ectomycorrhizal fungi than Gwaii Haanas ($F_{(1,40)} = 5.21$, $p = 0.028$), but the difference was small (Table 5.5) and likely due to outliers on the islands of South Skedans and West Skedans. There was a significant difference in the number of reads identified as ectomycorrhizal fungi between the soils of individual islands ($F_{(5,40)} = 3.14$, $p = 0.018$). Reads of OTUs assigned as ectomycorrhizal fungi represented only 42.3% and 56.3% of the total reads from identifiable OTUs in soils from South Skedans Island and West Skedans Island, respectively. Soils from all other islands had over 60% of reads assigned as ectomycorrhizal fungi.

Table 5.5: Relative abundance of ectomycorrhizal fungi in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the Gwaii Haanas and Laskeek Bay regions, respectively. Values are mean percentages of reads ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	72.6 ± 2.1	64.4 ± 3.8
	Deer-invaded	71.7 ± 2.0	67.9 ± 3.2

OTUs identified as ericoid mycorrhizal fungi represented 8.8% of the total identifiable reads (Table 5.6). As with other functional groups, there was no significant difference in the mean number of reads identified as ericoid mycorrhizal fungi between soils from non-invaded and deer-invaded islands, nor between soils from Gwaii Haanas and Laskeek Bay, nor between the soils of individual islands. Nonetheless, there were 39.7% fewer reads identified as ericoid mycorrhizal fungi in samples from the deer-invaded island of Gwaii Haanas (Ramsay Island) compared with non-invaded islands in that region (Tar Island, Agglomerate Island). In Laskeek Bay, samples from deer-invaded islands had 27% more reads identified as ericoid mycorrhizal fungi than samples from non-invaded islands (Figure 5.8).

Table 5.6: Relative abundance of ericoid mycorrhizal fungi in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the Gwaii Haanas and Laskeek Bay regions, respectively. Values are mean percentages of reads ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	8.6 ± 1.3	7.6 ± 2.2
	Deer-invaded	5.2 ± 0.9	9.6 ± 1.5

Table 5.7: Relative abundance saprotrophic, pathotrophic, ectomycorrhizal or ericoid mycorrhizal fungi in soils from individual islands. Values are mean percentages of reads ± SE.

Island	Saprotrophs	Pathotrophs	Ectomycorrhizal	Ericoid Mycorrhizal
Agglomerate	33.7 ± 2.2	13.7 ± 1.3	73.1 ± 2.4	10.6 ± 1.4
Lost	47.6 ± 4.5	19.2 ± 1.9	65.1 ± 4.1	4.4 ± 0.8
Louise	33.4 ± 3.5	16.2 ± 1.1	71.9 ± 2	11 ± 2.4
Low	46.6 ± 5.9	28.3 ± 6	63.9 ± 5.9	9.5 ± 3.3
Ramsay	33.9 ± 1.8	15.9 ± 1.4	71.7 ± 2.0	5.2 ± 0.9
Reef	32.9 ± 3.9	14.1 ± 1.6	72.7 ± 4.6	10.3 ± 2.8
South Skedans	72.4 ± NA	17.6 ± NA	42.3 ± NA	4.6 ± NA
Tar	34.8 ± 3.1	13.8 ± 2.5	72 ± 3.8	6.3 ± 1.9
West Skedans	50.4 ± 1.5	21.7 ± 0.9	56.3 ± 4.2	6.7 ± 2.1

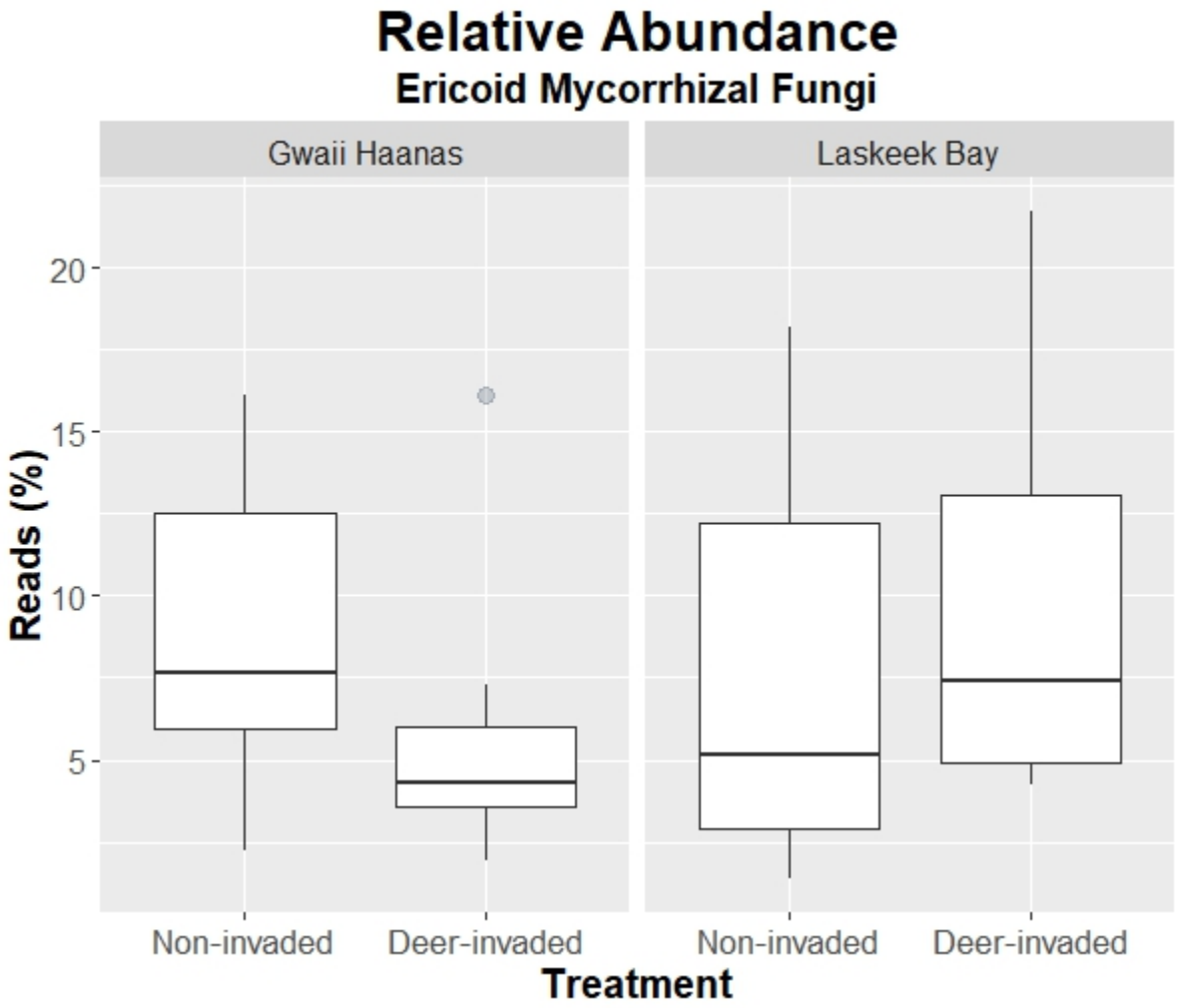

Figure 5.8: Relative abundance of OTUs identified as ericoid mycorrhizal fungi in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) in the Gwaii Haanas and Laskeek Bay regions, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

5.3.4 Taxonomic Groups

There were notable differences in the relative abundances of several classes of fungi between the soils of deer-invaded and non-invaded islands. Agaricomycetes were less abundant on deer-invaded islands, while Chytridiomycetes and Eurotiomycetes were more abundant on deer invaded islands (Figure 5.9). Glomeromycetes were conspicuously low in abundance and represented fewer than 1% of the total reads. Indicator analysis revealed 67 identifiable fungal species as significant indicators of non-invaded and deer-invaded islands ($p < 0.01$, Appendix B § Tables B.3).

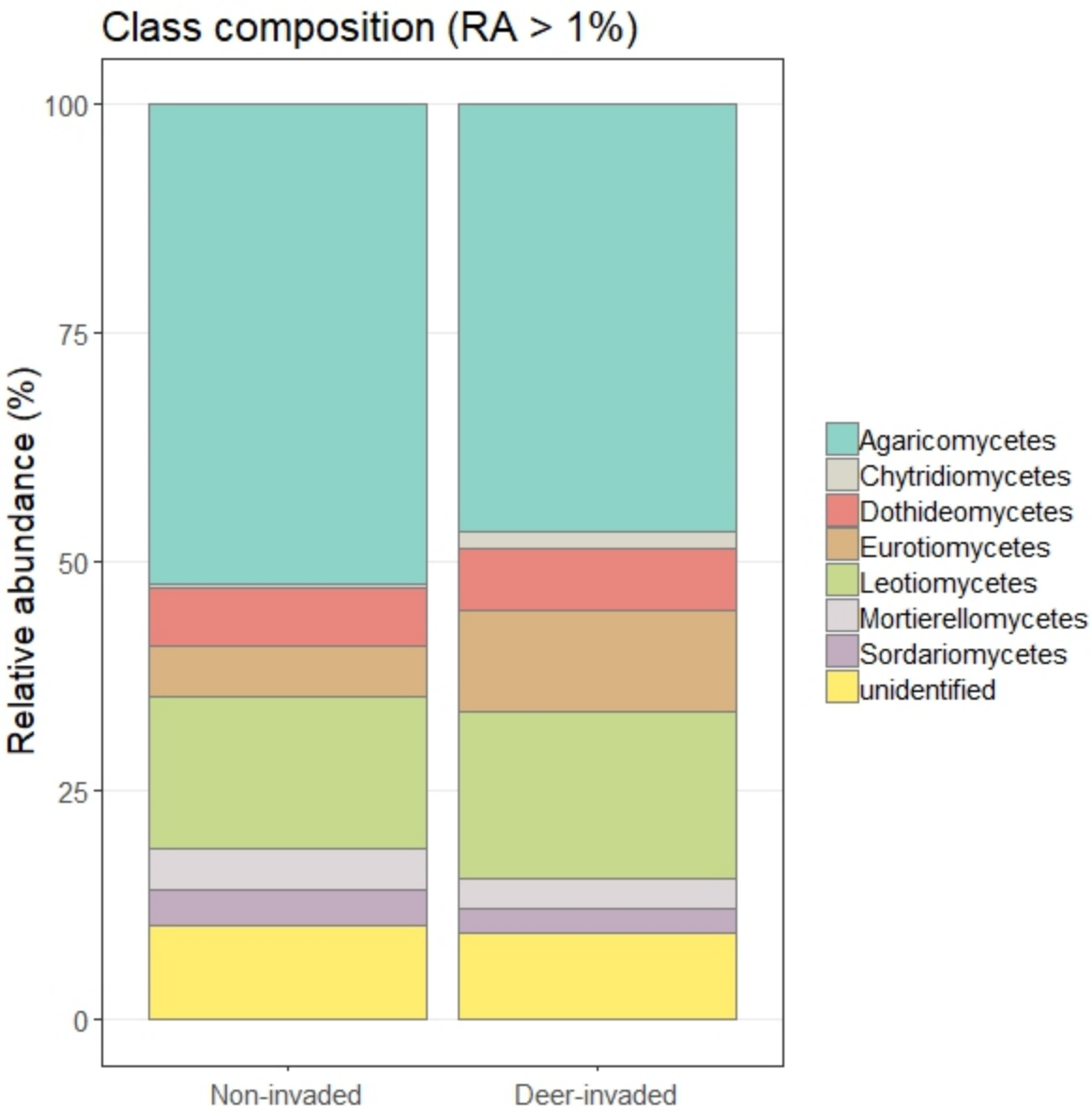

Figure 5.9: Relative abundance of fungal classes in soils from non-invaded islands (n = 21) and deer-invaded islands (n = 28). Only classes with relative abundances greater than 1% are displayed.

Several families in Agaricomycetes differed in relative abundance between soils from deer-invaded and non-invaded islands (Figure 5.10). The family Cantharellaceae was more abundant on deer-invaded islands, while Hydnodontaceae and Serendipitaceae were more abundant on non-invaded islands. Cantharellaceae was dominated by a single ectomycorrhizal species, *Craterellus tubaeformis* (Fr.) Quél. (formerly *Cantharellus tubaeformis*), a significant indicator of deer-invaded islands (indicator value = 0.85). *Cantharellus formosus* Corner occurred infrequently and at low abundances, present only

in a single sample from Agglomerate Island (non-invaded) and two samples from Louise Island (deer-invaded). Serendipitaceae, represented by 77 OTUs, was dominated by *Serendipita* species identified as orchid mycorrhizal fungi. Of the *Serendipita* OTUs, 25 were significant indicators of non-invaded islands and none were significant indicators of deer-invaded islands.

Chytridiomycetes were represented by a single species, *Rhizidium phycophilum* K.T. Picard, which, although present in all samples, was a significant indicator species of deer-invaded islands, with an indicator value of 0.91.

Several OTUs identified as species of Sordariomycetes were identified as significant indicators of non-invaded islands, including: *Verticillium leptobactrum W. Gams* (indicator value = 0.73), *Lecanicillium flavidum* (W. Gams & Zaayen) W. Gams & Zare (indicator value = 0.77) and *Pochonia cordycepisociata* H. Huang, Mu Wang & L. Cai (indicator value = 0.65).

In Eurotiomycetes, the most abundant OTU was identified as a *Penicillium* sp. and had an indicator value of 0.92 for deer-invaded islands. Overall, 75 OTUs were identified as *Penicillium* spp., including 23 significant indicator OTUs, the majority of which were indicators of deer-invaded islands. The next most abundant OTU in Eurotiomycetes was false-truffle (*Elaphomyces asperulus* Vitadd.), with an indicator value of 0.90 for deer-invaded islands.

All OTUs identified as ericoid mycorrhizal fungi were in the Leotiomycetes families of Myxotrichaceae and Helotiaceae, and included significant indicator OTUs for both deer-invaded and non-invaded islands. Of the ericoid mycorrhizal fungi identified, the most significant indicator OTUs of deer-invaded islands were *Meliniomyces* spp. which could not be identified to species. Significant indicator OTUs of non-invaded islands included the ericoid mycorrhizal species of *Oidiodendron echinulatum* G.L. Barron (indicator value = 0.80), *Oidiodendron maius* G.L. Barron (indicator value = 0.68) and *Oidiodendron pilicola* Kobayasi (indicator value = 0.64). Although an OTU identified as *Meliniomyces variabilis* Hambleton & Sigler was a significant indicator of deer-invaded islands (indicator value = 0.69), another OTU identified as the same species was similarly abundant between the two treatment groups and was not a significant indicator. The most abundant ericoid mycorrhizal species in Leotiomycetes was *Meliniomyces bicolor* Hambleton & Sigler, which was not a significant indicator OTU, being similarly abundant in the soils of both deer-invaded and non-invaded islands.

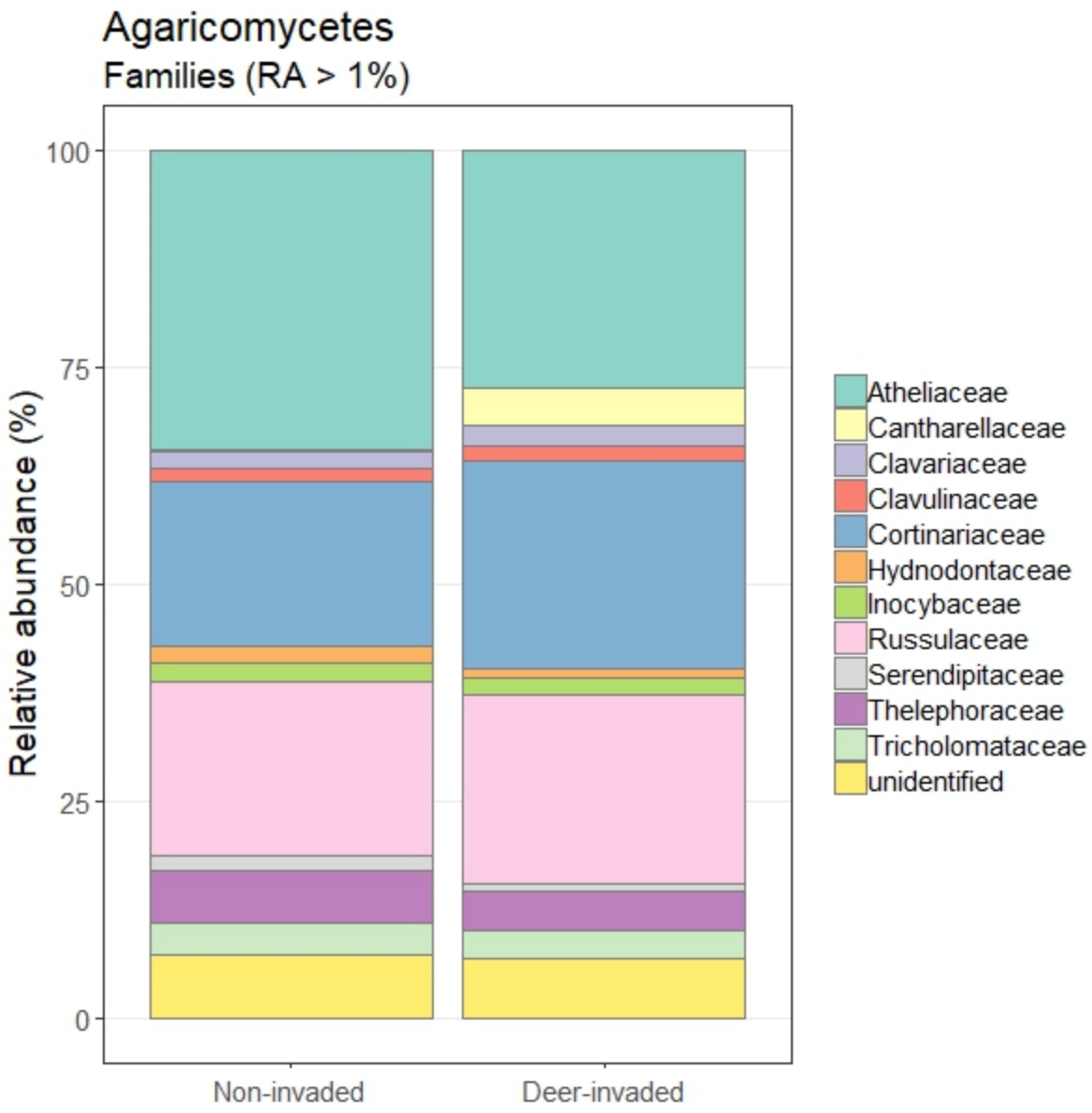

Figure 5.10: Relative abundance of families within Agaricomycetes in soils from non-invaded islands (n = 21) and deer-invaded islands (n = 28). Only families with relative abundances greater than 1% are displayed.

5.4 Discussion

5.4.1 Fungal Biomass

Ergosterol is a membrane lipid found almost exclusively in fungi and strongly correlates with living fungal biomass (Beni et al., 2014; Montgomery et al., 2000). Ergosterol is also present in other organisms such as microalgae but in forest soils, ergosterol is almost exclusively found in the

membranes of fungi (Beni et al., 2014). Although ergosterol tended to be lower in soils from deer-invaded islands compared with non-invaded islands, the difference was not significant and the pattern was largely restricted to Laskeek Bay. Because the regions of Laskeek Bay and Gwaii Haanas were sampled in different months and years, this study is not able to determine whether these patterns in ergosterol concentrations are due to legitimate spatial differences between Laskeek Bay and Gwaii Haanas or due to temporal variation in the fungal biomass in the soils of deer-invaded islands. Nonetheless, a colleague analyzing the phospholipid fatty acids (PLFA) of a similar set of soil samples from the islands of Laskeek Bay and Gwaii Haanas found a PLFA corresponding with fungal biomass to be lower in concentration in soils from deer-invaded islands (Catomeris, 2018). The similar trend between these two methods for estimating fungal biomass warrants further inquiry into the potential effects of Sitka black-tailed deer on fungal biomass, with care taken to parse potential spatial or temporal effects.

Conversion factors can be used to estimate fungal dry biomass based on ergosterol content of the soil, but the quantitative relationship between ergosterol and fungal biomass can vary between fungal taxa, methods of extraction and the growth phase of the fungi (Davis and Lamar, 1992; Montgomery et al., 2000; Olsson et al., 2003). Microwave-assisted extraction (MAE) techniques can extract up to 9x more ergosterol than saponification methods (Montgomery et al., 2000). A study using MAE methods to extract ergosterol from six species of fungi found 4 µg ergosterol per mg dry biomass (Montgomery et al., 2000). Another study using methanol extraction found the ratio ranged from 5 µg to 31 µg ergosterol per mg dry biomass (Stahl and Parkin, 1996). Using a mean of 5.1 µg ergosterol per mg dry biomass identified in a meta-analysis of ergosterol studies (Djajakirana et al., 1996), the concentration of fungal biomass per g dry soil on non-invaded and deer-invaded islands is estimated to be 8.31 mg/g and 7.49 mg/g, respectively. However, it is worth noting that while ergosterol is the dominant sterol found in the membranes of higher fungal taxa such as Ascomycota and Basidiomycota, it is largely absent in Glomeromycota and its presence varies between different taxa of Zygomycota (Olsson et al., 2003). Because the fungi identified through Illumina sequencing in the current study were dominated by Ascomycota and Basidiomycota, ergosterol is likely an appropriate biomarker for estimating the biomass of fungal taxa identified in this study.

5.4.2 Diversity and Structure of the Fungal Community

While there were significant differences in the species evenness and the Shannon diversity index between the fungal communities in soils from non-invaded and deer-invaded islands, the effect size was

small and driven by idiosyncratic differences in the fungal diversity of individual islands such as Agglomerate (non-invaded). Because fungal species richness was not significantly different between soils from non-invaded and deer-invaded islands, Shannon diversity largely reflected differences in the species evenness.

Multivariate analysis revealed the composition of the fungal community to be decoupled from the diversity and composition of the plant community. Neither the species richness, the species evenness nor the Shannon diversity index of the understory plant community served as significant predictors of fungal community composition. After including edaphic properties and the overall cover-abundance of understory plants in the db-RDA model, the cover-abundances of indicator plant species (Chapter 4) such as salal (*Gaultheria shallon*), red huckleberry (*Vaccinium parvifolium*), sword fern (*Polystichum munitum*) and deer fern (*Blechnum spicant*) were no longer found to be significant explanatory variables. Although fungal communities are often linked to the diversity and composition of plant communities, these results are not entirely surprising. Global patterns in the diversity and composition in soil fungal communities have also been found to be decoupled from the diversity and composition of plant communities, with climate factors, soil properties and spatial variables serving as better predictors (Tedersoo et al., 2014). Although phosphate concentrations were significantly higher in soils from deer-invaded islands (Chapter 3), phosphate did not significantly correlate with the composition of the fungal community after including plant cover in the model, suggesting that the availability of nitrogen is more limiting in these soils than phosphorus. Sitka black-tailed deer appear to influence the soil fungal community primarily through decreases in the overall abundance of understory plants and changes in soil properties such as pH, ammonium concentrations and soil depth.

Because soils from Gwaii Haanas were sampled in a different month and year from when soils from sampled in Laskeek Bay, this current study could not determine whether the regional differences between the fungal communities in soils from Gwaii Haanas and Laskeek Bay are due to the soil parent material of these two regions (Chapter 2) or due to seasonal and yearly variation in temperature and precipitation. For example, a field survey on aboveground fungal fruiting bodies found that the effect of climate on the species richness of fungi varied between different taxonomic and functional groups, with the species richness of Basidiomycota and ectomycorrhizal fungi strongly affected by differences in temperature and humidity while other taxa and functional groups were less affected by these climatic variation (Rudolph et al., 2018). In the current study, soil moisture content was significantly higher in the Laskeek Bay samples compared with the Gwaii Haanas samples, suggesting there were differences in the

amount of precipitation at the time of sampling, which in turn may have driven the differences in fungal community composition between the soils of these two regions. However, in the distance-based RDA of the fungal community, moisture content was found to not be a significant variable, which suggests that differences in soil parent material between Gwaii Haanas and Laskeek Bay are more likely to be responsible for the regional differences in the composition of the fungal community.

Distinct microbial communities form in the organic horizons under different tree species, with variation most pronounced in the F layer, (Prescott and Grayston, 2013) which was sampled in the current study. Heterogeneity in fungal communities may also be due to microsite conditions affected by individual trees. Variation in the quality of litter between different plant species contributes to heterogenous soil microbial communities in forests dominated by mixed tree species, with individual trees strongly influencing soil biota in their proximity (Saetre and Bååth, 2000). Thus, the heterogeneity of the fungal community may be due to random effects associated with individual trees in or around each plot.

Surprisingly, the relative abundance of ericoid mycorrhizal fungi was similar between non-invaded and deer-invaded islands. *Oidiodendron maius* G.L. Barron, an ericoid mycorrhizal species known to colonize the roots of salal (Xiao and Berch, 1995), represents an exception to this overall trend. *O. maius* was strongly associated with non-invaded islands, suggesting that it is particularly dependent on its ericaceous plant hosts which are consumed by aboveground herbivores. Overall, however, these results suggest that ericoid mycorrhizal fungi are resilient to the loss of ericaceous plants in the understory. In contrast to AM fungi, ericoid mycorrhizal fungi retain the ability to decompose organic matter (Tedersoo et al., 2014; van der Heijden et al., 2015), which may serve as a source of carbon for ErM fungi and buffer them from the loss of ericaceous plant hosts consumed by the invasive deer.

As an alternative hypothesis for how ericoid mycorrhizal fungi survive on islands invaded by deer, ErM fungi may form mutualisms with other plants that are able to persist or thrive on deer-invaded islands. Indeed, burgeoning evidence suggests that various species of ascomycetous ericoid mycorrhizal fungi are able to colonize the roots of non-ericaceous plants (Chambers et al., 2008). Bryophytes, which are especially abundant on deer-invaded islands (Chollet et al., 2013), may serve as a ubiquitous reservoir for ericoid mycorrhizal fungi (Brundrett, 2004). The *Hymenoscyphus ericae* aggregate, which forms ericoid mycorrhizae in plants from the genera of *Erica*, *Rhododendron* and *Vaccinium*, has been found to colonization the rhizoids of leafy liverworts (Duckett and Read, 1995).

Grasses and oak trees are other potential hosts for ericoid mycorrhizal fungi. A study on heathland plants in the Netherlands found that Helotiales ascomycetes isolated from the roots of a grass species (*Deschampsia flexuosa*) were able to colonize the roots and increase the nitrogen uptake of *Calluna vulgaris*, an ericaceous species (Zijlstra et al., 2005). Likewise a Helotiales ascomycete isolated from the roots of *Quercus ilex* in Italy formed hyphal coils characteristic of ericoid mycorrhizas in the hair roots of *Erica arborea* (Bergero et al., 2000). Furthermore, the most abundant ericoid mycorrhizal species identified in the current study, *Meliniomyces bicolor* Hambl. & Sigler (*Rhizoscyphus ericae* aggregate), is known to form ectomycorrhizas (Grelet et al., 2010). Further evidence of this phenomenon is presented in a Norwegian study in which ErM fungal DNA was extracted from the ectomycorrhizas of several tree species, including: *Picea abies*, *Pinus sylvestris*, *Betula pubescens*, *Populus tremula*, *Quercus robur* and *Salix phylicifolia* (Vrålstad et al., 2000). The *Piceirhiza bicolorata* morphotype shared 95% of its ITS1 region with *Hymenoscyphus ericae*, an ericoid mycorrhizal aggregate of the Helotiales order (Vrålstad et al., 2000). In a follow-up study, the *Hymenoscyphus ericae* aggregate formed ectomycorrhizas in both Norway spruce (*Picea abies*) and birch (*Betula pubescens*), and was found to include the dark septate endophyte otherwise known as *Phialophora finlandia* C.J.K. Wang & H.E. Wilcox (Vrålstad, Schumacher, & Taylor, 2002).

Although some species in Basidiomycota may form ericoid-type mycorrhizas (Allen et al., 2003), most of the currently recognized ErM fungi belong to Helotiales (van der Heijden et al., 2015). Likewise most dark septate endophytes belong to the Helotiales order within Leotiomycetes (Newsham, 2011), which also contains most of the ericoid mycorrhizal fungi identified in the current study. Most of the DSE identified were also noted to be ericoid mycorrhizal fungi in the FUNGuild database, providing evidence that ericaceous plant species such as salal may function as alternate hosts for fungi that are otherwise recognized as dark septate endophytes in other host plants on Haida Gwaii, such as *Thuja plicata* (Chapter 6). Indeed, dark septate endophytes have been previously identified in the roots of both salal (*Gaultheria shallon*) and western redcedar (*Thuja plicata*) in British Columbia (Ahlich and Sieber, 1996), raising the possibility that these species may share common mycelial networks of endophytic fungi.

Glomeromycota, the phylum containing arbuscular mycorrhizal fungi, was surprisingly low in abundance and frequency. OTUs representing Glomeromycota were completely absent from about a quarter of all the samples. However, results from the greenhouse bioassay (Chapter 6) would seem to confirm that AM fungi capable of colonizing the roots of *Thuja plicata* have a relatively low abundance in the soils that were sampled for this study. While, primer bias may have contributed to the

underrepresentation of Glomeromycota, it is unlikely to have resulted in the near absence of their DNA amplicons in the PCR products. The gITS7 forward primer used in this study matches 74% of Glomeromycota 5.8S sequences (Ihrmark et al., 2012), while the ITS4 reverse primer matches 99% of Glomeromycota LSU sequences (Toju et al., 2012). Therefore, the gITS7/ITS4 primer pair are likely to amplify the ITS2 region of most taxa from Glomeromycota.

Several entomopathogenic fungal species from Sordariomycetes were identified as being strongly associated with the soils of non-invaded islands. Functionally diverse, *Verticillium leptobactrum W. Gams* has been documented to parasitize nematodes (Regaieg et al., 2011) but is also capable of weathering chrysotile asbestos (Daghino et al., 2009). Two other species, *Pochonia cordycepisociata* H. Huang, Mu Wang & L. Cai and *Lecanicillium flavidum* (W. Gams & Zaayen) W. Gams & Zare, parasitize insects and nematodes, respectively (Gams and Zare, 2001; Huang et al., 2015). These entomopathogenic fungi were less abundant in the soils of deer-invaded islands, raising the question of how Sitka black-tailed deer indirectly affect the abundances of belowground insects, nematodes and their pathogens.

Rhizidium phycophilum K.T. Picard, the sole Chytridiomycetes species identified in the samples, is a saprotroph-symbiotroph that may form parasitic associations with algal cells, similar to other lichen-associated *Rhizidium* species (Picard et al., 2009). *R. phycophilum* was first described in soils from low elevation temperate rainforests in New South Wales dominated by Antarctic beech with an understory largely comprised of ferns and moss (Picard et al., 2009). Similar conditions are found on the deer-invaded islands of Haida Gwaii which tend to have a high abundance of moss and other bryophytes (Chollet et al., 2013). As *R. phycophilum* was a significant indicator of deer-invaded islands, these results demonstrate how Sitka black-tailed deer may be expanding the suitable habitat for this species by removing shrubs and other understory plants that would otherwise compete with lichens and bryophytes for light.

5.5 Conclusion

The objective of the research presented in this chapter was to determine the biomass, diversity and composition of the soil fungal communities on islands invaded or not invaded by Sitka black-tailed deer in Gwaii Haanas and Laskeek Bay. Small but significant differences in species (OTU) evenness and Shannon diversity were detected between the soil fungal communities of non-invaded and deer-invaded

islands. However, there were inconsistent patterns between the species evenness in Gwaii Haanas and Laskeek Bay, conflicting with hypotheses that fungal species would be distributed more evenly on deer-invaded islands. Species evenness likely varies between individual islands. Species (OTU) richness was not affected by the presence of Sitka black-tailed deer, conflicting with the hypothesis that fewer species would be present on deer-invaded islands. Multivariate analysis revealed significant differences in the composition of the fungal community that were driven largely by the total cover-abundance of understory plants and to a lesser degree by differences in soil pH, ammonium concentrations and the depth of the organic soil horizons. Functional guild assignments of the operational taxonomic units suggest that arbuscular mycorrhizal fungi are extremely low in abundance and often absent in these soils, with no significant difference in relative abundance between deer-invaded and non-invaded islands. There were also no significant differences in the overall relative abundances of saprotrophic or pathogenic fungi between non-invaded and deer-invaded islands. The majority of the identifiable OTUs were ectomycorrhizal fungi. While there was no significant difference in the relative abundance of reads representing ectomycorrhizal fungi between soils from deer-invaded and non-invaded islands, there were significantly fewer ectomycorrhizal fungi in Laskeek Bay than in Gwaii Haanas, however, differences were largely driven by the abundance of ectomycorrhizal fungi on individual islands. Soils from the deer-invaded island of Gwaii Haanas (Ramsay Island) had a lower relative abundance of identifiable ericoid mycorrhizal fungi than soils from non-invaded islands in Gwaii Haanas, but the overall difference between deer-invaded and non-invaded islands was not significant. In summary, differences in the soil properties and understory plant abundances on deer-invaded islands are correlated with significant shift in the composition of the fungal community, with species more evenly distributed at fine spatial scales. However, most variation in the fungal community cannot be attributed to the effects of Sitka black-tailed deer and major functional groups of mycorrhizal fungi were not significantly affected. Altogether, these data suggest that the soil fungal communities of Haida Gwaii are fairly resilient to the environmental changes associated with the invasion of Sitka black-tailed deer and that unique fungal communities form on individual islands.

CHAPTER 6 – Mycorrhizal Inoculum Potential of Deer-Invaded and Non-Invaded Islands

6.1 Introduction

6.1.1 Background

One of the functions of soil is to provide a source of inoculum to colonize the roots of newly germinated plants with mycorrhizal fungi which form symbiotic relationships with approximately 80% of terrestrial plant species (Smith and Read, 1997). By functioning as an extension of root systems, mycorrhizal fungi facilitate the uptake of water and soil nutrients (Vance, 2001). For example, in temperate forest ecosystems, mycorrhizal fungi are responsible for more than 75% of the nitrogen and phosphorus uptake in plants (van der Heijden et al., 2008). Aboveground herbivores can influence soil mycorrhizal fungi through long-term shifts in the functional composition of plant communities (Bardgett and Wardle, 2003; Gehring et al., 2002) or through the physiological responses of plants to grazing (Gehring and Whitham, 1994), however, it remains unclear how hyperabundant herbivores such as Sitka black-tailed deer affect the mycorrhizal inoculum potential of soils in temperate conifer forests such as those on Haida Gwaii.

The temperate conifer forests of Haida Gwaii are comprised of a variety of conifer trees, shrubs, ferns and herbs which themselves support various functional groups of mycorrhizal fungi. Western redcedar (*Thuja plicata*), one of the dominant tree species, forms mutualisms with arbuscular mycorrhizal (AM) fungi (Kough et al., 1985; Parke et al., 1983b). Dark septate endophytes (DSE) are also likely to be present in the roots of *Thuja plicata* on Haida Gwaii. DSE have been confirmed in the roots of other *Thuja* species globally (Nagaraj et al., 2015) and DSE have been isolated more locally from soils in forests dominated by *Thuja plicata* near Tofino, British Columbia (Narisawa et al., 2007). Western hemlock (*Tsuga heterophylla*), another dominant tree species on Haida Gwaii, forms relationships with dozens of ectomycorrhizal (EcM) fungal species (Kropp and Trappe, 1982). Although western hemlock (*Tsuga heterophylla*) is reported to form both arbuscular and ectomycorrhizae (Cázares and Smith, 1995), a study in British Columbia comparing mixed *Thuja plicata / Tsuga heterophylla* stands with mixed *Tsuga heterophylla / Abies amabilis* stands found that arbuscular mycorrhizae were absent in cedar trees grown in soil from the *Tsuga heterophylla / Abies amabilis* stands (Weber et al., 2005a) suggesting that either i) hemlock trees primarily form ectomycorrhizae, or ii) hemlock trees do not

support the arbuscular mycorrhizal fungi that colonize cedar trees. The understory plant communities of Haida Gwaii are dominated by ericaceous species such as salal (*Gaultheria shallon*), red huckleberry (*Vaccinium parvifolium*) and oval-leaved blueberry (*Vaccinium ovalifolium*, Banner et al., 2014) which likely form relationships with ericoid mycorrhizal (ErM) fungi (Brundrett, 2004). Salal may also serve as an alternate host for the dark septate endophytes and ectomycorrhizal fungi that colonize *Thuja plicata* and *Tsuga heterophylla*. Dark septate endophytes have been identified in the roots of both *Gaultheria shallon* and *Thuja plicata* in British Columbia (Ahlich and Sieber, 1996). Some species of ectomycorrhizal fungi that colonize plants in Pinaceae are capable of forming ericoid mycorrhizas in plants from *Ericaceae* (Lukešová et al., 2015). As plant species vary in the types of mycorrhizal fungi which they support, long-term shifts in the abundance, diversity and composition of plant communities associated with aboveground herbivores may alter the inoculum potential of different functional groups of mycorrhizal fungi (Gehring et al., 2002).

Differences in the inoculum potential of mycorrhizal fungi are associated with growth responses in plants, with higher root colonization generally associated with greater plant growth (Gehring et al., 2002; van der Heijden et al., 2015). Colonization by dark septate endophytes also tends to be associated with positive growth responses (Newsham, 2011). However, plant species vary in the degree to which their biomass and resource allocation respond to mycorrhizal fungi (Plenchette et al., 1983; van der Heijden et al., 1998). As a response to colonization by mycorrhizal fungi, plants have been observed to shift the proportion of their biomass in the roots and shoots, with both decreased root biomass (Koathari et al., 1990) or increase root biomass being reported (Porras-Soriano et al., 2009). Colonization by mycorrhizal fungi can also reduce various forms of stress in plants, including salt stress (Evelin et al., 2009) nutrient stress (Porras-Soriano et al., 2009) and drought stress (Ortega et al., 2004). The degree to which mycorrhizal fungi alleviate plant stress associated with drought conditions, nutrient deficiency or other environmental factors can be inferred by measuring the dark-adapted chlorophyll fluorescence of plant leaves (Borkowska, 2002; Parádi et al., 2003; Zuccarini and Okurowska, 2008), a sensitive indicator of the quantum efficiency of photosystem II (PSII) in the thylakoid membranes of plants (Murchie and Lawson, 2013). Generally, greater stress is associated with lower ratios of variable fluorescence to maximum fluorescence (Murchie and Lawson, 2013).

Although extensive research has evaluated the effects of herbivory on mycorrhizal fungi (Gehring and Whitham, 2002), most studies on herbivore-mycorrhizae interactions have measured the relative abundance of arbuscular mycorrhizal root colonization as physiological response to the grazing

of plants (Frank and Evans, 1997; Rossow et al., 1997; Saravesi et al., 2014) rather than the inoculum potential of mycorrhizal fungi. Furthermore, most of the previous research on the effects of herbivores on AM fungi has been conducted in grassland ecosystems, which are likely to be dominated by arbuscular mycorrhizal plant species, making these studies less relevant to temperate conifer forests, which are often dominated by ectomycorrhizae and other fungal endophytes (Tedersoo et al., 2014; Weber et al., 2005a). This chapter describes the results of a bioassay used to determine the capacity of soils from deer-invaded and non-invaded islands of Haida Gwaii to inoculate the roots of western redcedar (*Thuja plicata*) and western hemlock (*Tsuga heterophylla*) with mycorrhizal fungi and to evaluate the growth and stress responses of these tree species to the biotic and abiotic conditions of the soils. In a greenhouse experiment, *Thuja plicata* and *Tsuga heterophylla* were grown from seed in both living and sterilized soil from deer-invaded and non-invaded islands. I predicted that decreases in the abundance of understory plants on islands invaded by Sitka black-tailed deer would be associated with a decrease in the inoculum potential of arbuscular mycorrhizal fungi and dark septate endophytes capable of colonizing *Thuja plicata*, as well as a decrease in the inoculum potential of ectomycorrhizal fungi capable of colonizing *Tsuga heterophylla*. I expected that seedlings of both species grown in soil from deer-invaded islands would have lower biomass and higher root:shoot ratios as a result of less water and nutrient uptake through mycorrhizae. Furthermore, I predicted that lower mycorrhizal inoculum potential would be associated with greater stress under drought conditions, as inferred through lower F_v/F_m chlorophyll fluorescence values.

6.1.2 Objective and Hypotheses

The objective of the research presented in this chapter was to determine the growth response (biomass, root:shoot ratios), stress response (chlorophyll fluorescence) and inoculum potential of arbuscular mycorrhizal fungi, dark septate endophytes and ectomycorrhizal fungi in *Thuja plicata* and *Tsuga heterophylla* seedlings grown in soil from non-invaded and deer-invaded islands in the regions of Gwaii Haanas and Laskeek Bay.

For *Thuja plicata*, the following hypotheses were tested:

H_0 The presence of Sitka black-tailed deer on islands has no effect on the mycorrhizal inoculum potential of the soil, nor an effect on the biomass, root:shoot ratios and chlorophyll fluorescence of *Thuja plicata* grown in that soil.

H_1 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the inoculum potential of arbuscular mycorrhizal fungi in soil from those islands.

H_2 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the inoculum potential of dark septate endophytes in soil from those islands.

H_3 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the biomass of *Thuja plicata* grown in soil from those islands.

H_4 The presence of Sitka black-tailed deer on the islands is associated with an increase in the root:shoot ratios of *Thuja plicata* grown in soil from those islands.

H_5 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the chlorophyll fluorescence values (F_v/F_m) of *Thuja plicata* grown in soil from those islands.

For *Tsuga heterophylla*, the following hypotheses were tested:

H_0 The presence of Sitka black-tailed deer on the islands has no effect on the mycorrhizal inoculum potential of the soil, nor an effect on the biomass, root:shoot ratios and chlorophyll fluorescence of *Tsuga heterophylla* grown in that soil.

H_1 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the inoculum potential of ectomycorrhizal fungi in soil from those islands.

H_2 The presence of Sitka black-tailed deer on the islands is associated with a decrease in the biomass of *Tsuga heterophylla* grown in soil from those islands.

H_3 The presence of Sitka black-tailed deer on the islands is associated with an increase in the root:shoot ratios of *Tsuga heterophylla* grown in soil from those islands.

H_4 The presence of Sitka black-tailed deer on the islands is associated with a decrease in chlorophyll fluorescence values (F_v/F_m) of *Tsuga heterophylla* grown in soil from those islands.

6.2 Methods

6.2.1 Greenhouse Bioassay

Two greenhouse bioassays were conducted using *Tsuga heterophylla* and *Thuja plicata* grown from seed in sterilized media inoculated with soil from the 49 field sites using a fully crossed design with three factors: *treatment* (invaded islands vs non-invaded islands), *region* (Gwaii Haanas vs Laskeek Bay) and *soil* (living soil vs sterilized soil). Field soils used as fungal inoculum included: 21 samples from non-invaded islands and 28 samples from deer-invaded islands (*treatment*); 27 samples from Gwaii Haanas and 22 samples from Laskeek Bay (*region*). For each of the tree species, 98 seedlings were grown in paired samples, 49 inoculated with living field soil and 49 inoculated with sterilized field soil. However,

out of the 196 total containers planted with seeds, *Thuja plicata* seedlings failed to emerge in 15 of the containers and *Tsuga heterophylla* seedlings failed to emerge in 1 of the containers. The final sample sizes in each treatment group are summarized in tables 6.1 and 6.2.

Table 6.1: Sample size (plots) in each treatment group of the *Thuja plicata* bioassay.

	Living Soil		Sterilized Soil	
	Gwaii Haanas	Laskeek Bay	Gwaii Haanas	Laskeek Bay
Non-invaded	13	7	13	7
Deer-invaded	11	11	11	10

Table 6.2: Sample size (plots) in each treatment group of the *Tsuga heterophylla* bioassay.

	Living Soil		Sterilized Soil	
	Gwaii Haanas	Laskeek Bay	Gwaii Haanas	Laskeek Bay
Non-invaded	13	8	13	7
Deer-invaded	14	14	14	14

Seeds were obtained from the Tree Seed Centre. B class (wild type) seeds of *Tsuga heterophylla* and *Thuja plicata* were sourced from local provenances on Haida Gwaii within the same biogeoclimatic zone (CWHwh1) as the study site (Banner et al., 2014). The seeds of *Tsuga heterophylla* (seedlot# 04762) were collected in 1978 from a location 230 m in elevation, approximately 12 km west of the study site. The seeds of *Thuja plicata* (seedlot# 45052) were collected in 1999 from a location 100 m in elevation, approximately 20 km north of the study area. Seeds were prepared first by surface sterilizing them in a solution of 0.5% sodium hypochlorite for 10 minutes and then triple rinsing in deionized water. Seeds were further prepared using cold-wet stratification on sterilized peat-based media at 4°C for 3 months.

The greenhouse experiment was conducted at the University of British Columbia Horticulture Greenhouse over 247 days, from September 26, 2017 through May 30, 2018. Soil samples used as inoculum were kept separate from each other and stored at -20°C until use. Standard media was prepared by mixing a 1:1:2 ratio by volume of coarse sand, fine sand and a peat-based potting mix. The

standard media and a portion of each of the field samples were twice sterilized in an autoclave at 121°C for 45 minutes with a 48-hour interval in between the two sterilizations. For each sample, approximately 90 mL of standard media was mixed with 45 mL of field soil and put in cone-shaped containers (Greenhouse Megastore, product no. CN-SS-SC10R) which were approximately 3.8 cm in diameter, 21 cm in depth, and 164 mL in volume. Three seeds were sown per container and covered with an approximately 0.5 cm deep layer of fine sand. Containers were misted until seed germination. For containers with more than one germinating seedling, the shortest of the seedlings were culled after their cotyledons fully emerged, leaving only a single seedling per container. Seedlings were watered as needed to maintain field-capacity soil conditions for the duration of the experiment – generally 3 times per week. Plants were watered from above using tap water without any supplementary nutrients.

Near the conclusion of the experiment, the watering regime was ceased for 3 days to induce drought stress. Chlorophyll fluorescence was measured to evaluate how the drought conditions affected the photochemical efficiency of photosystem II in the plants (Maxwell and Johnson, 2000; Paknejad et al., 2007). A pulse-modulated chlorophyll fluorometer (Opti-Sciences, OS30p) was used to measure the variable fluorescence (F_v) and maximum fluorescence (F_m) of the foliage after dark-adapting the whole plants for 3 hours in total darkness. Three technical replicates were measured per plant. For subsequent analyses, the ratio of variable and maximum fluorescence (F_v/F_m) was used as indicator of stress in the plants (Fang-yuan and Guy, 2004; Paknejad et al., 2007).

Fresh biomass of roots and shoots was measured at the end of the experiment. The dry mass of shoots was measured after drying at 70°C for 48 hours. Roots were destructively harvested to determine mycorrhizal colonization rates and thus could not be directly measured for dry mass. Moisture content was measured on 10 subsamples of the roots in each of the *treatment* X *soil* groups by measuring the biomass before and after drying at 70°C for 48 hours. The mean moisture content of each *treatment* X *soil* group for each tree species was then used to estimate the total dry biomass of all roots.

Ectomycorrhizal root colonization of *Tsuga heterophylla* was estimated based on a modification of the protocol used by (Guichon, 2015), using morphotype criteria described by (Agerer, 1987). After cutting the roots of each sample into 2 cm segments, 10 random root segments were selected for observation under a microscope (Nikon Eclipse E400) at x40 magnification. Five random root tips were observed per root segment for a total of 50 root tips per sample. noting the presence or absence of mycorrhizae on each. For samples with fewer than 50 root tips, a complete survey of all root tips was conducted. Color, morphology, and the presence or absence of hyphae were used to determine whether or not root tips were colonized by ectomycorrhizal fungi.

To assess the colonization of arbuscular mycorrhizal fungi and dark septate endophytes in *Thuja plicata*, the procedure described by Vierheilig, Coughlan, Wyss, & Piche, (1998) was adapted to clear, stain and destain roots. Roots were cut into 1 cm segments and autoclaved at 121°C for 30 minutes in a solution of 10% KOH. The roots were then rinsed in deionized water that was acidified with a few drops of acetic acid and immersed in a solution of 5% ink (Sheaffer, product no. 94231) and 5% acetic acid at 95°C for 4 minutes. Roots were then destained in acidified water for 24 hours. Colonization rates were estimated using an adaptation of the line-intercept method (McGonigle et al., 1990). 20 random root segments were mounted on slides and observed under a compound microscope (Nikon Eclipse E400) at x400 magnification using three random 500 µm wide sections per root segment for a total of 60 observations per sample. The presence or absence of hyphae, vesicles, arbuscules, melanized septa and intracellular microsclerotia were noted to determine whether or not root sections were colonized with arbuscular mycorrhizal fungi, dark septate endophytes or both.

6.2.2 Statistical analysis

All data were analyzed in the R 3.4.2 programming language (R Core Team, 2017) with the *car* and *ggplot2* packages in RStudio (RStudio Team, 2016). Normality was tested with the Shapiro-Wilk test (Shapiro and Wilk, 1965) and equality of variances was tested with Levene's test (Levene, 1960). Three-factor ANOVA was used to analyze data based on the factors of *treatment* (non-invaded vs invaded by deer), *region* (Gwaii Haanas vs Laskeek Bay) and *soil* (living vs sterilized). For non-normal or heteroscedastic data, transformations noted below were used prior to ANOVA. For data that could not meet the assumptions of ANOVA, a Mann-Whitney U test was used instead (Mann and Whitney, 1947). Poisson regression was used to analyze the percent colonization of ectomycorrhizae in *Tsuga heterophylla*. Overdispersion was tested according to Cameron & Trivedi (1990).

For fluorescence data, a power transformation was used to correct for heteroscedasticity and non-normal distributions before applying the ANOVA:

$$X' = X^2$$

For biomass and root:shoot data, a logarithmic transformation was used to correct for heteroscedasticity and non-normal distributions before applying the ANOVA:

$$X' = \log_{10}(X)$$

To control for false discoveries, the Benjamini-Hochberg procedure was used with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995). To control the family-wise error rate associated with multiple comparisons, a Bonferroni correction was applied (Dunn, 1961) by adjusting the alpha using the following equation, with $\alpha = 0.05$ and k = the number of comparisons:

$$\alpha' = \alpha/k$$

6.3 Results

6.3.1 Mycorrhizal Inoculum Potential

There was no significant difference in the percent root colonization of arbuscular mycorrhizal (AM) fungi in *Thuja plicata* grown in soils from deer-invaded and non-invaded islands. AM fungi were observed in only 26% of the samples grown in living soil, with mean colonization rates driven largely by outliers. Where present, AM colonization in cedar seedlings grown in living soil ranged from 20% to 80%. Nonetheless, the median percent colonization was 0% for cedar seedlings grown in soil from both non-invaded and deer-invaded islands. There was also no significant difference in AM fungal colonization between cedar seedlings grown in soils from the Gwaii Haanas and Laskeek Bay regions. However, cedar seedlings grown in sterilized soil had significantly lower colonization of AM fungi compared with seedlings grown in living soil ($W = 1086.5$, $p = 0.001$, Figure 6.1). As the assumptions of ANOVA could not be met for the AM fungal root colonization data, the interactions between the factors of *treatment*, *region* and *soil* are not reported.

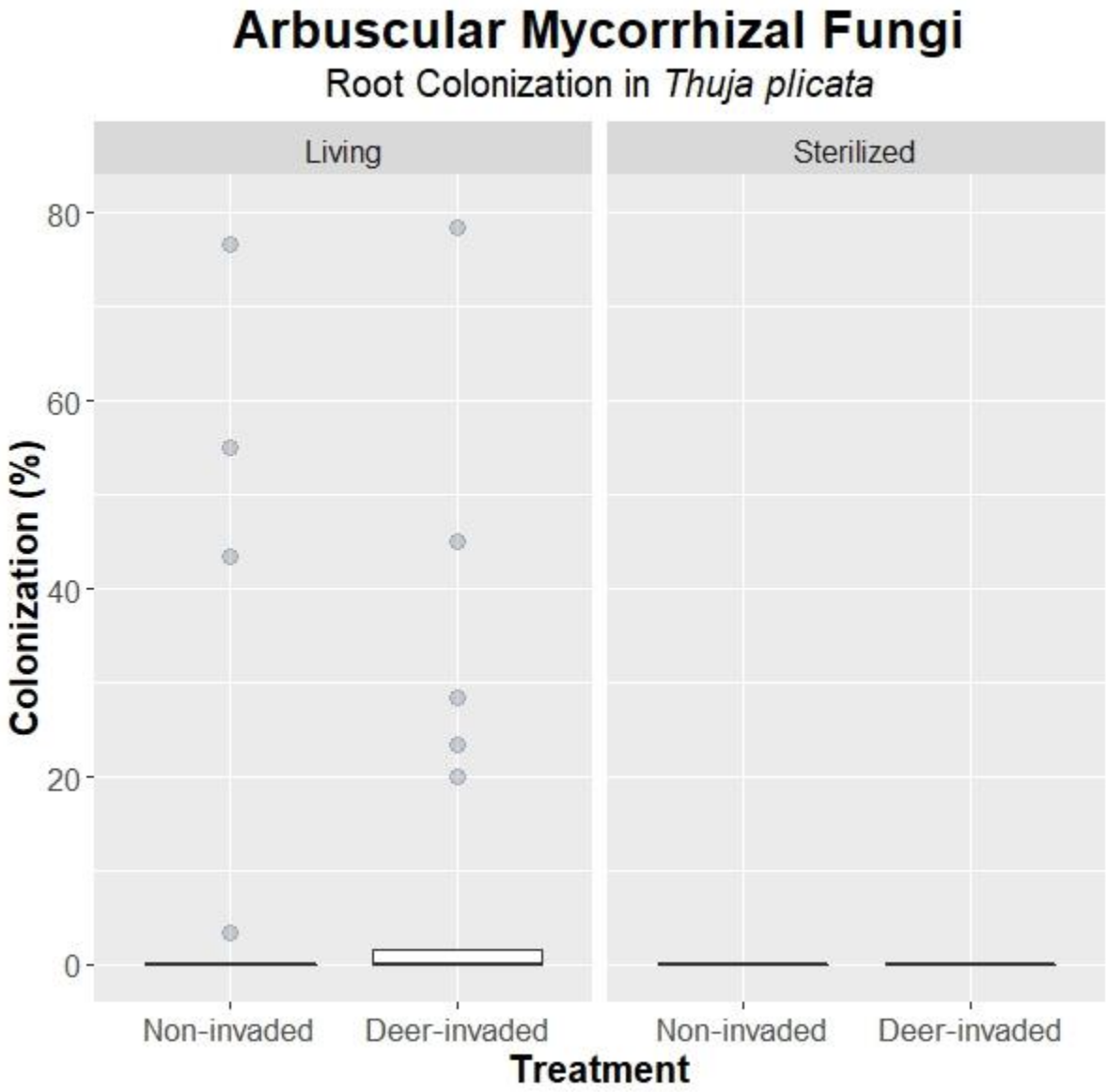

Figure 6.1: Root colonization of arbuscular mycorrhizal fungi in *Thuja plicata*, grown in living soil (n = 20, 22) and sterilized soil (n = 20, 21) from non-invaded and deer-invaded islands, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

Although the mean root colonization of dark septate endophytes (DSE) in *Thuja plicata* was lower in seedlings grown with living soil deer-invaded islands (17.6% ± 3.2 SE) compared with non-invaded islands (13.1% ± 2.2 SE), the difference was not significant (Table 6.3). Dark septate endophytes were highly abundant, being present in 93% of samples grown in living soil. There was no significant difference in DSE colonization between seedlings grown in soil from Gwaii Haanas and Laskeek Bay. The

mean colonization rate of seedlings grown in living and sterilized soil was 15.2% ± 1.9 SE and 0.1% ± 0.1 SE, respectively, a significant difference ($W = 1655$, $p < 0.001$). As the assumptions of ANOVA could not be met for the DSE root colonization data, the interactions between the factors of *treatment*, *region* and *soil* are not reported.

Table 6.3: Root colonization of dark septate endophytes in *Thuja plicata* grown in living soil from non-invaded islands (n = 13, 7) and deer-invaded islands (n = 11, 11) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Values are mean percentages ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	16.9 ± 4.9	18.8 ± 2.5
	Deer-invaded	11.9 ± 2.9	14.2 ± 3.4

Soils from islands invaded by Sitka black-tailed deer were not associated with a significant difference in the root colonization of ectomycorrhizal (EM) fungi in *Tsuga heterophylla*. Ectomycorrhizas were present in 76.1% ± 3.8 SE of root tips on western hemlock seedlings grown in living soil from non-invaded islands and 66.1% ± 3.6 SE of root tips of seedlings grown in living soil from the deer-invaded islands. All western hemlock seedlings grown in living soil had ectomycorrhizae present. Soil sterilization had a significant effect on the colonization of EM fungi ($Z = -16.268$, $p < 0.001$), with mean colonization rates of 70.4% ± 2.7 SE and 2.1% ± 1.0 SE in western hemlock seedlings grown in living and sterilized soil, respectively. The *region* factor did not have a significant effect on EM fungal colonization, but there was a significant interaction between the factors of *region* and *soil* ($Z = -3.329$, $p = 0.001$) as well as between all three factors of *treatment, region* and *soil* ($Z = 3.185$, $p = 0.001$), likely due to the random contamination of sterilized samples by ectomycorrhizal fungal spores in the greenhouse (Figure 6.2).

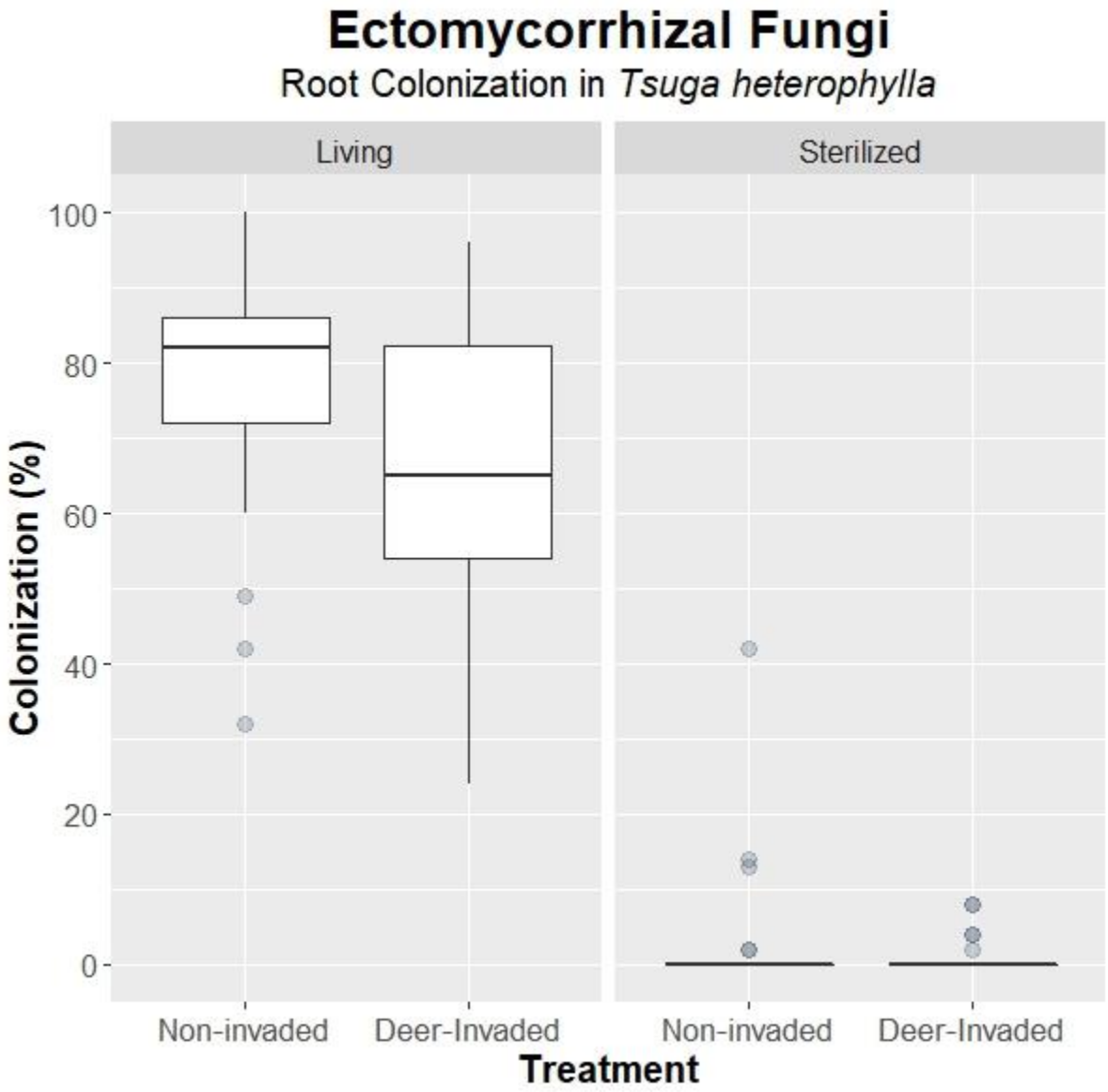

Figure 6.2: Root colonization of ectomycorrhizal fungi in *Tsuga heterophylla* grown in living soil (n = 21, 28) and sterilized soil (n = 20, 28) from non-invaded and deer-invaded islands, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

6.3.2 Chlorophyll Fluorescence

Thuja plicata seedlings grown in soil from islands invaded by the Sitka black-tailed deer had significantly lower chlorophyll fluorescence ratios (F_v/F_m values) than seedlings grown in soil from non-invaded islands ($F_{(1,75)} = 9.53$, $p = 0.003$, Table 6.4, Figure 6.3, Appendix C § Tables C.1). Mean F_v/F_m values of cedar seedlings grown in living soil from non-invaded and invaded islands were 0.65 ± 0.02 SE

and 0.58 ± 0.02 SE, respectively. There was also a significant difference between the mean F_v/F_m values of seedlings grown in living soil (0.61 ± 0.01 SE) and sterilized soil (0.57 ± 0.01 SE, $F_{(1,75)} = 9.68$, $p = 0.003$). There was no significant difference between the chlorophyll fluorescence ratios of cedar seedlings grown in soils from the regions of Gwaii Haanas and Laskeek Bay. There were no significant interactions between the factors of *treatment*, *soil* or *region*.

Table 6.4: F_v/F_m values of *Thuja plicata* grown in living soil from non-invaded islands (n = 13, 7) and deer-invaded islands (n = 11, 11) in the regions of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	0.63 ± 0.02	0.67 ± 0.01
	Deer-invaded	0.61 ± 0.02	0.55 ± 0.03

In contrast, *Tsuga heterophylla* seedlings grown in soil from non-invaded and deer-invaded islands did not have significantly different F_v/F_m values. However, western hemlock seedlings grown in sterilized soil had significantly lower F_v/F_m values than seedlings grown in living soil ($F_{(1,75)} = 91.93$, $p < 0.001$), with mean F_v/F_m values of 0.50 ± 0.01 SE and 0.64 ± 0.01 SE, respectively. There was no significant difference between the F_v/F_m values of western hemlock seedlings grown in soils from the regions of Gwaii Haanas and Laskeek Bay, and there were no significant interactions between the factors of *treatment*, *soil* or *region* (Figure 6.4).

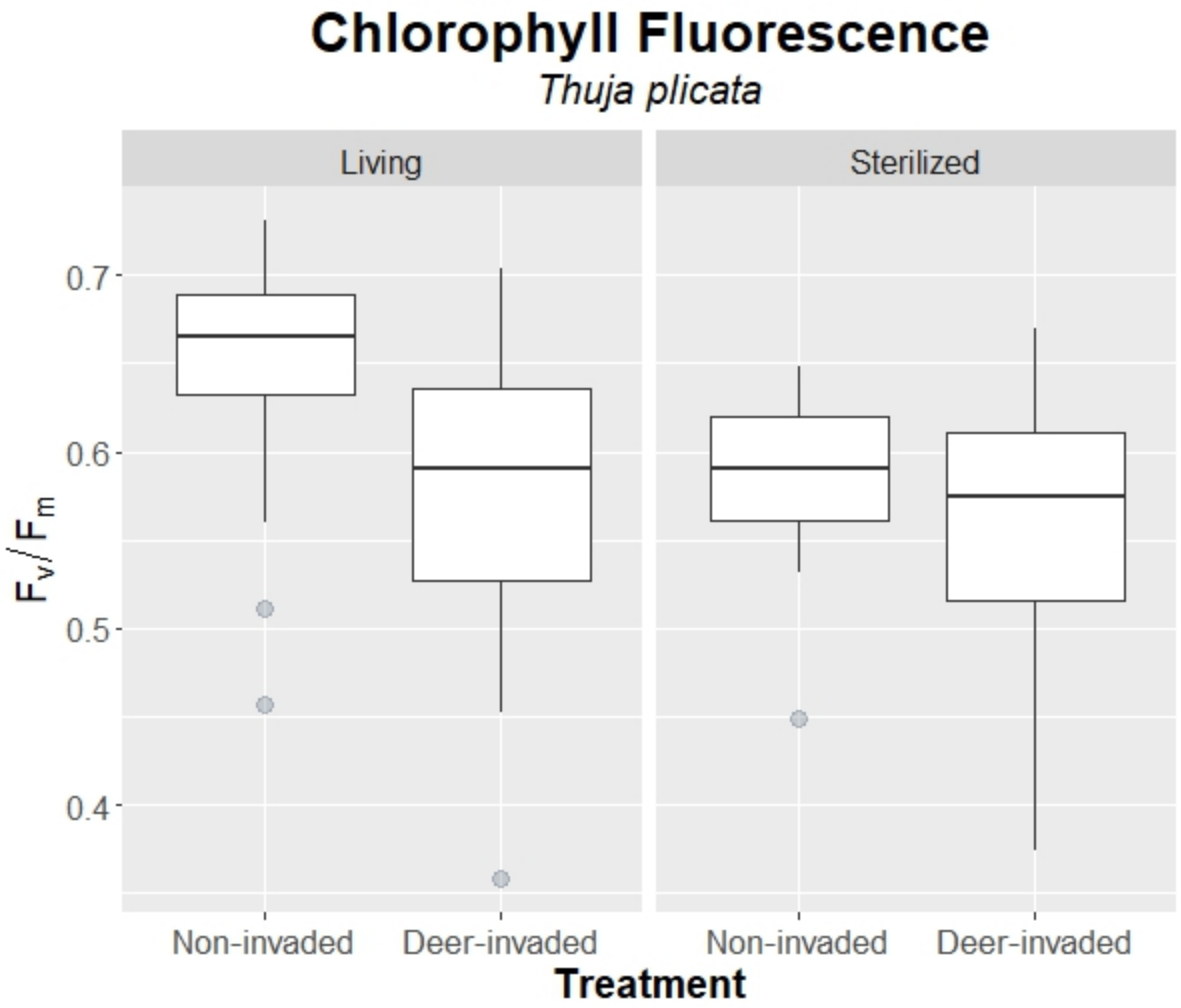

Figure 6.3: Chlorophyll fluorescence in *Thuja plicata* grown in living soil (n = 20, 22) and sterilized soil (n = 20, 21) from non-invaded and deer-invaded islands, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

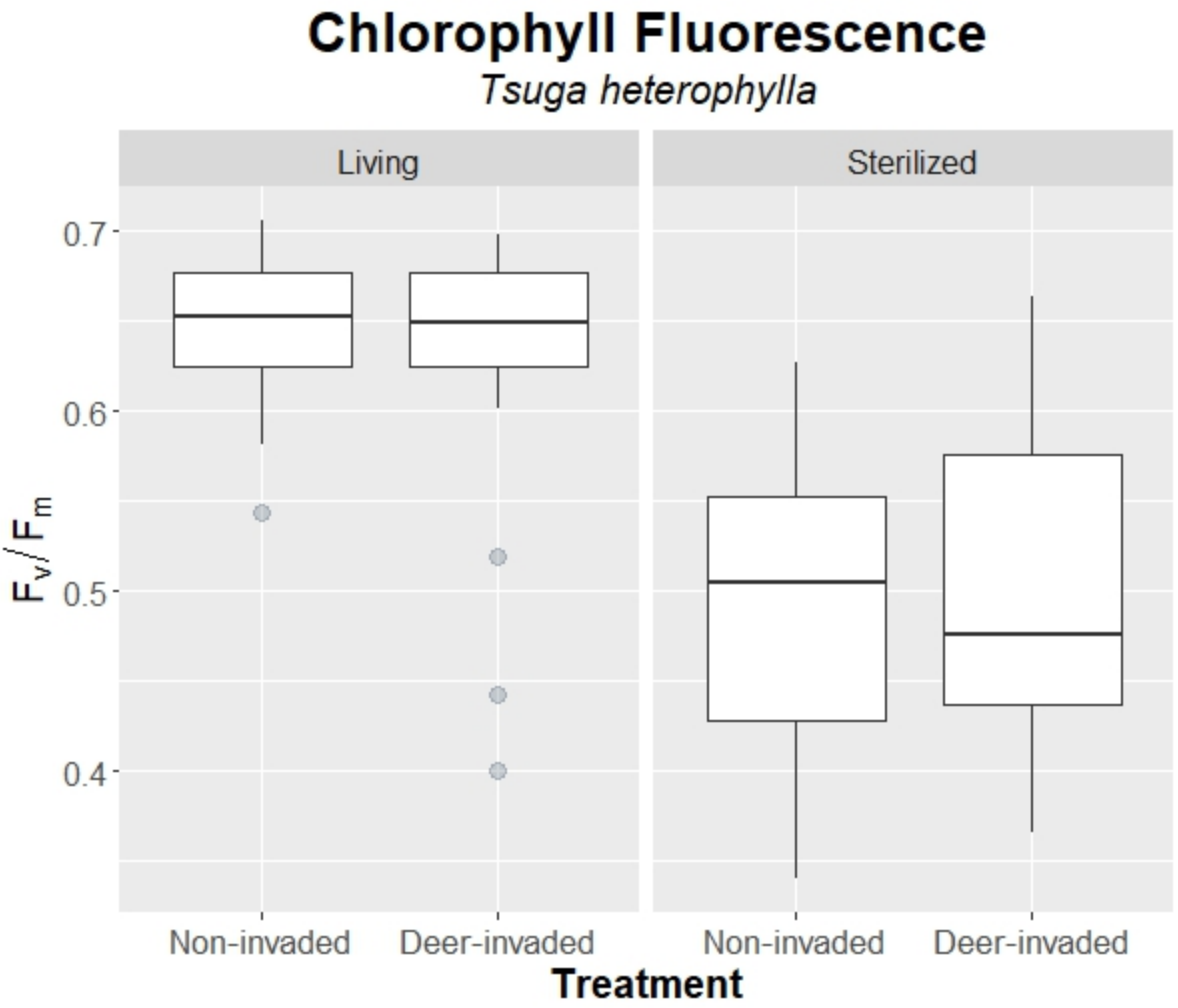

Figure 6.4: Chlorophyll fluorescence in *Tsuga heterophylla* grown in living soil (n = 21, 28) and sterilized soil (n = 20, 28) from non-invaded and deer-invaded islands, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

6.3.3 Biomass and Resource Allocation

There were no significant differences in the total dry biomass of *Thuja* plicata (roots and shoots) grown in soils from non-invaded and deer-invaded islands. Cedar seedlings grown in living and sterilized soil had a mean biomass of 0.176 g ± 0.025 SE and 0.124 g ± 0.023 SE, respectively, but the difference was not significant. The total biomass of *Thuja plicata* was similar between those grown in soils from the regions of Gwaii Haanas and Laskeek Bay. There were no significant interactions between the factors of *treatment*, *region* and *soil*.

Likewise, there were no significant differences in the total dry biomass of *Tsuga heterophylla* (roots and shoots) grown in soils from non-invaded and deer-invaded islands. The mean biomass of western hemlock seedlings grown in living and sterilized soil was 0.134 g ± 0.017 SE and 0.061 g ± 0.010 SE, respectively, a significant difference ($F_{(1,75)} = 24.95$, $p < 0.001$, Figure 6.5). Western hemlock seedlings grown in soils from the regions of Gwaii Haanas and Laskeek Bay did not have significantly different biomass, and there were no significant interactions between the factors of *treatment*, *region* and *soil*.

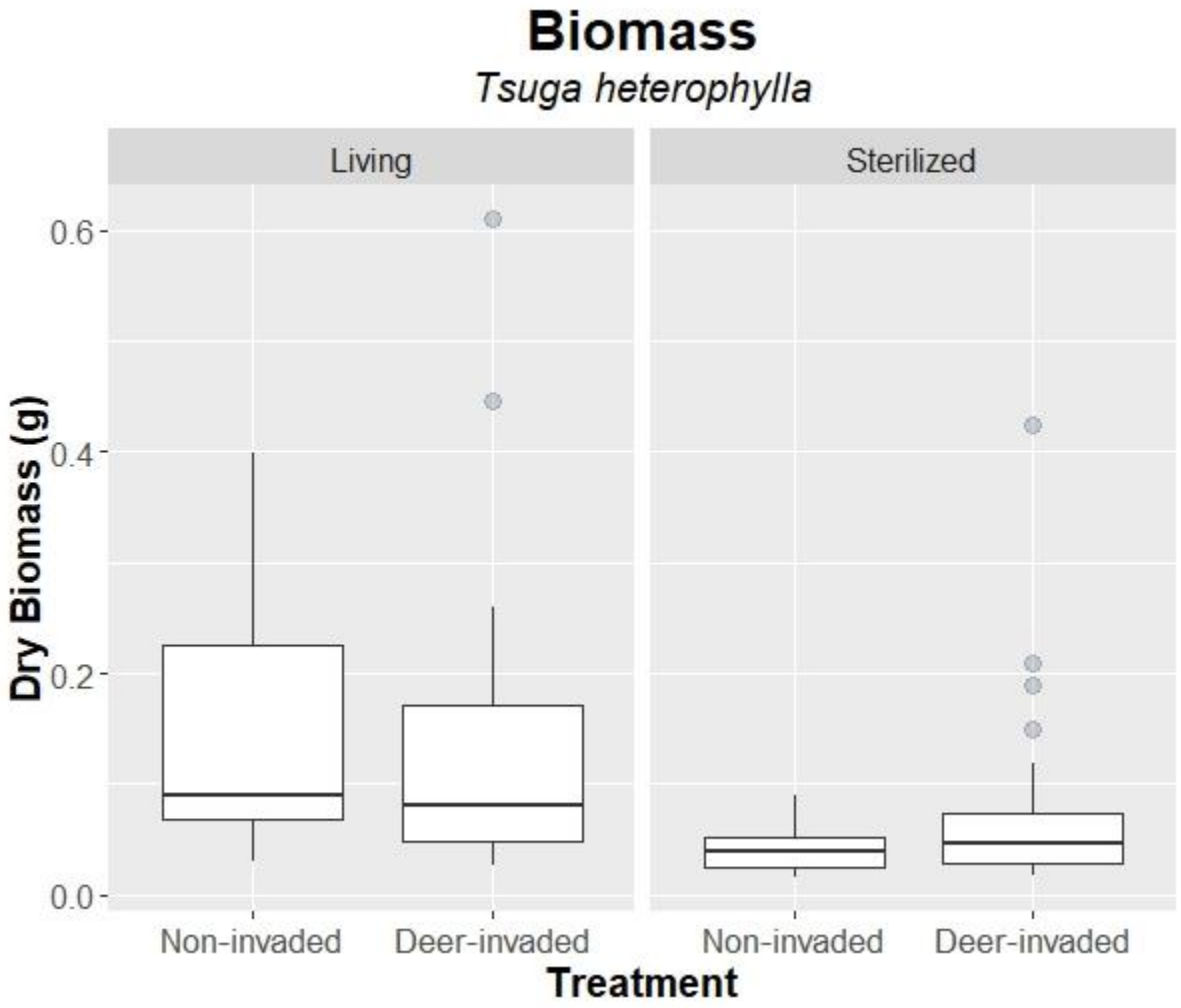

Figure 6.5: Biomass of *Tsuga heterophylla* (roots and shoots) grown in living soil (n = 21, 28) and sterilized soil (n = 20, 28) from non-invaded and deer-invaded islands, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

The mean root/shoot biomass ratio of *Thuja plicata* was not significantly different between those grown in soils from non-invaded and deer-invaded islands, nor between those grown in soils from the regions of Gwaii Haanas and Laskeek Bay. Cedar seedlings grown in living and sterilized soil mean root/shoot ratios of 1.18 ± 0.06 SE and 1.94 ± 0.06 SE, respectively, a significant difference ($F_{(1,75)}$ = 56.733, $p < 0.001$, Figure 6.6). There was a significant interaction between the factors of *treatment* and *region* ($F_{(1,75)}$ = 4.117, $p = 0.046$). Root/shoot ratios were higher in cedar seedling grown in living soils from the deer-invaded islands of Laskeek Bay compared with those grown in soils from the deer-invaded islands of Gwaii Haanas (Ramsay Island, Figure 6.7. However, the Benjamini-Hochberg procedure suggests this interaction may be a false discovery. There were no other significant interactions.

Similarly, sterilizing the soil resulted in a significant increase in the mean root/shoot ratios of *Tsuga heterophylla* ($F_{(1,75)}$ = 17.68, $p < 0.001$). Seedlings grown in living and sterilized soil mean root/shoot ratios of 0.97 ± 0.03 SE and 1.27 ± 0.06 SE, respectively. There were no significant effects by the *treatment* and *region* factors on the root/shoot ratios of *Tsuga heterophylla* and there were no significant interactions between factors (Figure 6.8).

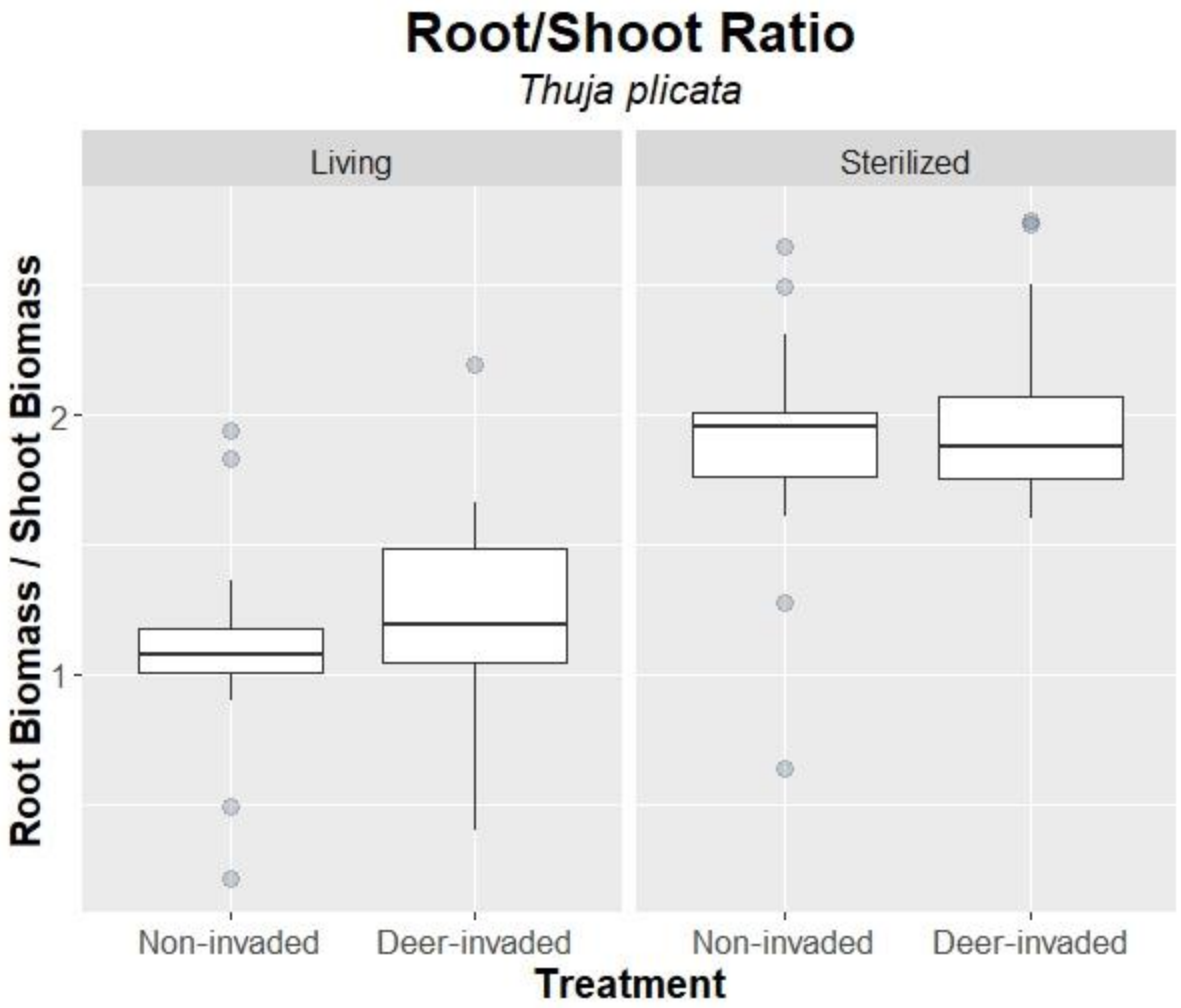

Figure 6.6: Root/shoot ratio of *Thuja plicata* grown in living soil (n = 20, 22) and sterilized soil (n = 20, 21) from non-invaded and deer-invaded islands, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

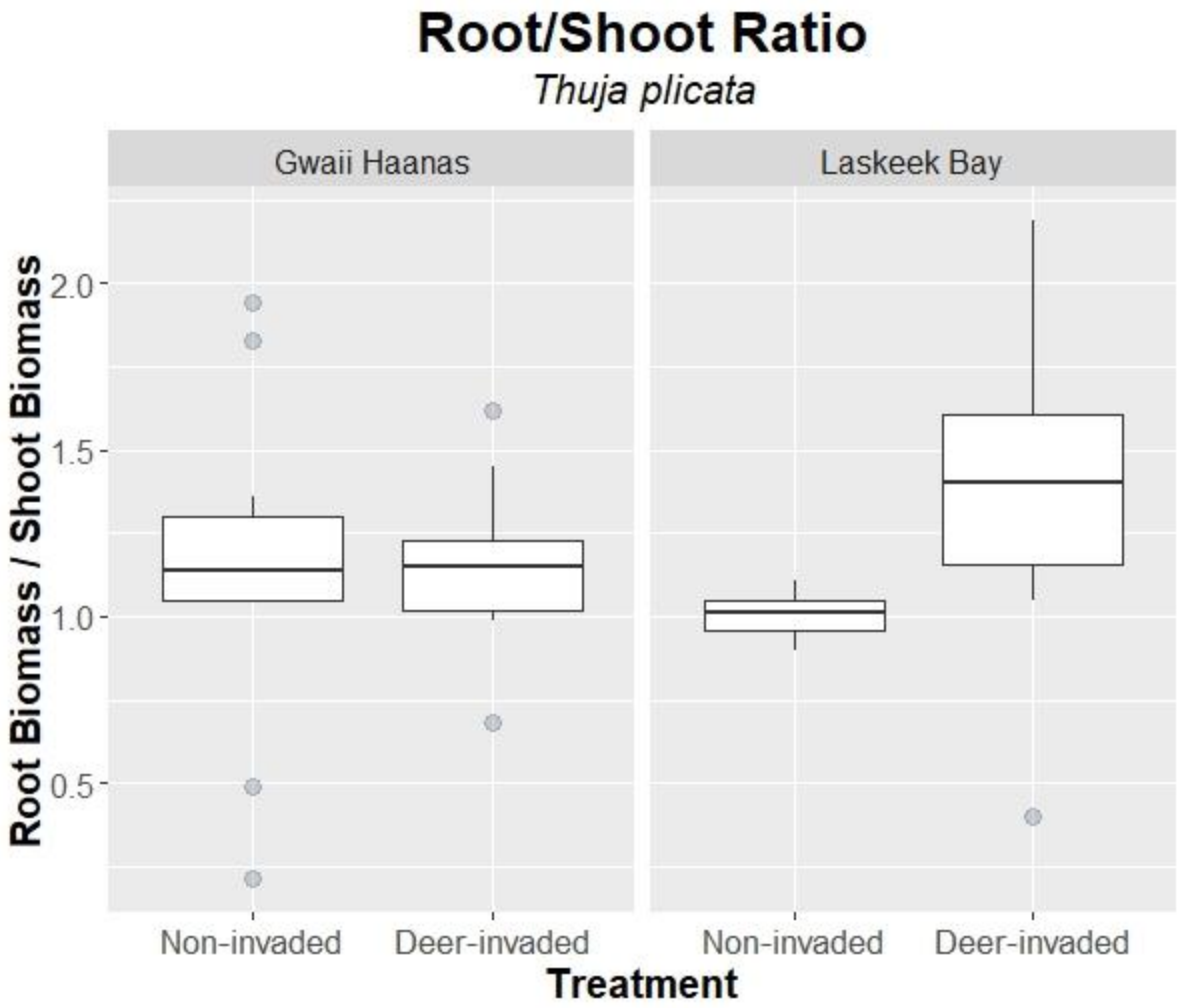

Figure 6.7: Root/shoot ratio of *Thuja plicata* grown in living soil from non-invaded and deer-invaded islands from the regions of Gwaii Haanas and Laskeek Bay. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

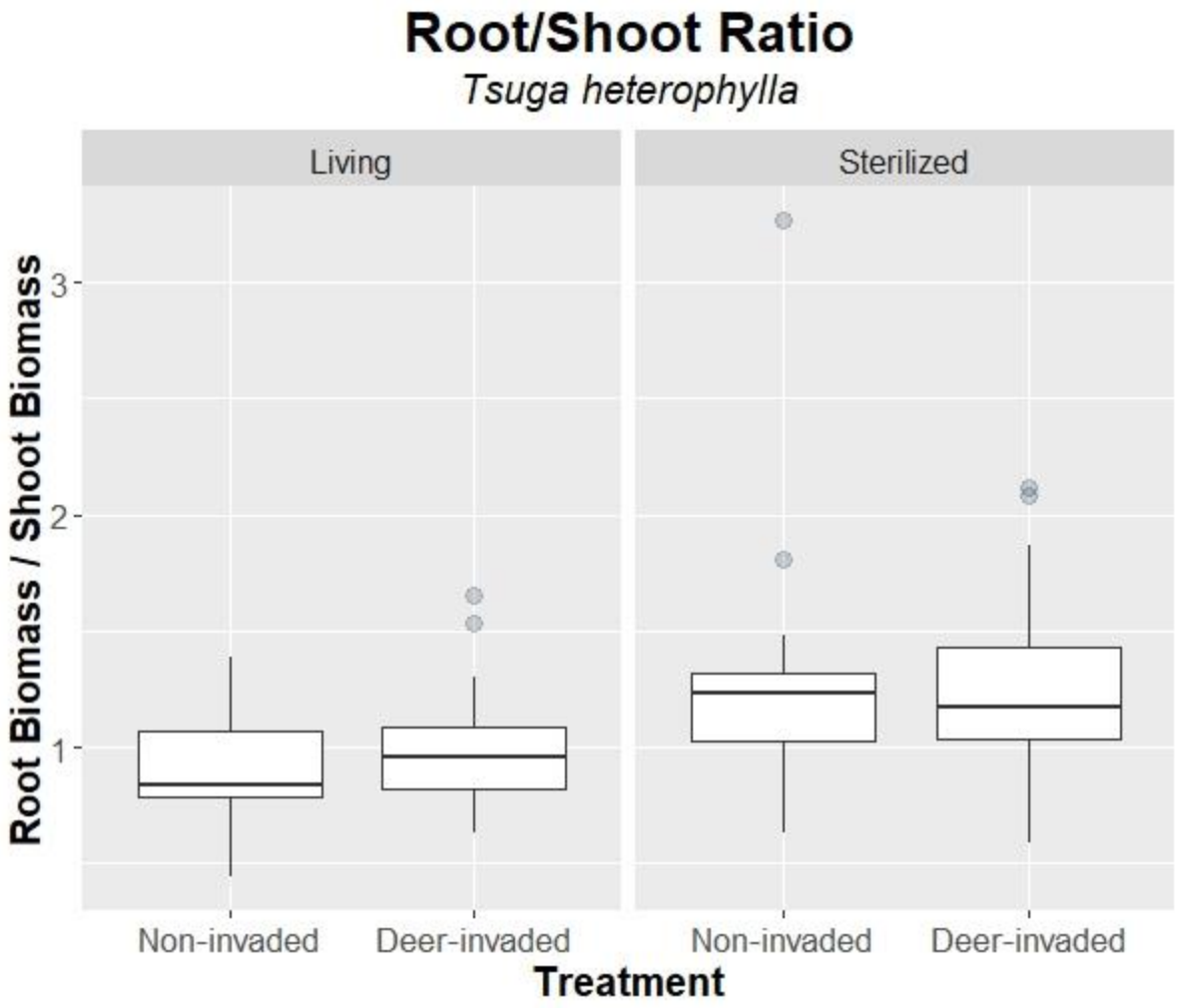

Figure 6.8: Root/shoot ratio of *Tsuga heterophylla* grown in living soil (n = 21, 28) and sterilized soil (n = 20, 28) from non-invaded and deer-invaded islands, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 * IQR; points represent values exceeding 1.5 * IQR.

6.4 Discussion

6.4.1 Mycorrhizal Inoculum Potential

Because arbuscular mycorrhizal (AM) fungi occurred infrequently in the *Thuja plicata* samples, the mean percent colonization of AM fungi may not be a representative of the typical *in situ* inoculum potential of arbuscular mycorrhizal fungi in most locations. Mean colonization was driven largely by outliers, with the percent colonization of one sample as high as 78%. As illustrated in Figure 6.1, median percent colonization by AM fungi was 0% in all treatment groups, suggesting that i) arbuscular

mycorrhizal fungi have a minor role in the organic soil horizons of Haida Gwaii, or ii) viable propagules of arbuscular mycorrhizal fungi generally did not survive the conditions and procedures associated with field sampling, laboratory preparation and long-term storage.

By comparison, dark septate endophytes (DSE) were ubiquitous among the samples and likely have a greater functional role than AM fungi in the organic soils of Haida Gwaii. Indeed, dark septate endophytes are commonly found in the soils of a wide range of environments including tropical, alpine and arctic ecosystems (Jumpponen and Trappe, 1998; Newsham, 2011). Most of the dark septate endophytes identified through next generation sequencing (Chapter 5) were noted to be ericoid mycorrhizal fungi in the FUNGuild database which suggests that the DSE observed in the roots of *Thuja plicata* in the greenhouse bioassay are the same fungi that colonize salal and other ericaceous plants in the understory of Haida Gwaii. Indeed, dark septate endophytes have been observed to colonize the roots of both ericaceous and coniferous plants (Lukešová et al., 2015). Although the difference was not statistically significant, there tended to be less DSE colonization of *Thuja plicata* grown in soils from deer-invaded islands, possibly a result of Sitka black-tailed deer reducing the abundance of ericaceous host plants.

6.4.2 Growth and Stress Responses to Soil Sterilization

The capacity of soil biota such as mycorrhizal fungi to mitigate drought stress in plants can be evaluated indirectly by measuring the degree that drought stress affects the efficiency of photosystem II (PSII) in the chloroplasts of dark-adapted leaves (Borkowska, 2002; Paknejad et al., 2007; Zhu et al., 2014). Specifically, the ratio of variable chlorophyll fluorescence (F_v) and maximum chlorophyll fluorescence (F_m) of leaves in a dark-adapted state indicates the efficiency of photosystem II, with optimum values around 0.83 (Flexas et al., 1999; Maxwell and Johnson, 2000). F_v/F_m values correlate with many environmental conditions that affect the photochemistry of PSII, such as severe drought or nutrient deficiency (Flexas et al., 1999; Murchie and Lawson, 2013; Paknejad et al., 2007). For example, lower F_v/F_m values in *Tsuga heterophylla* and *Thuja plicata* are associated with reductions in the photochemical efficiency of PSII induced by shading (Rehiman Khan et al., 2000). Severe drought stress in *Thuja plicata* is also associated with lower F_v/F_m values (Fang-yuan and Guy, 2004). Thus, by comparing the F_v/F_m values of plants under drought conditions, the degree of stress mitigated or facilitated by biotic and abiotic soil factors of the experiment may be inferred.

Varying degrees of stress were present in all treatment groups of both *Thuja plicata* and *Tsuga heterophylla*, as indicated by the F_v/F_m values which were generally below 0.7. However, as the cessation of the watering regime in this experiment was not a controlled treatment, other environment conditions besides low water availability may be responsible for the plant stress. Sterilizing the soil significantly increased stress in both species compared with seedlings grown in living soil, emphasizing the capacity of mutualistic soil biota such as DSE and EM fungi to provide water and nutrients to their host plants (Alvarez et al., 2009; Li et al., 2018; Parke et al., 1983a). Both species tended to have higher root:shoot ratios when grown in sterilized soil which suggests that in the absence of mutualistic soil biota, these tree species allocate greater resources to their root systems to increase the uptake of water and nutrients (Agren and Franklin, 2003; Xu et al., 2015).

Figure 6.9: Ectomycorrhizal root tip of *Tsuga heterophylla* grown in soil from LOW03. Bar represents 1 mm.

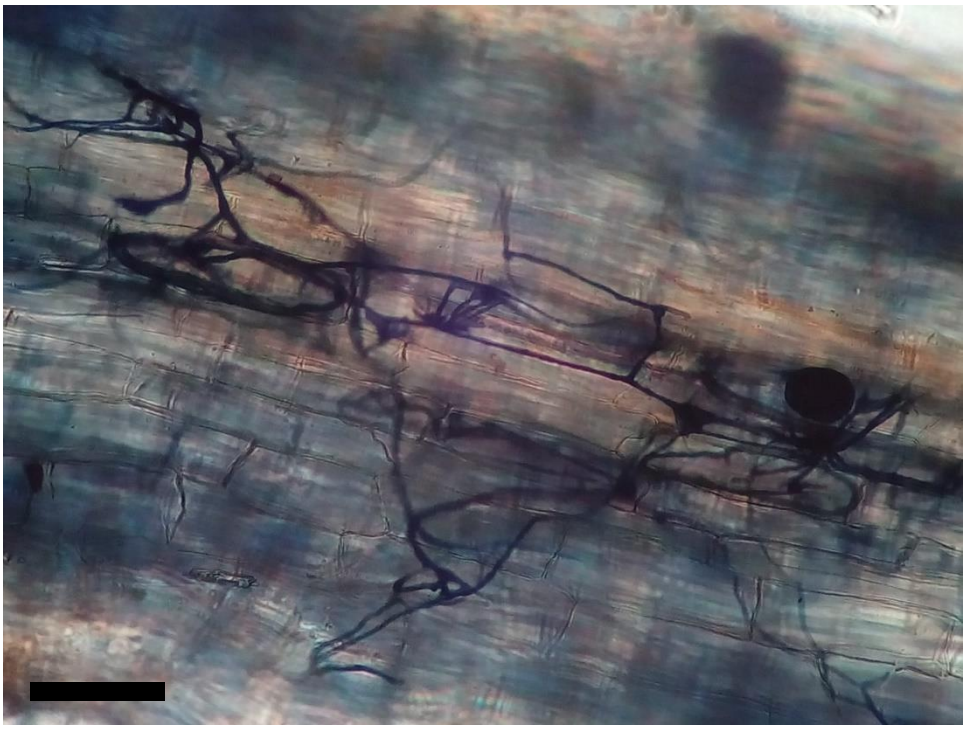

Figure 6.10: Arbuscular mycorrhizal fungi in *Thuja plicata* grown in living soil from RAM12. Bar represents 50 µm.

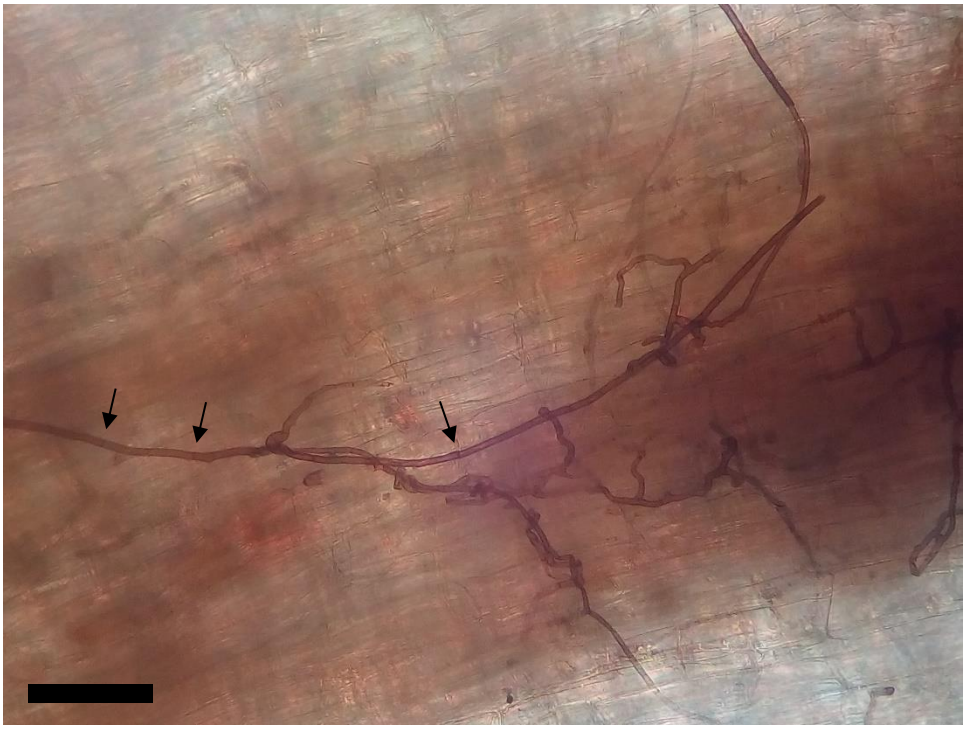

Figure 6.11: Dark septate endophyte in *Thuja plicata* grown in living soil from SSK01. Arrows indicate septa. Bar represents 50 µm.

6.4.3 Growth and Stress Responses to Soil from Deer-Invaded Islands

The stress response of *Tsuga heterophylla*, as inferred through chlorophyll fluorescence, was similar between seedlings grown in soils from deer-invaded and non-invaded islands. Likewise, the total dry biomass and root:shoot ratios of *Tsuga heterophylla* were unaffected by the soils of deer-invaded islands. Together, these observations suggest that western hemlock is resilient to the changes in the biotic and abiotic soil properties associated with islands invaded by Sitka black-tailed deer.

Stress in *Thuja plicata* was greater in seedlings grown in living soils from deer-invaded islands compared those grown in living soils from non-invaded islands. However, differences in F_v/F_m values between cedar seedlings grown in living soils from deer-invaded and non-invaded islands were largely driven by soils from Laskeek Bay (Table 6.1). Similarly, root:shoot ratios were higher in cedar seedlings grown in living soils from the deer-invaded islands of Laskeek Bay, an indication of poor nutrient and water uptake (Agren and Franklin, 2003; Xu et al., 2015). The root:shoot ratios of *Thuja plicata* were similar between sterilized soils from deer-invaded and non-invaded islands which suggests that a biological mechanism is responsible for the lower root:shoot ratios of seedlings grown in living soils from deer-invaded islands (Figure 6.8). The lower F_v/F_m values observed in *Thuja plicata* grown in living soil from deer-invaded islands may suggest that the dark septate endophytes in those soils were less effective at providing water and nutrients to their host plants compared with the DSE in the soils from non-invaded islands. Previous research has revealed that fertilizing soils with nitrogen, phosphorus and micronutrients can select for mycorrhizal fungi that have greater carbon demands and provide fewer benefits to their plant host (Johnson, 1993). Therefore, higher phosphorus availability in the soils of deer-invaded islands (Chapter 3) may have encouraged the proliferation of dark septate endophyte species that are less effective at providing water or nutrients to *Thuja plicata*. As an alternative explanation, other unidentified soil pathogens such as bacteria or viruses in soils from deer-invaded islands may have a pathogenic association with *Thuja plicata*, increasing its stress under drought conditions. Interactions between plants and mutualistic soil biota are often contingent on environmental conditions such as nutrient availability. For example, a study involving *Petunia hybrida* found that phosphorus additions to the soil resulted in no growth response and lower foliar nutrient content compared with control plants grown in sterilized soil, suggesting that the arbuscular mycorrhizal fungi present in the living soil had a parasitic relationship with the host plant, restricting access to soil phosphorus (Nouri et al., 2014). Although dark septate endophytes tend to have positive effects on the biomass and nutrient uptake in plants (Newsham, 2011), they are also known to also have pathogenic

associations in certain cases (García et al., 2012) raising the possibility that dark septate endophytes in the soils of deer-invaded islands have a parasitic relationship with *Thuja plicata* rather than a mutualistic one. Similarly, growth responses in plants to arbuscular mycorrhizal fungi can vary depending on the species of mycorrhizal fungi colonizing their roots (Wang et al., 2016). Indeed, mycorrhizal fungi are not limited to mutualistic plant associations; neutral or negative effects on plant growth have also been observed (Eo and Eom, 2009; Wang et al., 2016). However, as there were no significant differences in the percent colonization of arbuscular mycorrhizal fungi, dark septate endophytes nor ammonium concentrations between the soils of deer-invaded and non-invaded islands, and as only fungal communities were investigated in this bioassay, this research could not conclusively determine which soil organisms may have been responsible for the drought stress of cedar trees grown in soils from deer-invaded islands.

Although not as extreme as the differences observed in cedar seedlings grown in living soil, the F_v/F_m values of cedar seedlings grown in sterilized soil tended to be lower in seedlings grown with soil from deer-invaded islands compared with non-invaded islands, suggesting that an abiotic factor may contribute to the higher stress in *Thuja plicata* in addition to changes in the biota. Phosphate concentrations were higher in soils from deer-invaded islands (Chapter 3), but ammonium concentrations tended to be lower, especially in soils from the deer-invaded islands of Laskeek Bay. Thus, *Thuja plicata* may be more limited by nitrogen than phosphorus in the organic soil horizons which may have had a compounding effect on chlorophyll fluorescence in addition to the biotic factor.

The effects of soil biota from deer-invaded islands on the drought tolerance of *Thuja plicata* has implications for how the forests of Haida Gwaii respond to a changing climate. Since 1950, temperatures in BC have increased by 1 to 2°C and they are projected to increase by an additional 1 to 4°C by 2100 (Walker et al., 2007). Similar trends have been observed on Haida Gwaii. Daily minimum and maximum temperatures recorded at the Sandspit Airport weather station are increasing increased by 0.18°C and 0.21°C per decade, respectively (Walker et al., 2007). Climate models predict that Haida Gwaii will have decreased precipitation in the summer and more prolonged droughts by 2050 (Walker et al., 2007). Thus, by decreasing the drought tolerance of western redcedar, the soil biota associated with islands invaded by Sitka black-tailed deer may make western redcedar less resilient than western hemlock to the changing climate on Haida Gwaii.

6.4 Conclusion

The objective of the research presented in this chapter was to determine the inoculum potential of arbuscular mycorrhizal fungi, dark septate endophytes and ectomycorrhizal fungi in soil from non-invaded and deer-invaded islands in the regions of Gwaii Haanas and Laskeek Bay, and to determine the growth response (biomass, root:shoot ratios) and stress response (chlorophyll fluorescence) of western redcedar and western hemlock seedlings grown in those soils. Ectomycorrhizal root colonization of *Tsuga heterophylla* was similar between seedlings grown in soils from deer-invaded and non-invaded islands. Likewise, the biomass and root:shoot ratios of *Tsuga heterophylla* was similar between seedlings grown in soils from deer-invaded islands and non-invaded islands. Neither biotic nor abiotic soil properties associated with deer-invaded islands had an effect on the drought tolerance of western hemlock. However, soil biota from deer-invaded islands, particularly in the Laskeek Bay region, were associated with greater stress in *Thuja plicata* seedlings under drought conditions, with comparable F_v/F_m values to seedlings grown in sterilized soil. Root colonization by dark septate endophytes in *Thuja plicata* was lower in seedlings grown in soils from deer-invaded islands but the difference was not significant. Similarly, there were no significant differences between the total biomass and root:shoot ratios between *Thuja plicata* seedlings grown in soils from deer-invaded and non-invaded islands. In conclusion, soil microbial communities on islands invaded by Sitka black-tailed deer may make western redcedar more susceptible to drought conditions than western hemlock.

CHAPTER 7 – Potential Enzyme Activity of Deer-Invaded and Non-Invaded Islands

7.1 Introduction

7.1.1 Background

Soil processes such as nutrient cycling and the decomposition of organic matter are facilitated through the chemical reactions catalyzed by the extracellular enzymes produced by microorganisms (Burns et al., 2013). Soil organisms produce enzymes as a dynamic response to multiple environmental variables, including the quantity, quality and composition of organic substrates, as well as the concentrations of the enzymatic end-products or other limiting nutrients (Allison and Vitousek, 2005; Hernández and Hobbie, 2010). β-glucosidase and cellobiohydrolase are two enzymes involved in the final decomposition of cellulose, the most abundant polysaccharide in plant tissues (Lynd et al., 2002; Turner et al., 2002). Phosphatase is an enzyme produced by both plant roots and soil microorganisms to mineralize phosphorus from organic substrates (Olander and Vitousek, 2000). As β-glucosidase often correlates with soil respiration and total microbial biomass (Liang et al., 2015; Merino et al., 2016; Turner et al., 2002), it is a sensitive indicator of overall microbial activity. All three of these enzymes, β-glucosidase, cellobiohydrolase and phosphatase, can be induced or suppressed through changes in the quantity and quality of their respective substrates (Allison and Vitousek, 2005), making them useful for monitoring changes in litter decomposition and nutrient cycling associated with aboveground herbivores. Previous research has shown aboveground herbivores to have inconsistent effects on the activity of cellulose-degrading enzymes such as β-glucosidase and cellobiohydrolase, with both neutral effects (Acosta-Martínez et al., 2010; Burke et al., 2011; Francini et al., 2014) and negative effects reported (Stritar et al., 2010). Likewise, ungulates have been associated with neutral effects (Burke et al., 2011; Stritar et al., 2010), negative effects (Acosta-Martínez et al., 2010) and positive effects (Francini et al., 2014) on the activity of phosphatase. As these studies have focused on the enzyme activity of mineral soils in ecosystems types such as grasslands, pastures and temperate deciduous forests, their results are of limited utility in predicting how aboveground herbivores may affect the enzyme activity of organic soils in temperate conifer forests, such as those found on Haida Gwaii.

Aboveground herbivores both directly and indirectly alter the quantity, quality and composition of soil substrates (Bardgett and Wardle, 2003; Wardle et al., 2002), which in turn can either induce or suppress the activity of different extracellular enzymes (Hernández and Hobbie, 2010). By selectively

consuming more palatable plant species, aboveground herbivores such as deer can shift the function composition of plant communities towards less palatable species (Bardgett and Wardle, 2003) with leaf and root litter that is more recalcitrant to decomposition (Grime et al., 1996). Thus, Sitka black-tailed deer may be associated with decreases in carbon-acquiring enzymes such as β-glucosidase and cellobiohydrolase that are involved in the breakdown of relatively labile substrates. As a counteracting process, terrestrial herbivores can also increase the quantity of labile nutrient substrates for certain enzymes through inputs from their dung and urine which tend to stimulate microbial activity (Bardgett and Wardle, 2003). The production of phosphatase, for example, can be induced through additions of organic phosphorus-containing substrates (Allison and Vitousek, 2005). In agricultural settings, applications of manure tend to induce greater activity in phosphatase and cellulolytic enzymes (Garg and Bahl, 2008; Saha et al., 2008). By increasing the availability of substrates rich in phosphorus, Sitka black-tailed deer may be associated with increased phosphatase activity in the soils of Haida Gwaii. In addition to being constrained by the quantity of their substrates, cellulose-degrading enzymes such as β-glucosidase can also be limited by nitrogen availability (Allison and Vitousek, 2005). Indeed, inputs of nitrogen in fertilization experiments tend to stimulate the activity of β-glucosidase and cellobiohydrolase (Allison et al., 2008; Saiya-Cork et al., 2002). However, herbivores have inconsistent effects on the availability of soil nitrogen, and are often associated with net losses of nitrogen due to higher volatilization rates (Douglas A. Frank and Groffman, 1998; McInnes et al., 1986). Decreases in available nitrogen associated with Sitka black-tailed deer would be expected to suppress the activities of β-glucosidase and cellobiohydrolase.

In addition to changes in the quantity or quality of available substrates, the diversity of plant communities is also linked to enzyme activity (Maltz et al., 2017), possibly due to interspecific variation in the composition of root exudates (Grayston et al., 1997) or the ecophysiological traits of plant litter that affect decomposition rates (Zukswert and Prescott, 2017). For example, lower plant species richness in coastal shrublands of southern California was associated with lower β-glucosidase and cellobiohydrolase activity in decomposing leaf litter (Maltz et al., 2017). On Haida Gwaii, Sitka black-tailed deer are associated with significantly lower understory plant diversity (Allombert et al., 2005; Chollet et al., 2016), which may reduce the diversity of organic substrates entering the soil through leaf and root litter, further contributing to decreased β-glucosidase and cellobiohydrolase activity.

To determine how the invasion of Sitka black-tailed deer may affect the activity of extracellular enzymes involved with nutrient acquisition and carbon cycling, this chapter presents the results of an

experiment comparing the potential activities of phosphatase, β-glucosidase and cellobiohydrolase from deer-invaded and non-invaded islands in the regions of Gwaii Haanas and Laskeek Bay. I predicted that increases in labile inputs of organic phosphorus from the dung of Sitka black-tailed deer would induce greater phosphatase activity. In contrast, I expected that decreases in available nitrogen associated with Sitka black-tailed deer would suppress the activity of the cellulose-degrading enzymes, β-glucosidase and cellobiohydrolase. I also predicted that shifts in the functional composition of the plant communities towards species producing poorer quality litter would further contribute to a decline in the activity of β-glucosidase and cellobiohydrolase.

7.1.2 Objective and Hypotheses

The objective of the research presented in this chapter was to determine the extracellular enzyme activities of phosphatase, β-glucosidase and cellobiohydrolase in soils from islands invaded or not invaded by Sitka black-tailed deer in the regions of Gwaii Haanas and Laskeek Bay. The following hypotheses were tested:

- H_0 The presence of Sitka black-tailed deer on islands has no effect on the extracellular enzyme activity in the soil.
- H_1 The presence of Sitka black-tailed deer on islands is associated with an increase in potential phosphatase activity in the soil.
- H_2 The presence of Sitka black-tailed deer on islands is associated with a decrease in potential β-glucosidase activity in the soil.
- H_3 The presence of Sitka black-tailed deer on islands is associated with a decrease in potential cellobiohydrolase activity in the soil.

7.2 Methods

7.2.1 Enzyme Assays

To determine potential enzyme activity, the following protocol was adapted based on the methods used by Brockett et al. (2012) and Saiya-Cork et al. (2002). Enzyme activity was measured fluorometrically in flat-bottomed black polystyrene 96-well, 300-µl microplates (Corning, product no. C3904) using substrates ester-linked to the 4-methylumbelliferone (MUB) fluorophore. The activities of phosphatase, β-glucosidase and cellobiohydrolase were assayed using substrates acquired from Sigma-

Aldrich, including MUB-phosphate (product no. M8883), MUB-β-D-glucopyranoside (product no. M3633) and MUB-β-D-cellobioside (product # M6018), respectively. 4-methylumbelliferone (product no. M1381) was used as a reference standard.

Soils were sampled, processed and stored as described in Chapter 2. Briefly, after storing soil samples at approximately 4°C for two to four weeks, samples were homogenized by sieving to 4 mm and then stored at -20°C until further use. Soil samples were then freeze-dried and ground to a fine powder using a mortar and pestle. In each sterilized polypropylene bottle, 75 mg of soil were added along with 50 mL of sterilized 50 mM sodium acetate buffer (pH 5) and approximately 30 sterilized glass beads. The soil slurry was shaken for 1 hour, after which an additional 50 mL of buffer was added to make the final soil slurry. Substrates were prepared daily in 200 µM solutions using deionized water. Positive controls were prepared by making a 10 mM stock solution of 4-methylumbelliferone in methanol which was diluted to a working solution of 10 µM MUB in deionized water. Microplates were acid washed in a solution of 3% HCl and triple-rinsed in deionized water. Samples were arranged on microplates using a random block design using the following procedure. For each sample, 200 µL of soil slurry and 50 µL of the respective substrate solution were added to each assay well, with eight technical replicates per sample. Several negative and positive controls were used to estimate the background fluorescence emission of the substrates, soil and buffer solution, and to adjust for signal quenching by the soil. To estimate the background fluorescence of the substrates, 50 µL of substrate solution and 200 µL of buffer solution were added to each substrate-buffer well. To estimate the background fluorescence of the soil slurries, 50 µL of buffer solution and 200 µL soil slurry were added to each soil-buffer well. To estimate the background fluorescence of the buffer solution, 250 µL of buffer solution were to each buffer well. 4-methylumbelliferone was used as a positive control to estimate quenching, by adding 50 µL of MUB and 200 µL of soil slurry to each soil-standard well, and by adding 50 µL of 10 µM MUB and 200 µL buffer to each standard well. A separate standard curve was constructed for each enzyme assay using a gradient of 0 µL to 50 µL of 10 µM MUB in 5 µL increments, with a supplementary volume of buffer solution for a total of 250 µL in each well. Eight technical replicates were used each control group. Microplates were incubated in a cupboard in total darkness at 20°C for two, four or seven hours for phosphatase, glucosidase and cellobiohydrolase, respectively. Reactions were stopped by adding 20 µL of 0.5 µM NaOH to each well. Fluorescence was measured on a TECAN Spark 10M spectrophotometer using an excitation wavelength of 340(20) nm, an emission wavelength of 460(35) nm and gain set to 45. Microplates were shaken for 5 seconds and then 5 readings were taken per well, with 15 flashes per reading.

Enzyme activity was calculated as nmol of product per hour per gram dry soil by comparing the fluorescence signal to a standard curve of 4-methylumbelliferone after adjusting the fluorescence values for quenching and the background fluorescence associated with the substrates and soil using the following equation.

$$\text{Fluorescence Signal} = [F_{assay} / (E_c \cdot Q_c)] - [F_{sub} / (E_c)] - [F_{soil} / (E_c \cdot Q_c)] + [F_{buffer} / (E_c \cdot Q_c)]$$

E_c = fluorescence of standard wells / mean fluorescence of standard wells

Q_c = fluorescence of soil-standard wells / fluorescence of standard wells

F_{assay} = fluorescence of assay wells

F_{sub} = fluorescence of substrate-buffer wells

F_{soil} = fluorescence of soil-buffer wells

F_{buffer} = fluorescence of buffer wells

7.2.2 Statistical analysis

Normality was tested with the Shapiro-Wilk test (Shapiro and Wilk, 1965) and equality of variances was tested with Levene's test (Levene, 1960). Two-factor ANOVA was used to analyze data based on the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay). To control for false discoveries, the Benjamini-Hochberg procedure was used with a false discovery rate of 0.05 (Benjamini and Hochberg, 1995). Data were analyzed in the R 3.4.2 programming language (R Core Team, 2017) with the *car* and *ggplot2* packages in RStudio (RStudio Team, 2016).

7.3 Results

Potential phosphatase activity was 30.8% higher in soil from deer-invaded islands compared with soil from non-invaded islands ($F_{(1,45)} = 13.959$, $p = 0.001$), with means of 2143.2 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 119.2 SE and 2803.9 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 121.6 SE in soils from non-invaded and deer-invaded island, respectively. There were there was no significant differences in potential phosphatase activity between soils from the

regions of Laskeek Bay and Gwaii Haanas, nor a significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay, Table 7.1, Figure 7.1)

Table 7.1: Phosphatase activity ($nmol \cdot hr^{-1} \cdot g^{-1}$) in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	2216.3 ± 134.7	2024.5 ± 230.0
	Deer-invaded	2746.9 ± 158.0	2860.9 ± 189.7

In contrast, the potential activity of β-glucosidase was 18.9% lower in soils from deer-invaded islands compared with soils from non-invaded islands, a significant difference ($F_{(1,45)} = 10.43$, $p = 0.002$), with a mean of 1055.0 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 56.8 SE in soils from deer-invaded islands and a mean of 1301.6 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 66.6 SE in soils from non-invaded islands. Potential β-glucosidase activity was also significant higher ($F_{(1,45)} = 6.69$, $p = 0.013$) in soils from Laskeek Bay (1252.2 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 56.1 SE) than in soils from Gwaii Haanas (1086.2 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 68.0 SE), a difference of 15.3%. There was a significant interaction between the *region* and *treatment* factors ($F_{(1,45)} = 9.86$, $p = 0.003$, Table 7.2). In Gwaii Haanas, potential β-glucosidase activity was 36.0% lower in soils from Ramsay Island (deer-invaded, 855.1 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 53.0 SE) than in the soils from the non-invaded islands (1335.0 $nmol \cdot hr^{-1} \cdot g^{-1}$ ± 87.2 SE). In contrast, the potential β-glucosidase activity in soils from deer-invaded and non-invaded islands in Laskeek Bay was relatively similar (Figure 7.2).

Table 7.2: β-glucosidase activity ($nmol \cdot hr^{-1} \cdot g^{-1}$) in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	1335.0 ± 87.2	1247.3 ± 107.0
	Deer-invaded	855.1 ± 53.0	1255.0 ± 66.7

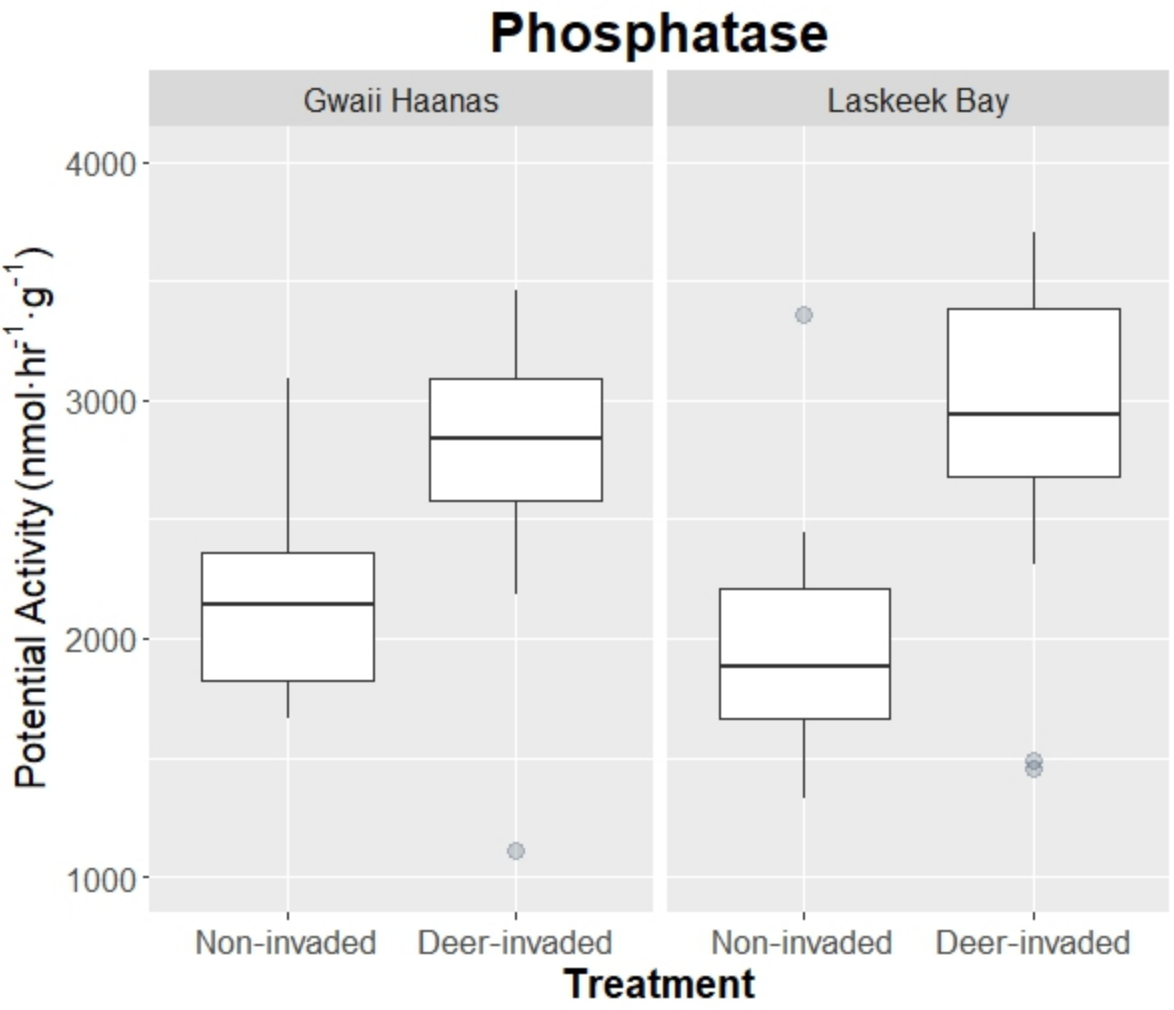

Figure 7.1: Phosphatase activity in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 · IQR; points represent values exceeding 1.5 · IQR.

Likewise, the potential activity of cellobiohydrolase was significantly lower ($F_{(1,45)}$ = 5.442, p = 0.024) in soils from deer-invaded islands (328.7 nmol·hr^{-1}·g^{-1} ± 22.5 SE) compared with soils from non-invaded islands (406.1 nmol·hr^{-1}·g^{-1} ± 29.4 SE). Potential cellobiohydrolase activity was also significantly higher ($F_{(1,45)}$ = 8.234, p = 0.006) in the soils from Laskeek Bay region (408.7 nmol·hr^{-1}·g^{-1} ± 26.5 SE) compared with Gwaii Haanas (323.7 nmol·hr^{-1}·g^{-1} ± 24.1 SE), a difference of 26.2%. There was no significant interaction between the factors of *treatment* (non-invaded vs deer-invaded) and *region* (Gwaii Haanas vs Laskeek Bay, $F_{(1,45)}$ = 3.303, p = 0.076).

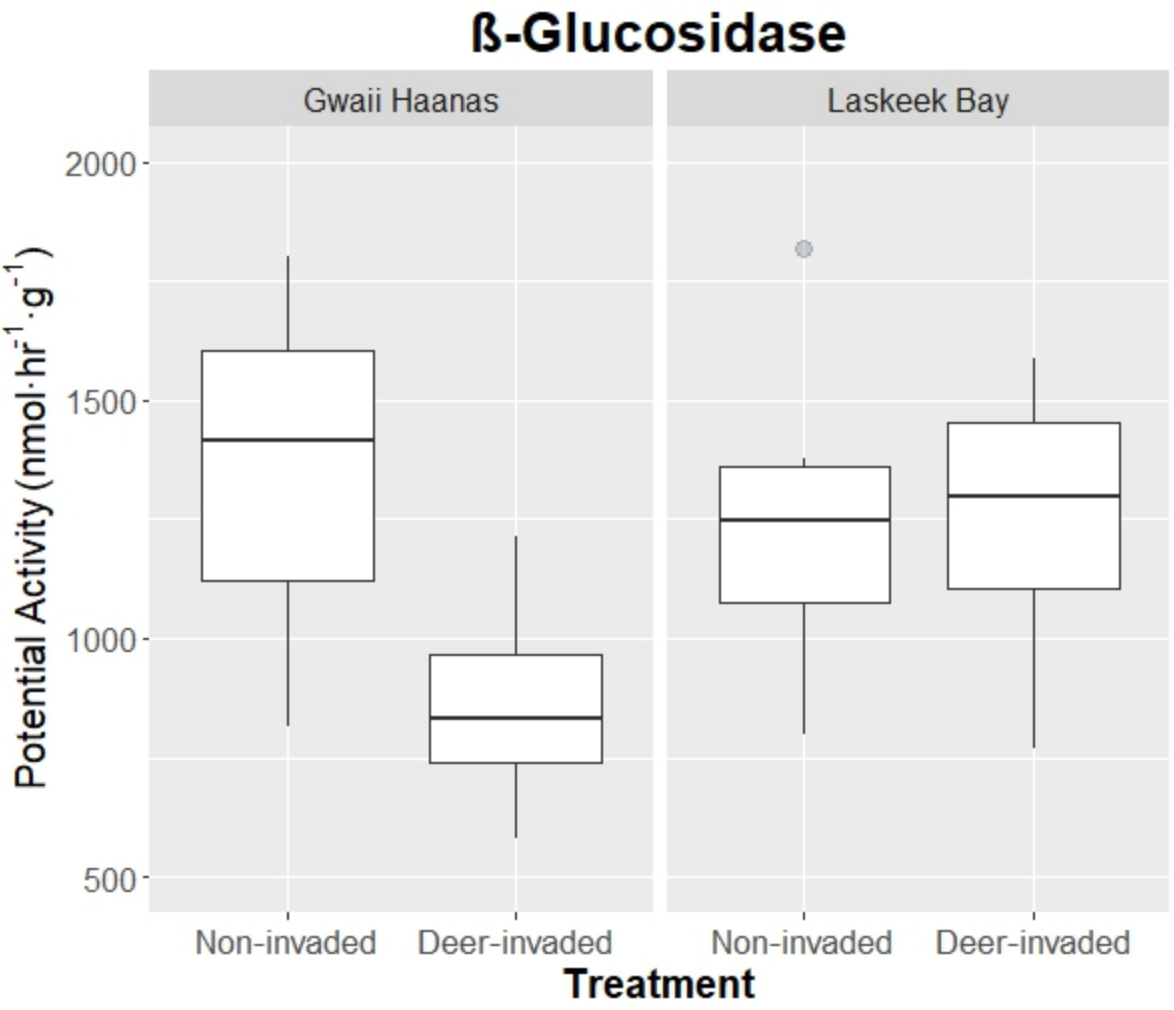

Figure 7.2: β-glucosidase activity in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 · IQR; points represent values exceeding 1.5 · IQR.

Table 7.3: Cellobiohydrolase activity ($nmol \cdot hr^{-1} \cdot g^{-1}$) in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. Values are means ± SE.

Region		Gwaii Haanas	Laskeek Bay
Treatment	Non-invaded	397.1 ± 30.4	420.7 ± 62.1
	Deer-invaded	255.6 ± 26.5	401.8 ± 24.2

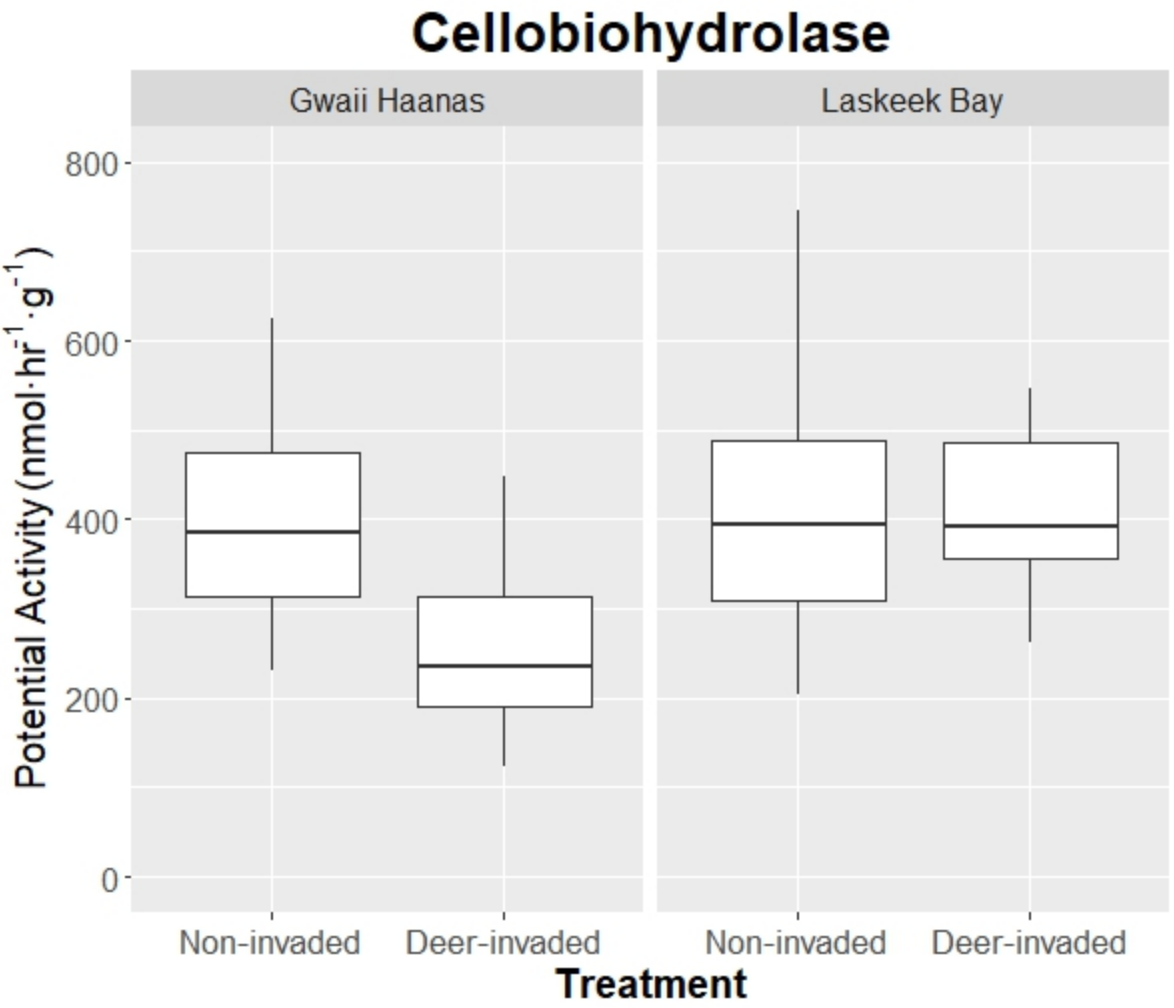

Figure 7.3: Cellobiohydrolase activity in soils from non-invaded islands (n = 13, 8) and deer-invaded islands (n = 14, 14) of Gwaii Haanas and Laskeek Bay, respectively. The bold horizonal line represents the median value; the box represents the interquartile range (IQR); vertical lines represent minimum and maximum values up to 1.5 · IQR; points represent values exceeding 1.5 · IQR.

7.4 Discussion

7.4.1 Phosphatase Activity

Phosphatase, an enzyme produced by bacteria, fungi and plant roots, plays a crucial role in the transformation of organic phosphorus into the more available mineral form of phosphate (Margalef et al., 2017). Potential phosphatase activity was consistently higher in soils from deer-invaded islands compared with soils from non-invaded islands, in both Gwaii Haanas and Laskeek bay. These results conflict with previous studies that have found terrestrial herbivores to be associated with decreases in

phosphatase activity (Acosta-Martínez et al., 2010; Stritar et al., 2010). More surprisingly, higher phosphatase activity in soils from deer-invaded islands was associated with higher concentrations of extractable phosphate (Chapter 3). Numerous studies have found phosphatase activity to be negatively correlated with available phosphorus (Allison and Vitousek, 2005; Halstead, 1964). For example, phosphorus additions to organic forest soils in Maine resulted in decreased phosphatase activity, while nitrogen additions had the opposite effect and stimulated greater phosphatase activity (Fatemi et al., 2016). Likewise, in a chronosequence of differently aged volcanic soils in Hawaii, phosphorus additions resulted in decreased phosphatase activity, while nitrogen additions in the younger nitrogen-limited soils resulted in increased phosphatase activity (Olander and Vitousek, 2000). These studies suggest that phosphatase activity is strongly regulated by end-product suppression.

However, phosphatase activity is not always strongly linked to phosphate concentrations (Jones and Oburger, 2011). Indeed, organic phosphorus may be a better predictor of phosphatase activity (Margalef et al., 2017), with additions of labile organic phosphorus associated with the induction of greater phosphatase activity (Allison and Vitousek, 2005). In agricultural experiments, applications of manure tend to induce greater phosphatase activity, which can result in positive correlations between phosphatase and extractable phosphorus (Garg and Bahl, 2008; Saha et al., 2008). Similarly, in the ectomycorrhizospheres of spruce seedlings grown in the soils of either Engelmann spruce forests or nearby clearcuts, phosphatase activity was higher in the forest soils despite there also being higher concentrations of extractable phosphorus, which may have been due to the forest soils having higher organic matter content and lower pH than in the clearcut soils (Walker et al., 2016). Lower soil pH tends to be associated with greater phosphatase activity (Sinsabaugh et al., 2008). Thus, in the current study, the increased phosphatase activity in soils from deer-invaded islands may have been facilitated by the lower pH in those soils (Chapter 2). These results suggest that, rather than suppressing phosphatase activity, higher concentrations of phosphate in soils from deer-invaded islands are likely the result of the phosphatase activity itself. Islands invaded by Sitka black-tailed deer have accelerated phosphorus cycles associated with greater phosphatase activity compared with non-invaded islands, likely a result of induction by changes in pH and substrate availability.

7.4.2 β-Glucosidase and Cellobiohydrolase Activity

β-glucosidase and cellobiohydrolase are important enzymes involved in the final decomposition of cellulose (Allison and Vitousek, 2005; Lynd et al., 2002). β-glucosidase activity is particularly sensitive

to the quantity and quality of organic soil substrates (Turner et al., 2002) and often correlates with soil respiration (Liang et al., 2015; Merino et al., 2016). As such, β-glucosidase represents a dynamic indicator of overall soil microbial activity. The potential activities of both β-glucosidase and cellobiohydrolase were lower in soils from deer-invaded islands, suggesting that decomposition rates and overall microbial activity may be suppressed on deer-invaded islands. The decreased activity of these cellulose-degrading enzymes may be due to changes in the diversity of organic matter entering the soil through leaf litter, root litter and rhizosphere deposition, the quality and composition of which can vary widely between different plant species (Bardgett and Wardle, 2003; Grayston et al., 1997). Grazing and browsing by aboveground herbivores can drive long-term shifts in the functional composition of plant communities towards species that produce more recalcitrant substrates, indirectly suppressing microbial activity (Bardgett and Wardle, 2003). Thus, by preferentially consuming more palatable understory plants, the Sitka black-tailed deer likely deprive the soil of labile substrates that are easier to decompose (Wardle et al., 2002), resulting in a decrease in the production of β-glucosidase and cellobiohydrolase. However, the decreased activity in β-glucosidase and cellobiohydrolase was largely restricted to the single deer-invaded island that was sampled in the Gwaii Haanas region, Ramsay Island; the activity of β-glucosidase and cellobiohydrolase was similar between the soils of non-invaded and deer-invaded islands of Laskeek Bay. Therefore, connections between Sitka black-tailed deer and the activity of β-glucosidase and cellobiohydrolase are difficult to attribute to differences in substrate availability.

The production of carbon-acquiring enzymes such as β-glucosidase and cellobiohydrolase can also be affected by the availability of nitrogen and phosphorus (Allison et al., 2008; Saiya-Cork et al., 2002), especially in organic forest soils where additions of phosphorus have been found to induce the activity of β-glucosidase (Fatemi et al., 2016). The net immobilization of nutrients by soil microorganisms tends to occur when the proportion of carbon to nitrogen or phosphorus far exceeds the proportions found in microbial biomass. Generally, net immobilization of nitrogen occurs when C:N ratios are greater than 30:1, while net immobilization of phosphorus occurs when C:P ratios are greater than 300:1 (Waring et al., 2007). The organic soils in the Gwaii Haanas and Laskeek Bay regions have C:N ratios greater than 40:1, and C:P ratios in excess of 2000:1 (Catomeris, 2018), suggesting that nitrogen and phosphorus are extremely limiting to microbial activity in these soils. However, while extractable ammonium was indeed lower in soils from deer-invaded islands (Chapter 3), this trend was largely restricted to Laskeek Bay and therefore cannot explain the differences in enzyme activity found in Gwaii Haanas where ammonium concentrations were similar between the non-invaded island and the deer-

invaded island. In this study, phosphorus availability is also unable to explain the activity of β-glucosidase and cellobiohydrolase. Extractable phosphate was higher in soils from deer-invaded islands, which would be predicted to induce the activity of β-glucosidase and cellobiohydrolase, rather than suppress it. Thus, the activity of these two cellulose-degrading enzymes cannot be attributed to differences in the concentrations of these nutrients.

Because the regions of Gwaii Haanas and Laskeek Bay were sampled in different months and years, temporal differences in enzyme activity could also potentially explain why β-glucosidase and cellobiohydrolase activity varied between the two regions. A study examining the extracellular enzyme activities of soils in a temperate deciduous forest in Pennsylvania found that phosphatase, β-glucosidase and cellobiohydrolase activities were significantly different when sampled in August compared with June (Burke et al., 2011), demonstrating the dynamic production of extracellular enzymes in the soil. Alternatively, decreases in the production of β-glucosidase and cellobiohydrolase on Ramsay Island may be the result of a temporary disturbance to microbial activity that eventually disappears as soil microbial communities adapt to the conditions of deer-invaded islands. Sitka black-tailed deer may have reached hyperabundant densities earlier in Laskeek Bay than in Gwaii Haanas (Vila et al., 2005, 2004b). Therefore, disturbances to soil microbial community of Ramsay Island may be more recent and reflect early stages of post-invasion succession. With enough time for the soil microbial communities to adapt to changes in the physiochemical soil properties (Chapter 3), the loss of understory plants (Chapter 4) and shifts in the taxonomic structure of the fungal community (Chapter 5) associated with the deer invasion, the production of β-glucosidase and cellobiohydrolase may eventually recover to levels comparable with soils on non-invaded islands.

Nonetheless, the most parsimonious explanation for the decreased activity of β-glucosidase and cellobiohydrolase in soils from the deer-invaded island of Gwaii Haanas is simply that there are idiosyncratic spatial or temporal differences in enzyme activity between individual islands due to differences in soil substrates or other confounding factors with no causal connection to the presence or absence of Sitka black-tailed deer.

7.5 Conclusion

The objective of the experiment presented in this chapter was to determine the extracellular enzyme activities of phosphatase, β-glucosidase and cellobiohydrolase on islands invaded or not invaded

by Sitka black-tailed deer in Gwaii Haanas and Laskeek Bay. Potential phosphatase activity was consistently higher in soils from deer-invaded islands in both Gwaii Haanas and Laskeek Bay, supporting the hypothesis that labile organic phosphorus substrates deposited into the soil by deer would induce phosphatase activity. Surprisingly, phosphatase activity was associated with higher phosphate concentrations in soils from deer-invaded islands, suggesting that in the organic soil horizons of Haida Gwaii, production of this enzyme is driven by substrate availability rather than end-product concentrations. Soils from islands invaded by Sitka black-tailed deer were associated with significant decreases in the potential activity of β-glucosidase and cellobiohydrolase, but this pattern was largely restricted to Gwaii Haanas. The potential activity of β-glucosidase and cellobiohydrolase was similar between the deer-invaded and non-invaded islands of Laskeek Bay. Therefore, caution should be taken in interpreting these results, which may reflect i) spatial differences the quality and composition of organic substrates between individual islands, or ii) temporal differences in microbial activity between when the soils of these two regions were sampled. Lastly, the regional differences in these results emphasize the importance of considering spatial and temporal effects when using a natural experiment to study dynamic processes such as enzyme activity. Further study is warranted on how the invasion of Sitka black-tailed deer may directly or indirectly affect enzyme activity in the organic soils of Haida Gwaii.

Chapter 8 – Conclusion

8.1 Summary of Findings

As little is known about the effects of deer and other terrestrial herbivores on the soil fungi of temperate conifer forests, this research contributed to the scientific body of knowledge on how aboveground trophic cascades may extend to belowground organisms and processes. On Haida Gwaii, where Sitka black-tailed deer have nearly eradicated the understory shrubs and ferns in much of the archipelago, any modification to the feedback between soil biota and western redcedar (*Thuja plicata*) has implications for the management of this culturally and economically vital tree species. The purpose of this thesis was to determine the potential effects of invasive Sitka black-tailed deer on the soils, plants and fungi of Haida Gwaii. I used a natural experiment to compare islands that were either invaded or not invaded by Sitka black-tailed deer. After sampling the organic soil horizons and surveying the plant community, I analyzed various physical and chemical properties of the soil and compared the diversity, abundance and composition of the plant communities between deer-invaded and non-invaded islands. I used ergosterol as a biomarker for fungal biomass and molecular techniques to sequence DNA and identify taxonomic and functional groups of soil fungi. To further explore the effects of Sitka black-tailed deer on the mycorrhizal inoculum potential of the soil, I conducted two greenhouse bioassays using *Tsuga heterophylla* and *Thuja plicata* in which I measured the percent root colonization of ectomycorrhizal fungi, arbuscular mycorrhizal fungi and dark septate endophytes of seedlings grown in soils from the field. In addition to measuring the biomass and root/shoot ratios of the seedlings, I used chlorophyll fluorescence as an indicator of plant stress. Lastly, I conducted enzyme assays to estimate the potential activity of three soil enzymes involved in nutrient acquisition and soil decomposition: phosphatase, β-glucosidase and cellobiohydrolase.

In Chapter 3, I showed how soil compaction was significantly higher and the depth of the organic horizons was significantly shallower on deer-invaded islands. Extractable phosphate was significantly higher while pH was significantly lower in soils from deer-invaded islands. While extractable ammonium tended to be lower in soils from deer-invaded islands, the difference was not significant. Nitrate concentrations were similar between the soils of deer-invaded and non-invaded islands.

In Chapter 4, I revealed that the cover-abundance of understory plants was significantly lower on deer-invaded islands. Although the species richness of the understory plant community was similar

between deer-invaded and non-invaded islands, species evenness was significantly higher on deer-invaded islands, demonstrating how the extreme grazing pressure by Sitka black-tailed deer homogenizes the remaining understory plants. The Shannon diversity index of the plant community was also significantly higher on deer-invaded islands, likely a result of differences in species evenness. Multivariate analysis revealed significant differences in the composition of the plant communities on deer-invaded and non-invaded islands. However, the smallest deer-invaded islands, West Skedans and South Skedans, had plant communities more similar to the non-invaded islands. Indicator species analysis revealed salal (*Gaultheria shallon*), sword fern (*Polystichum munitum*) and twinberry (*Lonicera involucrata*) to be among the significant indicator species of non-invaded islands, while false azalea (*Menziesia feruginea*), deer fern (*Blechnum spicant*), single delight (*Moneses uniflora*), two orchid species (*Neottia banksiana*, *Neottia cordata*) and other forbs and grasses were significant indicator species of deer-invaded islands.

Likewise, in Chapter 5, I demonstrated how the species (OTU) richness of soil fungi was similar between deer-invaded and non-invaded islands. Although species evenness and the Shannon diversity index of the fungal communities were significantly different between deer-invaded and non-invaded islands, there was no consistent pattern across the two regions of Gwaii Haanas and Laskeek Bay, suggesting that fungal communities vary in their diversity between individual islands. Fungal biomass, as inferred through ergosterol concentrations, was similar between deer-invaded and non-invaded islands. However, fungal biomass tended to be lower in soils from deer-invaded islands in Laskeek Bay. Multivariant analysis revealed significant differences between the fungal communities of deer-invaded and non-invaded islands, however, the presence of Sitka black-tailed deer on an island only explained a small fraction of the variation in the fungal communities, with regional differences and individual islands explaining a greater amount of the variation. The fungal communities on West Skedans and South Skedans were outliers among the deer-invaded islands and were more similar to the fungal communities on non-invaded islands. The composition of the fungal community was significantly correlated with the total cover-abundance of understory plants and edaphic properties such as ammonium concentrations, pH and the depth of the organic horizons. Other variables, such as the cover-abundances of the individual indicator plant species or soil properties such as soil compaction and phosphate concentrations, explained additional variation in the fungal community but were not significant terms in the model. There were no significant differences in the relative abundance of any of the functional groups examined, including: AM fungi, EcM fungi, ErM fungi, saprotrophic fungi and pathogenic fungi. Taxonomic differences in the fungal community were subtle. Glomeromycota were extremely low in

abundance and were absent in approximately one quarter of the samples. Eurotiomycetes and Chytridiomycetes were greater in relative abundance on deer-invaded islands, while Agaricomycetes was lower in abundance. Significant indicator species (OTUs) were identified for both deer-invaded and non-invaded islands.

Chapter 6 showed how the inoculum potential of ectomycorrhizal fungi in *Tsuga heterophylla* was similar between seedlings grown in soils from deer-invaded and non-invaded islands; likewise, there were no significant differences in the inoculum potential of AM fungi or dark septate endophytes in *Thuja plicata* seedlings grown in soils from deer-invaded and non-invaded islands, though the percent root colonization of DSE was notably lower in seedlings grown in soils from deer-invaded islands. AM fungi occurred infrequently in the roots of *Thuja plicata*, whereas DSE were present in the majority of samples. The biomass and root/shoot ratios of *Tsuga heterophylla* and *Thuja plicata* were similar between those grown in soils from deer-invaded and non-invaded islands. However, while the chlorophyll fluorescence (Fv/Fm) ratio of *Tsuga heterophylla* seedlings was similar between those grown in soils from deer-invaded and non-invaded islands, the chlorophyll fluorescence ratios of *Thuja plicata* were significantly lower in seedlings grown in soils from deer-invaded islands. By using sterilized negative controls to account for abiotic soil properties, the differences in chlorophyll fluorescence could be attributed to a biotic factor, suggesting that the soil microbial communities on deer-invaded islands contribute to greater stress in *Thuja plicata* under drought conditions. However, the greater stress in *Thuja plicata* did not correlate with the root colonization of AM fungi or DSE.

Finally, in chapter 7, I reported that potential phosphatase activity was significantly higher in soils from deer-invaded islands and may explain the higher phosphate concentrations found in those soils. The potential activities of β-glucosidase and cellobiohydrolase were significantly lower in soils from deer-invaded islands, but only in Gwaii Haanas. This pattern was not observed in Laskeek Bay, suggesting that cellulolytic enzyme activity may vary between individual islands with no causal link to the presence or absence of Sitka black-tailed deer.

In conclusion, Sitka black-tailed deer are associated with small but significant changes in the composition of the soil fungal communities on Haida Gwaii, likely driven by severe decreases in the abundance of understory plants consumed by the deer and shifts in edaphic properties such as pH and the depth of organic horizons. Shifts in the composition in the fungal community were also associated with significant differences in extracellular enzyme activity and stress responses in western redcedar (*Thuja plicata*). The soil biota on deer-invaded island may decrease the drought tolerance of *Thuja*

plicata, compounding the effects of climate change on the forests of Haida Gwaii. Nonetheless, these results suggest that the soil fungal communities of organic soils in late successional temperate conifer forests are largely resilient to the effects of invasive herbivores during the first 75 years after their initial colonization.

8.2 Limitations of this Study

8.2.1 Limitations of a Natural Experiment

As a natural experiment, the research presented in this thesis is fundamentally limited in its ability to make definitive conclusions about causal connections between Sitka black-tailed deer and the soils, plants and fungi of Haida Gwaii. Comparisons were made between islands invaded or not invaded by the Sitka black-tailed deer based on anecdotal and empirical evidence of their presence or absence. Inferential statistics allow for correlations and associations to be determined between the presence of Sitka black-tailed deer and the variables of interest, such as diversity metrics of the plant and fungal communities. However, there remains the possibility that unidentified confounding factors may have influenced the association of deer with some of the phenomena that were significantly different between deer-invaded and non-invaded islands. With the exception of South Skedans Island, multiple plots were established on each island, reducing the number of statistically independent units to the number of islands compared (9), further limiting the explanatory power of this experiment and the potential influence of autocorrelated outliers.

8.2.2 Spatial and Temporal Dynamics

Because the soils of Laskeek Bay and Gwaii Haanas were sampled in two separate years, one of the limitations of this study is an inability to identify whether regional differences in the data are due to spatial processes associated with each region or temporal processes associated with when the samples were taken. Spatial differences between Gwaii Haanas and Laskeek Bay could be related to differences in i) soil parent material (Brown, 1968), ii) browsing histories or residence times since initial colonization by Sitka black-tailed deer (Vila et al., 2005, 2004b), iii) factors related to island biogeography such as the area or isolation of individual islands (MacArthur and Wilson, 1967), or iv) stochastic processes such as storms. Of particular relevance to this thesis, Sitka black-tailed deer were introduced at the north end of

the archipelago (Golumbia et al., 2008), and because Laskeek Bay is further north than Gwaii Haanas, the Sitka black-tailed deer likely invaded Laskeek Bay prior to reaching Gwaii Haanas. The age-class distributions of *Vaccinium parvifolium* and *Gaultheria shallon* suggest that Sitka black-tailed deer reached hyperabundant densities 10 to 20 years earlier in Laskeek Bay than in Gwaii Haanas (Vila et al., 2005, 2004b). Thus, Gwaii Haanas may be more influenced by biological legacies or other forms of ecological inertia associated with its pre-invasion state (Von Holle et al., 2003). Similarly, time lags associated with the exponential population growth of Sitka black-tailed deer may delay the impacts of the species invasion in Gwaii Haanas relative to Laskeek Bay (Crooks, 2005). For example, fungal biomass tended to be lower in the soils of deer-invaded islands of Laskeek Bay, which may be a more long-term impact of the deer. As an alternative explanation for regional differences in the soils, plants and fungi, some of the effects of Sitka black-tailed deer may be temporary and only associated with earlier stages of succession following the initial disturbance. Three potential temporal effects should also be considered. The abundance, activity and composition of soil fungi are highly dynamic and fluctuate through the seasons (Voříšková et al., 2014). For example, the genetic identity of ectomycorrhizal and saprotrophic communities in the same location can be significantly different in as little as 3 months (Burke et al., 2011). Therefore, differences in the community composition of soil fungi between the Gwaii Haanas and Laskeek Bay samples could be attributable to the different months or years when the field work was conducted. Secondly, as noted in Chapter 3, soil moisture was significantly greater in the Laskeek Bay samples due to greater precipitation than when the Gwaii Haanas samples were taken. Because soil moisture is a major driver of fungal diversity and community composition (Erlandson et al., 2016; Frąc et al., 2018), this factor alone could account for the purported regional differences in the fungal community. Lastly, the samples from Laskeek Bay were stored at 5°C for almost twice as long as the Gwaii Haanas samples, the implications of which are discussed below.

8.2.3 Potential Effects of Next Generation Sequencing Methods

The molecular methods used in this study successfully identified thousands of fungal taxa in the soils on non-invaded and deer-invaded islands, confirming that a wide diversity of soil fungi can be identified through sequencing of the ITS region (Buée et al., 2009). More specifically, the ITS2 region meets several criteria as an ideal DNA barcoding marker: high interspecific variability, conserved priming sites and reliable PCR amplification (Han et al., 2013). Although the primer pair gITS7/ITS4 has been demonstrated to amplify the ITS2 region of Glomeromycota (Ihrmark et al., 2012), in this study, OTUs

identified as arbuscular mycorrhizal fungi were infrequent and had an extremely low relative abundance. This could be the result of primer bias in favor of other phyla such as Ascomycota or Basidiomycota, or due high length variation in the ITS region of Glomeromycota which can be up to 3935 bp in length (Krüger et al., 2009). The Illumina MiSeq system used in this current study uses paired end reads of 300 bp which thus may discriminate against taxa with ITS2 regions longer than 500 bp. However, results from the greenhouse bioassay (Chapter 6) lend evidence that arbuscular mycorrhizal fungi are indeed infrequent and low in abundance in these soils. Furthermore, the fungal DNA amplicons tended to range between 300 and 500 bp in length, which suggests that the read-length of the Illumina platform was adequate for representing this fungal community.

The choice of using ITS2 rather than ITS1 likely skewed the analysis of the fungal community by increasing the relative abundance of DNA amplicons associated with Ascomycota and reducing the overall diversity of the fungal community. A study using 454-sequencing compared the results of using the ITS1 and ITS2 regions for characterizing the fungal community and found no significant differences in the total number of reads or operational taxonomic units but they found a decrease in diversity when using ITS2 (Monard et al., 2013). ITS1 may result in the overrepresentation of Basidiomycota and ITS2 may result in an overrepresentation of Ascomycota, likely due to primer bias (Monard et al., 2013). Another study compared the effectiveness of using ITS1 and ITS2 by amplifying the DNA of a known fungal community and found that the two ITS regions produce similar results, concluding that a 97% similarity cut-off was a reasonable threshold for estimating the number of known species in the data sets (Blaalid et al., 2013). However, caution must be taken when interpreting the sequencing results at fine-scale taxonomic resolutions (genus and species) because the ITS1 and ITS2 regions vary in which taxa that they overrepresent or underrepresent through the clustering of sequences into OTUs (Blaalid et al., 2013). As a method for identifying fungal taxa, genetic sequencing is also limited by the accuracy and representation of fungal sequences in databases such as UNITE or FUNGuild. For example, the UNITE database itself was initially established for the purpose of identifying ectomycorrhizal fungi (Kõljalg et al., 2005) but has since grown to include the ITS sequences of fungal taxa from other functional groups. Nonetheless, biased representation of Agaricomycetes in UNITE may limit the utility of this database for describing fungal communities at a fine-scale taxonomic resolution. Rare or obscure taxonomic groups are also likely to be underrepresented. For example, dark septate endophytes often lack taxonomically distinct features, making them difficult to identify (Narisawa et al., 2007). For this reason, dark septate endophytes may be underrepresented in databases such as UNITE, despite being highly abundant in soils around the world (Mandyam and Jumpponen, 2005).

8.2.4 Potential Effects of the Storage and Pretreatment Methods

The storage and pretreatment methods used in this study may have increased or decreased the potential activity of the enzymes being studied. As described in Chapter 7, field samples used in the enzyme assays were stored at approximately 5°C for 2 to 4 weeks before being sieved and frozen at -20°C for longer term storage. Samples were then free-dried at -20°C and ground with a mortar and pestle as a final pretreatment technique. Depending on the particular enzymes being studied, both drying and freezing at -20°C can alter the potential activity of soil enzymes (Peoples and Koide, 2012). Studies on the effects of different storage and pretreatment methods on β-glucosidase vary considerably in their results. For example, freeze-drying forest soils at -20°C decreased β-glucosidase activity by 73% compared with fresh samples, while storing fresh samples at 5°C for 4 weeks resulted in a 25% decrease in β-glucosidase activity (Yoshikura et al., 1980). In contrast, another study found that while drying and rewetting forest soil had no significant effect on the activity of β-glucosidase activity, four weeks of storage at 4°C and four weeks of storage at -20°C both significantly increased the activity of β-glucosidase compared with fresh samples (Lee et al., 2007). Yet another study found that freezing soil had no effect on β-glucosidase activity compared with fresh samples (Daou et al., 2016). Few studies have explored the effects of storage methods on cellobiohydrolase activity, but one study found that storage at -20°C for 28 days significantly decreased the potential activity of cellobiohydrolase (Peoples and Koide, 2012). Overall, these studies suggest that it is difficult to predict how the potential activity of β-glucosidase and cellobiohydrolase were affected by the storage and pretreatment methods used in this study but degradation of the enzymes likely occurred to a certain degree.

In contrast to β-glucosidase and cellobiohydrolase, acid phosphatase is extremely resilient to handling and storage conditions. For example, sterilizing soils in an autoclave using steam at 121°C for up to an hour did not completely inactivate acid phosphatase activity (Eivazi and Tabatabai, 1977). Similarly, another study found that three different methods of storing soil, including i) drying and rewetting, ii) four weeks of storage at 4°C, and iii) four weeks of storage at -20°C, did not significantly affect acid phosphatase activity (Lee et al., 2007). Likewise, repeatedly freezing and thawing Mediterranean forest soils seven times at -20°C had no significant effect on acid phosphatase activity (Daou et al., 2016). Although one study did find that storage at -20°C significantly reduced acid phosphatase activity (Peoples and Koide, 2012), overall these studies suggest that the potential phosphatase activity reported in this thesis was not substantially affected by the storage and

pretreatment methods. Nonetheless, the effects of storage and pretreatment on enzyme activity can be assumed to have affected all samples proportionally. It is acceptable for most studies on enzyme activity to compare the relative differences between treatment groups (Peoples and Koide, 2012).

Because western redcedar (*Thuja plicata*), an arbuscular plant species (Kough et al., 1985; Parke et al., 1983b), is such an abundant tree on Haida Gwaii (Banner et al., 2014) and was commonly found in the plots, it was surprisingly that the inoculum potential of AM fungi was so low in abundance and frequency in the greenhouse bioassay (Chapter 6). Arbuscular mycorrhizal fungi are obligate biotrophs (Bago and Bécard, 2002; van der Heijden et al., 2015), therefore separation of their hyphae from their host plant could conceivably reduce their viability by removing their source of carbon. However, several studies have shown AM fungal inoculum to be remarkably resilient to various conditions and lengths of storage. For example, the intraradical vesicles of *Glomus intraradices* encapsulated within alginate beads remained viable after being stored for over 5 years at 4°C (Plenchette and Strullu, 2003). Another study found that AM fungal spores functioned as viable inoculum for plants when stored in moist soil (water potentials between −0.04 MPa and −0.8 MPa), but declined in soils that were dried (Ruiz-Lozano and Azcón, 1996). Kuszala, Gianinazzi, and Gianinazzi-Pearson (2001) stored isolates of four AM fungal genera at a variety of temperatures, from 24°C to -80°C and found that all storage temperatures resulted in successful sporulation. Another study found that after removing the shoots of plant hosts, the belowground extraradical mycelium of two AMF species, *Funneliformis mosseae* and *Rhizoglomus irregulare*, continued to form viable symbioses with new host plants for as long as five months after shoot removal (Pepe et al., 2018). These studies suggest that propagules of arbuscular mycorrhizal fungi are resilient to the storage conditions used in this thesis. However, in addition to being stored at 5°C and later at -20°C, samples were wet-sieved to 4 mm as a pretreatment technique intended to homogenize the samples and to remove rocks or large root fragments from the samples. Decayed roots can serve as a greater reservoir for AM fungal propagules than the surrounding soil matrix (Müller et al., 2017), suggesting that sample preparation techniques that remove decayed roots through sieving could deplete the soil of a major inoculum source for AM fungi. Therefore, arbuscular mycorrhizal fungi could potentially occur more frequently in the soils of Haida Gwaii or have a greater inoculum potential *in situ* than demonstrated in the bioassay described in Chapter 6. Likewise, the low abundance and frequency of AM fungi reported in the sequencing results of Chapter 5 may underrepresent the true abundance of Glomeromycota on Haida Gwaii.

8.3 Further Research

As a result of this study, several new questions and hypotheses were generated that warrant further study. The greenhouse bioassay revealed that soil biota from deer-invaded islands is associated with greater stress in western redcedar (*Thuja plicata*) under drought conditions. However, chlorophyll fluorescence (F_v/F_m values) of western redcedar was neither correlated with the percent root colonization of arbuscular mycorrhizal fungi nor with dark septate endophytes. I offer two mutually inclusive hypotheses that may explain this pattern: i) the dark septate endophytes or other organisms in the soils of the deer-invaded islands function as pathogens or parasites of *Thuja plicata*, ii) the dark septate endophytes that proliferate in the soils of deer-invaded islands provide negligible benefits to *Thuja plicata* compared with the DSE of non-invaded islands.

Although the specific taxa colonizing the roots of *Thuja plicata* were not identified, most of the dark septate endophytes identified in the soil through next generation sequencing were ericoid mycorrhizal fungi. Thus, the dark septate endophytes that colonized the roots of *Thuja plicata* in the greenhouse bioassay were likely ericoid mycorrhizal fungi. As previous researchers have noted, dark septate endophytes have been observed to form ericoid mycorrhizae and ectomycorrhizae in conifers (Lukešová et al., 2015), while known ericoid mycorrhizal fungi have been observed to colonize bryophytes and other non-ericaceous vascular plants (Straker, 1996; van der Heijden et al., 2015). I hypothesize that i) *Thuja plicata* and other plants that persist on deer-invaded islands serve as alternative hosts for the same ErM fungi that form mutualisms with salal (*Gaultheria shallon*) and *Vaccinium* species on Haida Gwaii, and ii) ericoid mycorrhizal fungi and/or dark septate endophytes form common mycelial networks between ericaceous shrubs and overstory conifer trees.

Several differences between the soils of deer-invaded and non-invaded were inconsistent between the regions of Gwaii Haanas and Laskeek Bay. For example, ammonium concentrations tended to be lower in soils from the deer-invaded islands of Laskeek Bay, but this pattern was not observed in the soils of Gwaii Haanas. Similarly, the potential enzyme activities of β-glucosidase and cellobiohydrolase were lower in the soils from the deer-invaded island of Gwaii Haanas (Ramsay Island), but not in soils from the deer-invaded islands of Laskeek Bay. In the *Thuja plicata* bioassay, differences in chlorophyll fluorescence (F_v/F_m values) were largely driven by seedlings grown in living soil from deer-invaded islands of Laskeek Bay, not Gwaii Haanas. As these two regions were sampled during different months and years, this study was unable to distinguish between spatial or temporal variation between

the samples from these regions. Future research should employ experimental designs that are robust to potential spatial or temporal differences in the edaphic properties of Haida Gwaii, for example by considering differences in soil parent material or seasonal variation in climate.

Finally, we observed many examples of culturally modified trees serving as refugia for native plant species that are preferentially browsed by Sitka black-tailed deer. Large spruce and cedar trees, spared through selective harvesting, continue to provide refugia for native plants such as salal (*Gaultheria shallon*) and red huckleberry (*Vaccinium parvifolium*) in their canopy soils or in deep furrows along their bark. Coarse woody debris also appeared to provide refugia for plant species that cannot persist under intense browsing pressure. Future research could: i) investigate the role of refugia in maintaining the abundance and diversity of understory plant communities in the wake of the deer invasion, and ii) evaluate how the resilience of these communities is affected by contemporary forestry practices compared with the historical resource management techniques of the Haida.

Figure 8.1: Culturally modified tree as a refuge for salal (*Gaultheria shallon*) on a deer-invaded island

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Appendices

Appendix A

Table A.1: Cover-abundance (%), species richness, Pielou's evenness (%) and Shannon's diversity of the understory plant communities in plots on Agglomerate Island (n = 7), Lost Island (n = 3), Louise Island (n = 5), Low Island (n = 5), Ramsay Island (n = 14), Reef Island (n = 6), South Skedans Island (n = 1), Tar Island (n = 6), and West Skedans Island (n = 2). Values are means ± SE.

Island	Cover-Abundance (%)	Species Richness	Pielou's Evenness (%)	Shannon's Diversity
Agglomerate	46.45 ± 11.38	7.00 ± 1.11	33.80 ± 6.31	0.65 ± 0.15
Lost	118.5 ± 25.07	10.67 ± 1.76	59.56 ± 7.92	1.42 ± 0.27
Louise	4.60 ± 1.63	10.60 ± 1.50	71.84 ± 6.62	1.64 ± 0.16
Low	101.7 ± 8.87	8.40 ± 1.60	30.47 ± 7.87	0.66 ± 0.21
Ramsay	2.64 ± 0.44	11.64 ± 1.31	84.27 ± 4.65	2.00 ± 0.14
Reef	2.23 ± 0.63	9.17 ± 0.87	82.83 ± 9.52	1.81 ± 0.22
South Skedans	43.50 ± NA	14.00 ± NA	24.43 ± NA	0.64 ± NA
Tar	92.04 ± 11.06	10.33 ± 0.80	28.59 ± 5.73	0.65 ± 0.12
West Skedans	84.31 ± 14.69	6.50 ± 0.50	14.33 ± 8.84	0.27 ± 0.18

Table A.2: Summary of analysis of variance (ANOVA) models for the total cover-abundance, species richness, Pielou's evenness and Shannon's diversity of understory plants using the factors of treatment (non-invaded islands vs deer-invaded), region (Gwaii Haanas vs Laskeek Bay), and island nested within the region factor. Significant effects are indicated in bold ($p < 0.05$).

Cover-Abundance					
Factor	Df	SS	Mean SS	F	p
Treatment	1	21.3875	21.38753	298.603	**<0.001**
Region	1	1.221	1.22101	17.047	**<0.001**
Treatment × Region	1	0.0202	0.02024	0.283	0.59793
Island (Region)	5	5.5776	1.11553	15.574	**<0.001**
Residuals	40	2.865	0.07163		
Species Richness					
Treatment	1	40.3333	40.33333	3.097	0.08609
Region	1	9.1823	9.18232	0.705	0.40608
Treatment × Region	1	21.325	21.32501	1.637	0.20805
Island (Region)	5	90.2117	18.04234	1.385	0.25036
Residuals	40	520.9476	13.02369		
Pielou's Evenness					
Treatment	1	1.8635	1.86351	62.305	**<0.001**
Region	1	0.0616	0.06158	2.059	0.15910
Treatment × Region	1	0.249	0.24895	8.323	**0.00628**
Island (Region)	5	1.0597	0.21194	7.086	**<0.001**
Residuals	40	1.1964	0.02991		
Shannon's Diversity					
Treatment	1	11.0308	11.03083	53.716	**<0.001**
Region	1	0.4731	0.47313	2.304	0.13691
Treatment × Region	1	2.0928	2.09282	10.191	**0.00275**
Island (Region)	5	5.418	1.0836	5.277	**<0.001**
Residuals	40	8.2142	0.20535		

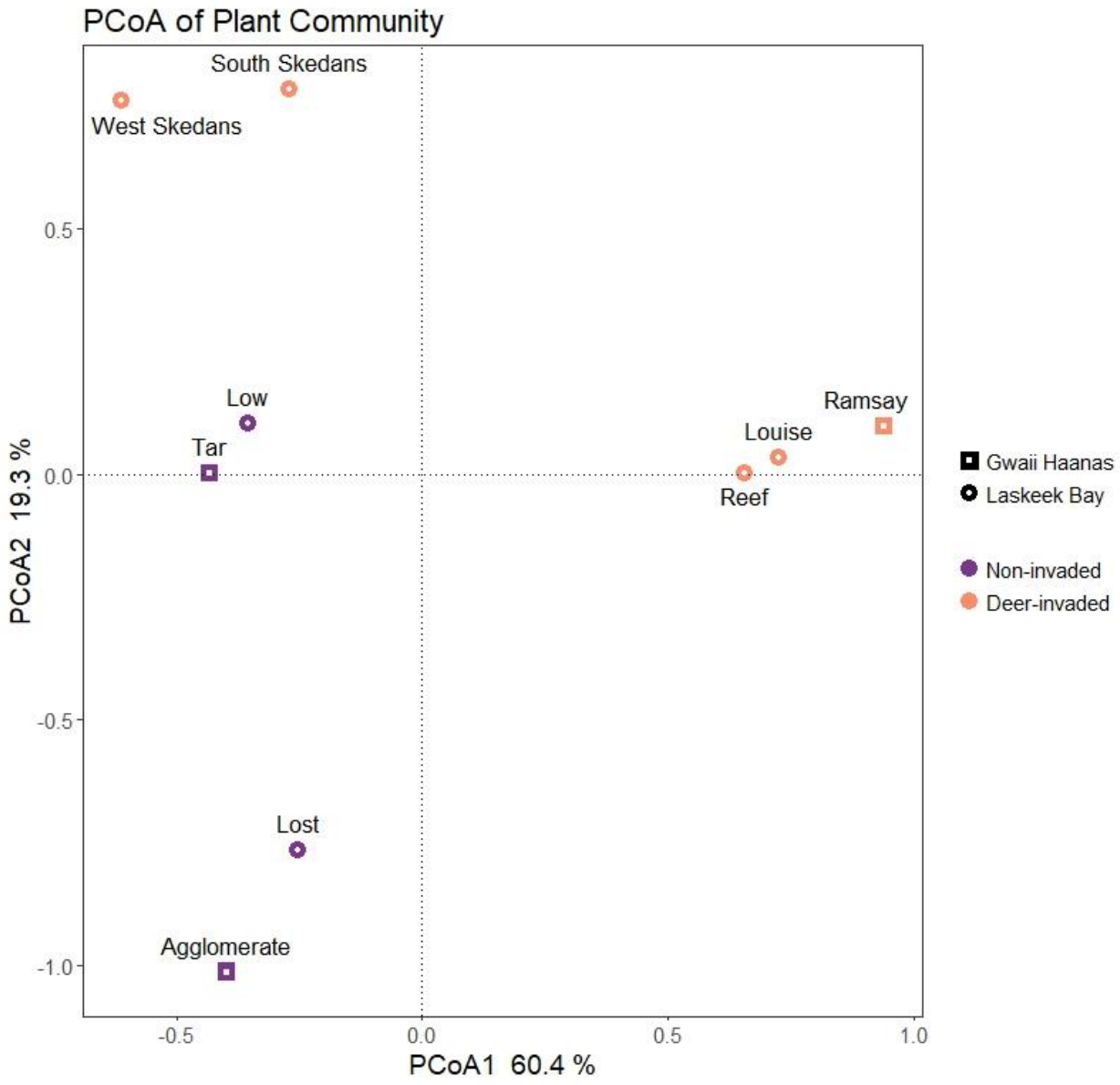

Figure A.1: Principal coordinates analysis (PCoA) of the plant communities on individual islands using Bray-Curtis dissimilarities of Hellinger-transformed cover-abundances, comparing non-invaded islands (n = 2, 2) and deer-invaded islands (n = 1, 4) in the regions of Gwaii Haanas and Laskeek Bay, respectively.

Table A.3: Mean absolute abundance (%), mean relative abundance (%) and frequency of presence (%) of understory plant species in plots on non-invaded islands (n = 21) and deer-invaded islands (n = 28). Species that were not present in a treatment group are represented by a dash (-). Standard error is listed in parentheses.

Species	Absolute Abundance (%)		Relative Abundance (%)		Frequency (%)	
	Non-Invaded	Deer-Invaded	Non-Invaded	Deer-Invaded	Non-Invaded	Deer-Invaded
Adiantum pedatum	-	<0.01 (<0.01)	-	0.15 (0.15)	-	4
Agrostis exarata	-	<0.01 (<0.01)	-	0.10 (0.10)	-	4
Athyrium filix-femina	0.01 (<0.01)	0.03 (<0.01)	0.01 (<0.01)	0.71 (0.28)	10	21
Blechnum spicant	0.08 (0.04)	0.09 (0.01)	0.07 (0.03)	3.89 (0.83)	33	68
Boschniakia hookeri	0.02 (<0.01)	-	0.02 (<0.01)	-	14	-
Bromus pacificus	-	0.02 (0.01)	-	0.40 (0.28)	-	11
Bromus sitchensis	-	0.03 (<0.01)	-	0.78 (0.35)	-	21
Carex laeviculmis	-	0.01 (<0.01)	-	0.41 (0.24)	-	11
Calamagrostis nutkaensis	0.30 (0.20)	0.01 (0.01)	0.74 (0.55)	0.45 (0.45)	19	4
Chimaphila menziesii	-	<0.01 (<0.01)	-	0.62 (0.43)	-	7
Cornus canadensis	-	<0.01 (<0.01)	-	0.09 (0.09)	-	4
Corallorhiza maculata	-	<0.01 (<0.01)	-	0.36 (0.36)	-	4
Driopteris expansa	0.03 (0.02)	0.05 (0.01)	0.05 (0.04)	2.03 (0.70)	14	39
Elymus glaucus	<0.01 (<0.01)	-	<0.01 (<0.01)	-	5	-

Species	Absolute Abundance (%)		Relative Abundance (%)		Frequency (%)	
	Non-Invaded	Deer-Invaded	Non-Invaded	Deer-Invaded	Non-Invaded	Deer-Invaded
Chamerion angustifolium	<0.01 (<0.01)	-	<0.01 (<0.01)	-	5	-
Equisetum arvense	-	<0.01 (<0.01)	-	0.19 (0.19)	-	4
Gaultheria shallon	55.05 (8.51)	7.70 (4.18)	58.88 (7.24)	26.03 (6.09)	95	82
Galium triflorum	0.01 (<0.01)	0.01 (<0.01)	0.01 (<0.01)	0.35 (0.24)	10	11
Goodyera pubescens	<0.01 (<0.01)	0.01 (<0.01)	<0.01 (<0.01)	1.15 (0.68)	5	11
Juncus effusus	-	0.02 (0.01)	-	0.63 (0.48)	-	7
Lonicera involucrata	1.36 (0.65)	0.16 (0.11)	1.40 (0.66)	0.69 (0.32)	95	21
Luzula multiflora	-	<0.01 (<0.01)	-	0.30 (0.30)	-	4
Luzula parviflora	0.02 (0.01)	0.04 (0.01)	0.05 (0.03)	1.45 (0.51)	19	29
Lysichiton americanus	-	<0.01 (<0.01)	-	0.09 (0.09)	-	4
Lycopodium selago	-	<0.01 (<0.01)	-	0.22 (0.22)	-	4
Maianthemum dilatatum	2.37 (1.77)	0.03 (<0.01)	2.07 (1.31)	0.97 (0.53)	81	21
Malus fusca	-	<0.01 (<0.01)	-	0.08 (0.08)	-	4
Menziesia feruginea	-	0.25 (0.11)	-	7.36 (1.24)	-	86
Moneses uniflora	0.02 (0.01)	0.10 (0.01)	0.07 (0.04)	4.70 (0.84)	19	75
Neottia banksiana	0.01 (<0.01)	0.08 (0.01)	0.04 (0.03)	3.91 (0.86)	10	64

Species	Absolute Abundance (%)		Relative Abundance (%)		Frequency (%)	
	Non-Invaded	Deer-Invaded	Non-Invaded	Deer-Invaded	Non-Invaded	Deer-Invaded
Neottia cordata	-	0.07 (0.01)	-	4.03 (0.94)	-	57
Polypodium glycyrrhiza	0.12 (0.03)	0.11 (0.01)	0.20 (0.05)	4.24 (0.87)	71	79
Polystichum munitum	11.71 (2.66)	0.08 (0.03)	22.87 (6.53)	1.77 (0.86)	100	36
Prenanthes alata	0.02 (<0.01)	-	0.04 (0.02)	-	14	-
Pteridium aquilinum	0.36 (0.20)	-	0.40 (0.22)	-	33	-
Ribes laxiflorum	0.15 (0.14)	0.03 (0.01)	0.13 (0.12)	0.57 (0.33)	10	14
Rubus parviflorus	0.57 (0.26)	-	0.53 (0.26)	-	19	-
Rubus spectabilis	5.79 (2.51)	0.33 (0.14)	5.87 (2.79)	6.24 (0.88)	67	96
Sambucus racemosa	0.01 (<0.01)	0.01 (<0.01)	0.01 (<0.01)	0.28 (0.20)	10	11
Salix scouleriana	0.95 (0.95)	-	0.58 (0.58)	-	5	-
Stellaria crispa	-	0.03 (0.01)	-	1.06 (0.43)	-	25
Symphoricarpos albus	0.29 (0.20)	-	0.22 (0.15)	-	14	-
Tellima grandiflora	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	0.15 (0.15)	5	4
Vaccinium ovalifolium	-	0.02 (0.01)	-	0.56 (0.39)	-	7
Vaccinium parvifolium	4.84 (1.81)	0.80 (0.20)	5.75 (1.94)	23.01 (4.15)	100	100
Vicia gigantea	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	5	4

Table A.4: Mean absolute abundance (%) of understory plant species in plots on Agglomerate Island (AGG, n = 7), Lost Island (LOS, n = 3), Louise Island (LOU, n = 5), Low Island (LOW, n = 5), Ramsay Island (RAM, n = 14), Reef Island (REE, n = 6), South Skedans Island (SSK, n = 1), Tar Island (TAR, n = 6), and West Skedans Island (WSK, n = 2). Species that were not present on an island are represented by a dash (-). Standard error is listed in parentheses.

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK
Adiantum pedatum	-	-	-	-	<0.01 (<0.01)	-	-	-	-
Agrostis exarata	-	-	-	-	<0.01 (<0.01)	-	-	-	-
Athyrium filix-femina	-	0.04 (0.04)	0.03 (0.03)	0.03 (0.03)	0.04 (0.02)	0.02 (0.02)	-	-	-
Blechnum spicant	0.02 (0.02)	0.38 (0.19)	0.08 (0.03)	-	0.12 (<0.01)	0.04 (0.03)	0.13 (NA)	0.06 (0.03)	-
Boschniakia hookeri	-	-	-	-	-	-	-	0.06 (0.03)	-
Bromus pacificus	-	-	0.13 (0.07)	-	-	-	-	-	-
Bromus sitchensis	-	-	-	-	0.04 (0.02)	-	0.13 (NA)	-	-
Carex laeviculmis	-	-	-	-	0.03 (0.01)	-	-	-	-
Calamagrostis nutkaensis	0.88 (0.55)	-	-	-	0.03 (0.03)	-	-	0.02 (0.02)	-
Chimaphila menziesii	-	-	0.03 (0.03)	-	-	0.02 (0.02)	-	-	-
Cornus canadensis	-	-	-	-	<0.01 (<0.01)	-	-	-	-
Corallorhiza maculata	-	-	0.03 (0.03)	-	-	-	-	-	-
Driopteris expansa	-	-	0.05 (0.03)	-	0.06 (0.02)	0.02 (0.02)	0.13 (NA)	0.10 (0.06)	-
Elymus glaucus	-	-	-	-	-	-	-	0.02 (0.02)	-

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK
Chamerion angustifolium	-	-	-	-	-	-	-	0.02 (0.02)	-
Equisetum arvense	-	-	-	-	<0.01 (<0.01)	-	-	-	-
Gaultheria shallon	28.71 (12.8)	40.00 (12.33)	1.28 (0.70)	80.00 (17.5)	0.38 (0.21)	1.08 (0.61)	37.5 (NA)	72.50 (14.16)	80.00 (17.5)
Galium triflorum	-	0.04 (0.04)	-	0.03 (0.03)	0.02 (0.01)	-	0.13 (NA)	-	-
Goodyera pubescens	-	-	-	0.03 (0.03)	0.03 (0.01)	-	-	-	-
Juncus effusus	-	-	-	-	0.04 (0.03)	-	-	-	-
Lonicera involucrata	0.14 (0.02)	7.67 (2.33)	0.03 (0.03)	0.68 (0.58)	0.02 (0.01)	-	0.75 (NA)	0.21 (0.05)	1.69 (1.31)
Luzula multiflora	-	-	0.03 (0.03)	-	-	-	-	-	-
Luzula parviflora	0.05 (0.03)	-	-	-	0.05 (0.02)	0.04 (0.03)	-	0.02 (0.02)	-
Lysichiton americanus	-	-	-	-	<0.01 (<0.01)	-	-	-	-
Lycopodium selago	-	-	-	-	<0.01 (<0.01)	-	-	-	-
Maianthemum dilatatum	0.11 (0.05)	14.50 (11.50)	0.05 (0.03)	0.88 (0.54)	0.03 (0.01)	-	0.13 (NA)	0.19 (0.06)	-
Malus fusca	-	-	-	-	<0.01 (<0.01)	-	-	-	-
Menziesia feruginea	-	-	0.68 (0.58)	-	0.20 (0.05)	0.13 (<0.01)	0.13 (NA)	-	-
Moneses uniflora	0.05 (0.03)	-	0.15 (0.06)	0.03 (0.03)	0.10 (0.01)	0.13 (<0.01)	-	-	-
Neottia banksiana	0.02 (0.02)	-	0.13 (<0.01)	-	0.06 (0.02)	0.13 (<0.01)	-	0.02 (0.02)	-

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK
Neottia cordata	-	-	0.05 (0.03)	-	0.09 (0.02)	0.08 (0.03)	-	-	-
Polypodium glycyrrhiza	0.07 (0.03)	0.33 (0.21)	0.10 (0.03)	0.05 (0.03)	0.13 (0.02)	0.08 (0.03)	-	0.13 (<0.01)	0.13 (<0.01)
Polystichum munitum	14.36 (4.29)	22.5 (8.04)	0.05 (0.03)	3.30 (1.78)	0.04 (0.02)	0.08 (0.06)	0.75 (NA)	10.23 (5.74)	0.06 (0.06)
Prenanthes alata	0.04 (0.02)	-	-	-	-	-	-	0.02 (0.02)	-
Pteridium aquilinum	-	1.13 (0.94)	-	0.20 (0.14)	-	-	-	0.52 (0.50)	-
Ribes laxiflorum	-	-	-	0.60 (0.60)	0.02 (0.01)	0.02 (0.02)	0.38 (NA)	0.02 (0.02)	-
Rubus parviflorus	-	2.00 (1.00)	-	1.20 (0.73)	-	-	-	-	-
Rubus spectabilis	0.02 (0.02)	16.83 (10.53)	0.13 (<0.01)	8.28 (7.33)	0.12 (<0.01)	0.13 (<0.01)	3.00 (NA)	4.92 (3.07)	1.56 (1.44)
Sambucus racemosa	-	-	-	0.03 (0.03)	0.02 (0.01)	-	0.13 (NA)	0.02 (0.02)	-
Salix scouleriana	-	6.67 (6.67)	-	-	-	-	-	-	-
Stellaria crispa	-	-	0.05 (0.03)	-	0.03 (0.01)	0.02 (0.02)	0.13 (NA)	-	-
Symphoricarpos albus	-	1.00 (1.00)	-	0.60 (0.60)	-	-	-	0.02 (0.02)	-
Tellima grandiflora	-	-	-	-	<0.01 (<0.01)	-	-	0.02 (0.02)	-
Vaccinium ovalifolium	-	-	-	-	0.04 (0.03)	-	-	-	-
Vaccinium parvifolium	1.98 (1.39)	13.75 (11.89)	1.58 (0.59)	5.80 (1.71)	0.87 (0.31)	0.21 (0.05)	0.13 (NA)	2.92 (1.50)	0.44 (0.31)
Vicia gigantea	-	0.04 (0.04)	-	-	-	-	-	-	0.06 (0.06)

Table A.5: Mean relative abundance (%) of understory plant species in plots on Agglomerate Island (AGG, n = 7), Lost Island (LOS, n = 3), Louise Island (LOU, n = 5), Low Island (LOW, n = 5), Ramsay Island (RAM, n = 14), Reef Island (REE, n = 6), South Skedans Island (SSK, n = 1), Tar Island (TAR, n = 6), and West Skedans Island (WSK, n = 2). Species that were not present on an island are represented by a dash (-). Standard error is listed in parentheses.

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK
Adiantum pedatum	-	-	-	-	0.30 (0.30)	-	-	-	-
Agrostis exarata	-	-	-	-	0.20 (0.20)	-	-	-	-
Athyrium filix-femina	-	0.03 (0.03)	0.50 (0.50)	0.04 (0.04)	1.03 (0.48)	0.49 (0.49)	-	-	-
Blechnum spicant	0.02 (0.02)	0.29 (0.13)	1.24 (0.56)	-	5.96 (1.17)	3.18 (2.02)	0.29 (NA)	0.06 (0.03)	-
Boschniakia hookeri	-	-	-	-	-	-	-	0.06 (0.03)	-
Bromus pacificus	-	-	2.24 (1.39)	-	-	-	-	-	-
Bromus sitchensis	-	-	-	-	1.54 (0.64)	-	0.29 (NA)	-	-
Carex laeviculmis	-	-	-	-	0.83 (0.47)	-	-	-	-
Calamagrostis nutkaensis	2.19 (1.59)	-	-	-	0.89 (0.89)	-	-	0.02 (0.02)	-
Chimaphila menziesii	-	-	1.67 (1.67)	-	-	1.52 (1.52)	-	-	-
Cornus canadensis	-	-	-	-	0.17 (0.17)	-	-	-	-
Corallorhiza maculata	-	-	2.00 (2.00)	-	-	-	-	-	-
Driopteris expansa	-	-	1.00 (0.61)	-	3.13 (1.24)	1.28 (1.28)	0.29 (NA)	0.17 (0.11)	-
Elymus glaucus	-	-	-	-	-	-	-	0.02 (0.02)	-

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK
Chamerion angustifolium	-	-	-	-	-	-	-	0.02 (0.02)	-
Equisetum arvense	-	-	-	-	0.38 (0.38)	-	-	-	-
Gaultheria shallon	44.44 (13.95)	35.08 (11.50)	22.02 (10.51)	73.94 (14.95)	11.17 (4.95)	31.13 (12.88)	86.21 (NA)	75.09 (9.47)	94.61 (3.87)
Galium triflorum	-	0.05 (0.05)	-	0.02 (0.02)	0.67 (0.46)	-	0.29 (NA)	-	-
Goodyera pubescens	-	-	-	0.02 (0.02)	2.29 (1.31)	-	-	-	-
Juncus effuses	-	-	-	-	1.27 (0.94)	-	-	-	-
Lonicera involucrata	0.47 (0.13)	7.24 (3.09)	0.24 (0.24)	0.60 (0.49)	0.82 (0.56)	-	1.72 (NA)	0.23 (0.04)	2.37 (1.99)
Luzula multiflora	-	-	1.67 (1.67)	-	-	-	-	-	-
Luzula parviflora	0.13 (0.07)	-	-	-	2.04 (0.76)	2.01 (1.50)	-	0.02 (0.02)	-
Lysichiton americanus	-	-	-	-	0.17 (0.17)	-	-	-	-
Lycopodium selago	-	-	-	-	0.45 (0.45)	-	-	-	-
Maianthemum dilatatum	0.47 (0.25)	11.10 (8.33)	0.74 (0.50)	1.13 (0.83)	1.66 (1.03)	-	0.29 (NA)	0.21 (0.06)	-
Malus fusca	-	-	-	-	0.16 (0.16)	-	-	-	-
Menziesia feruginea	-	-	8.52 (5.36)	-	8.13 (1.23)	8.24 (2.08)	0.29 (NA)	-	-
Moneses uniflora	0.17 (0.10)	-	3.91 (1.69)	0.04 (0.04)	4.48 (1.06)	8.24 (2.08)	-	-	-
Neottia banksiana	0.09 (0.09)	-	4.91 (1.77)	-	2.53 (1.00)	8.24 (2.08)	-	0.04 (0.04)	-

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK
Neottia cordata	-	-	0.74 (0.5)	-	5.66 (1.50)	4.97 (1.85)	-	-	-
Polypodium glycyrrhiza	0.3 (0.14)	0.26 (0.15)	2.91 (1.43)	0.06 (0.04)	5.79 (1.41)	3.79 (1.56)	-	0.15 (0.02)	0.15 (0.03)
Polystichum munitum	44.87 (15.02)	18.36 (5.53)	0.74 (0.5)	4.12 (2.72)	1.28 (0.60)	4.34 (3.78)	1.72 (NA)	15.09 (9.15)	0.06 (0.06)
Prenanthes alata	0.09 (0.07)	-	-	-	-	-	-	0.02 (0.02)	-
Pteridium aquilinum	-	1.33 (1.22)	-	0.27 (0.21)	-	-	-	0.51 (0.47)	-
Ribes laxiflorum	-	-	-	0.52 (0.52)	0.53 (0.40)	1.28 (1.28)	0.86 (NA)	0.02 (0.02)	-
Rubus parviflorus	-	1.34 (0.68)	-	1.4 (0.91)	-	-	-	-	-
Rubus spectabilis	0.02 (0.02)	11.27 (5.79)	4.91 (1.77)	11.74 (10.91)	6.38 (1.31)	8.24 (2.08)	6.90 (NA)	5.09 (2.53)	2.24 (2.11)
Sambucus racemosa	-	-	-	0.02 (0.02)	0.53 (0.40)	-	0.29 (NA)	0.02 (0.02)	-
Salix scouleriana	-	4.03 (4.03)	-	-	-	-	-	-	-
Stellaria crispa	-	-	2.17 (1.62)	-	1.12 (0.60)	0.49 (0.49)	0.29 (NA)	-	-
Symphoricarpos albus	-	0.60 (0.60)	-	0.52 (0.52)	-	-	-	0.02 (0.02)	-
Tellima grandiflora	-	-	-	-	0.30 (0.30)	-	-	0.02 (0.02)	-
Vaccinium ovalifolium	-	-	-	-	1.12 (0.76)	-	-	-	-
Vaccinium parvifolium	6.72 (5.14)	8.99 (6.9)	37.85 (9.33)	5.58 (1.41)	27.02 (6.34)	12.58 (3.92)	0.29 (NA)	3.13 (1.38)	0.47 (0.29)
Vicia gigantea	-	0.03 (0.03)	-	-	-	-	-	-	0.09 (0.09)

Appendix B

Table B.1: Ergosterol concentrations ($\mu g \cdot g^{-1}$), OTU richness, Pielou's evenness (%) and Shannon's diversity of the fungal communities is soils from plots on Agglomerate Island (n = 7), Lost Island (n = 3), Louise Island (n = 5), Low Island (n = 5), Ramsay Island (n = 14), Reef Island (n = 6), South Skedans Island (n = 1), Tar Island (n = 6), and West Skedans Island (n = 2). Values are means ± SE.

Island	Ergosterol ($\mu g \cdot g^{-1}$)	OTU Richness	Pielou's Evenness (%)	Shannon's Diversity
Agglomerate	34.13 ± 2.06	1317 ± 29	63.09 ± 1.02	4.53 ± 0.09
Lost	53.19 ± 13.43	1677 ± 71	70.48 ± 0.47	5.23 ± 0.06
Louise	33.96 ± 4.01	1559 ± 98	67.57 ± 1.15	4.96 ± 0.13
Low	59.13 ± 11.65	1525 ± 38	67.19 ± 1.12	4.93 ± 0.10
Ramsay	33.87 ± 3.56	1544 ± 62	68.59 ± 0.48	5.03 ± 0.05
Reef	35.51 ± 8.17	1417 ± 99	65.25 ± 1.07	4.73 ± 0.12
South Skedans	54.95 ± NA	1773 ± NA	69.73 ± NA	5.22 ± NA
Tar	32.62 ± 2.83	1451 ± 91	67.24 ± 0.77	4.89 ± 0.09
West Skedans	78.86 ± 38.23	1790 ± 21	71.20 ± 0.68	5.33 ± 0.06

Table B.2: Summary of the permutational analysis of variance (PERMANOVA) models of the fungal community. The island model analyzes factors of treatment (non-invaded vs deer-invaded), region (Gwaii Haanas vs Laskeek Bay), and island nested within the region factor. The environmental gradients model analyzes continuous variables: the abundance of understory plants, pH, the depth of the organic horizons and the concentrations of ammonium (NH_4^+). Significant effects indicated in bold ($p < 0.05$).

Island Model						
Factor	Df	SS	Mean SS	F_{pseudo}	R^2	p
Treatment	1	1.3452	1.34515	6.5512	0.10485	**0.001**
Region	1	0.6922	0.69217	3.3686	0.05391	**0.001**
Treatment × Region	1	0.4671	0.46714	2.2718	0.03636	**0.007**
Island (Region)	5	2.1088	0.42177	2.0584	0.16471	**0.001**
Residuals	10	8.2136	0.20534		0.64017	
Total	48	12.8269				
Environmental Gradients Model						
Abundance	1	1.81000	1.81000	8.3877	0.141	**0.001**
pH	1	0.6367	0.63669	2.9505	0.050	**0.001**
Depth	1	0.4494	0.44943	2.0827	0.045	**0.007**
NH_4^+	1	0.4329	0.43286	2.0059	0.033	**0.014**
Residuals	44	9.4949	0.21579		0.740	
Total	48	12.8238				

Degrees of freedom (Df), Sum of squares (SS); pseudo-F value by permutation; significant effects indicated in bold ($p < 0.05$); p-values based on 999 permutations.

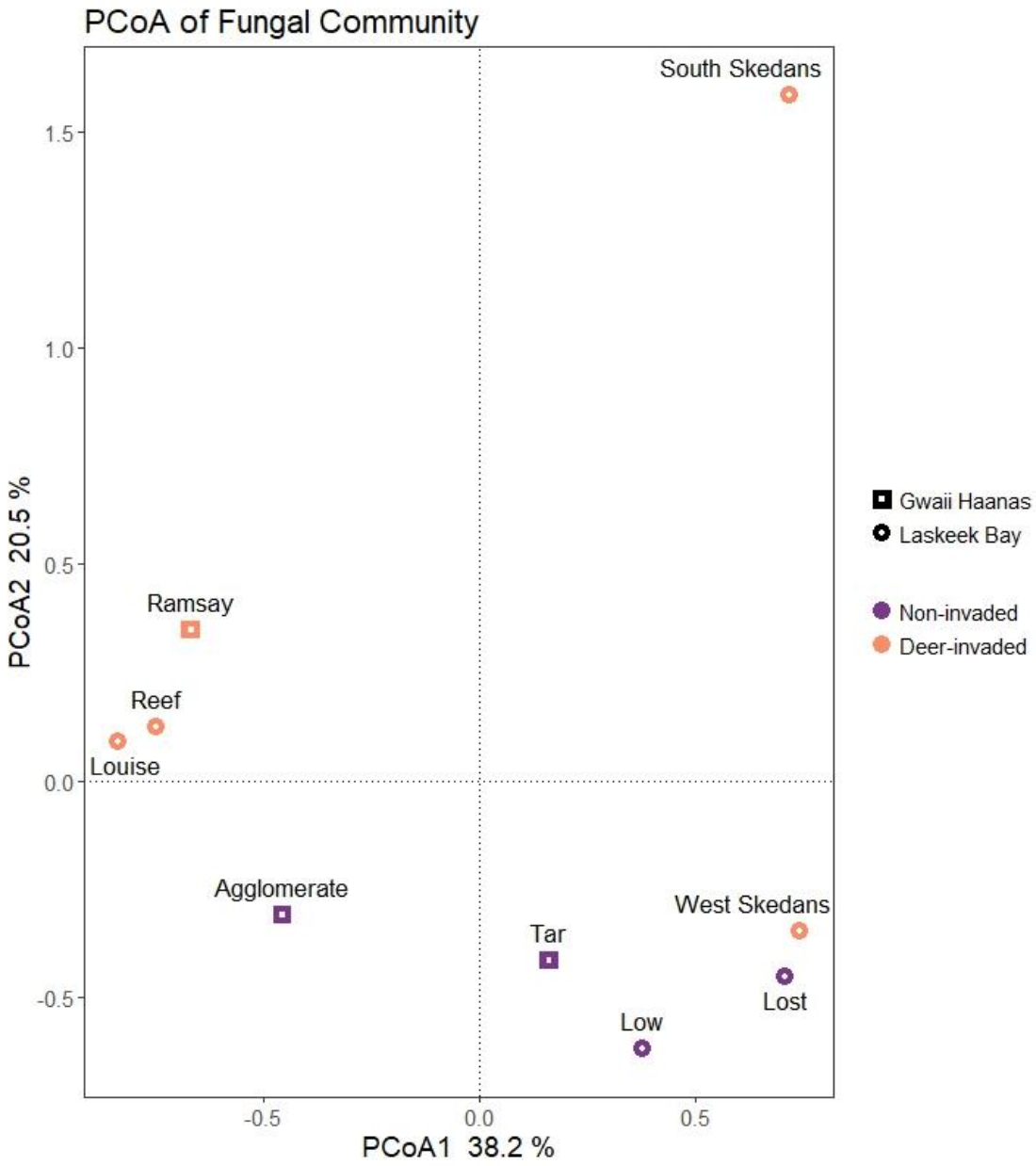

Figure B.1: Principal coordinates analysis (PCoA) of the fungal communities on individual islands using Bray-Curtis dissimilarities of rarefied reads, comparing non-invaded islands (n = 2, 2) and deer-invaded islands (n = 1, 4) in the regions of Gwaii Haanas and Laskeek Bay, respectively.

Table B.3: Mean relative abundance (%), frequency of presence (%) and indicator values of identifiable fungal species in soils from plots on non-invaded islands (n = 21), plots on deer-invaded islands (n = 28) and all plots (n = 49). Only species with significant indicator values ($p < 0.01$) are included. Species that were not present in a treatment group are represented by a dash (-). Standard error is listed in parentheses.

Species	Relative Abundance (%)			Frequency (%)			Indicator Values	
	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded
Russula favrei	2.65 (0.66)	0.28 (0.16)	1.30 (0.34)	100	43	67	0.90	0.04
Craterellus tubaeformis	0.19 (0.08)	1.97 (0.40)	1.21 (0.26)	52	93	76	0.05	0.85
Cenococcum geophilum	0.59 (0.14)	1.58 (0.22)	1.16 (0.15)	86	100	94	0.23	0.73
Rhizidium phycophilum	0.32 (0.06)	1.78 (0.35)	1.15 (0.23)	100	100	100	0.15	0.85
Mortierella pulchella	0.61 (0.08)	1.39 (0.17)	1.05 (0.11)	100	100	100	0.30	0.70
Cortinarius vanduzerensis	0.28 (0.15)	1.10 (0.28)	0.75 (0.18)	67	93	82	0.14	0.74
Elaphomyces asperulus	0.03 (0.02)	0.95 (0.30)	0.55 (0.19)	29	82	59	0.01	0.80
Russula xerampelina	0.12 (0.07)	0.59 (0.15)	0.39 (0.10)	76	100	90	0.13	0.83
Oidiodendron maius	0.53 (0.09)	0.18 (0.04)	0.33 (0.05)	100	100	100	0.75	0.25
Amanita flavoconia	0.55 (0.18)	0.06 (0.03)	0.27 (0.08)	90	71	80	0.82	0.06
Cistella acuum	0.60 (0.29)	0.01 (<0.01)	0.26 (0.13)	100	61	78	0.98	0.01
Lecanicillium flavidum	0.33 (0.07)	0.10 (0.02)	0.20 (0.04)	100	100	100	0.77	0.23
Tricholoma inamoenum	0.36 (0.20)	<0.01 (<0.01)	0.15 (0.09)	52	7	27	0.52	<0.01
Russula decolorans	0.02 (0.01)	0.25 (0.13)	0.15 (0.07)	14	61	41	0.01	0.57
Amphinema byssoides	0.22 (0.06)	0.05 (0.02)	0.12 (0.03)	86	54	67	0.71	0.09

Species	Relative Abundance (%)			Frequency (%)			Indicator Values	
	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded
Tricholoma sejunctum	<0.01 (<0.01)	0.19 (0.11)	0.11 (0.06)	10	54	35	<0.01	0.54
Cortinarius comptulus	0.15 (0.05)	0.04 (0.03)	0.09 (0.03)	81	21	47	0.64	0.05
Mortierella amoeboidea	0.12 (0.02)	0.04 (0.02)	0.08 (0.02)	100	82	90	0.74	0.21
Russula crassotunicata	<0.01 (<0.01)	0.11 (0.04)	0.07 (0.02)	19	86	57	<0.01	0.85
Ganoderma lucidum	<0.01 (<0.01)	0.09 (0.01)	0.05 (<0.01)	100	100	100	0.10	0.90
Inocybe calamistrata	-	0.09 (0.09)	0.05 (0.05)	-	46	27	-	0.46
Cortinarius brunneoalbus	<0.01 (<0.01)	0.08 (0.04)	0.05 (0.02)	19	61	43	<0.01	0.60
Thelebolus globosus	<0.01 (<0.01)	0.08 (0.07)	0.04 (0.04)	10	68	43	<0.01	0.68
Mortierella parvispora	0.08 (0.02)	0.02 (<0.01)	0.04 (0.01)	95	86	90	0.80	0.14
Chalara piceae-abietis	0.09 (0.03)	<0.01 (<0.01)	0.04 (0.02)	100	75	86	0.93	0.06
Hygrophorus discoideus	0.07 (0.06)	-	0.03 (0.03)	29	-	12	0.29	-
Verticillium leptobactrum	0.05 (0.01)	0.02 (<0.01)	0.03 (<0.01)	100	96	98	0.74	0.25
Tomentella subclavigera	0.06 (0.02)	<0.01 (<0.01)	0.03 (<0.01)	43	11	24	0.40	0.01
Chalara pseudoaffinis	0.04 (0.02)	<0.01 (<0.01)	0.02 (<0.01)	90	54	69	0.76	0.09
Cortinarius angelesianus	-	0.04 (0.01)	0.02 (<0.01)	-	46	27	-	0.46
Cortinarius casimiri	0.04 (0.02)	<0.01 (<0.01)	0.02 (<0.01)	48	11	27	0.40	0.02
Mortierella pseudozygospora	<0.01 (<0.01)	0.03 (<0.01)	0.02 (<0.01)	38	86	65	0.03	0.79

Species	Relative Abundance (%)			Frequency (%)			Indicator Values	
	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded
Cortinarius alboviolaceus	-	0.03 (0.02)	0.02 (<0.01)	-	43	24	-	0.43
Galerina fallax	<0.01 (<0.01)	0.03 (<0.01)	0.02 (<0.01)	38	82	63	0.02	0.78
Aureoboletus mirabilis	-	0.03 (0.02)	0.02 (0.01)	-	43	24	-	0.43
Hormonema macrosporum	0.03 (0.02)	<0.01 (<0.01)	0.02 (<0.01)	86	50	65	0.77	0.05
Cortinarius aurantiobasis	<0.01 (<0.01)	0.02 (<0.01)	0.02 (<0.01)	33	79	59	0.04	0.70
Cortinarius diasemospermus	<0.01 (<0.01)	0.03 (<0.01)	0.02 (<0.01)	19	54	39	0.01	0.51
Mycena strobilinoidea	0.03 (0.01)	<0.01 (<0.01)	0.01 (<0.01)	76	25	47	0.68	0.03
Heterobasidion abietinum	<0.01 (<0.01)	0.02 (<0.01)	0.01 (<0.01)	81	100	92	0.15	0.81
Pochonia cordycepisociata	0.02 (<0.01)	<0.01 (<0.01)	0.01 (<0.01)	86	61	71	0.66	0.14
Mycena pearsoniana	0.02 (0.01)	<0.01 (<0.01)	0.01 (<0.01)	71	14	39	0.62	0.02
Entoloma cetratum	<0.01 (<0.01)	0.02 (<0.01)	<0.01 (<0.01)	19	96	63	<0.01	0.94
Alatospora acuminata	0.02 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	52	21	35	0.50	0.01
Cortinarius paleifer	-	<0.01 (<0.01)	<0.01 (<0.01)	-	39	22	-	0.39
Amanita constricta	-	<0.01 (<0.01)	<0.01 (<0.01)	-	39	22	-	0.39
Cortinarius turibulosus	-	<0.01 (<0.01)	0.01 (<0.01)	-	39	22	-	0.39
Eucasphaeria capensis	0.01 (<0.01)	0.01 (<0.01)	<0.01 (<0.01)	86	18	47	0.79	<0.01
Plectania melastoma	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	62	14	35	0.60	<0.01

Species	Relative Abundance (%)			Frequency (%)			Indicator Values	
	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded
Umbelopsis autotrophica	0.01 (<0.01)	0.01 (<0.01)	<0.01 (<0.01)	38	4	18	0.38	<0.01
Mucor aligarensis	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	67	18	39	0.61	0.02
Hypholoma capnoides	<0.01 (<0.01)	<0.01 (<0.01)	0.01 (<0.01)	29	86	61	0.05	0.70
Gymnopilus punctifolius	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	24	79	55	0.02	0.73
Mortierella turficola	-	<0.01 (<0.01)	<0.01 (<0.01)	-	46	27	-	0.46
Alpova diplophloeus	-	<0.01 (<0.01)	<0.01 (<0.01)	-	50	29	-	0.50
Slooffia cresolica	-	<0.01 (<0.01)	<0.01 (<0.01)	-	36	20	-	0.36
Digitodesmium intermedium	<0.01 (<0.01)	<0.01 (<0.01)	0.01 (<0.01)	43	7	22	0.41	<0.01
Penicillium sublectaticum	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	10	46	31	0.01	0.43
Entoloma rhodocylix	-	<0.01 (<0.01)	0.01 (<0.01)	-	68	39	-	0.68
Hyphodontia aspera	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	10	61	39	<0.01	0.58
Keratinomyces ceretanicus	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	52	7	27	0.50	<0.01
Hirsutella rhossiliensis	<0.01 (<0.01)	<0.01 (<0.01)	0.01 (<0.01)	33	4	16	0.32	<0.01
Eremomyces bilateralis	<0.01 (<0.01)	-	<0.01 (<0.01)	52	-	22	0.52	-
Nadsonia fulvescens	-	<0.01 (<0.01)	<0.01 (<0.01)	-	39	22	-	0.39
Umbelopsis vinacea	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	29	4	14	0.27	<0.01
Hyphodontiella multiseptata	<0.01 (<0.01)	-	<0.01 (<0.01)	24	-	10	0.24	-

Species	Relative Abundance (%)			Frequency (%)			Indicator Values	
	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded	All	Non-Invaded	Deer-Invaded
Metacordyceps chlamydosporia	<0.01 (<0.01)	-	<0.01 (<0.01)	29	-	12	0.29	-

Table B.4: Mean relative abundance (%) of identifiable fungal species in soils from plots on Agglomerate Island (AGG, n = 7), Lost Island (LOS, n = 3), Louise Island (LOU, n = 5), Low Island (LOW, n = 5), Ramsay Island (RAM, n = 14), Reef Island (REE, n = 6), South Skedans Island (SSK, n = 1), Tar Island (TAR, n = 6), and West Skedans Island (WSK, n = 2). The mean relative abundance (%) and frequency of presence (%) of identifiable fungal species among all plots (n = 49) are also reported. Only species with relative abundances greater than 0.1% are included. Standard error is listed in parentheses.

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK	Relative Abundance (%)	Frequency (%)
Meliniomyces bicolor	3.20 (0.73)	0.47 (0.12)	3.96 (1.25)	1.79 (0.79)	1.17 (0.19)	3.05 (1.00)	0.21 (NA)	1.43 (0.47)	0.45 (<0.01)	1.98 (0.27)	100
Russula fragilis	2.28 (1.34)	0.06 (0.06)	4.13 (0.62)	1.81 (0.97)	2.09 (0.50)	2.85 (1.19)	<0.01 (NA)	0.48 (0.25)	<0.01 (<0.01)	1.94 (0.33)	88
Russula vinosa	4.09 (1.54)	0.32 (0.3)	2.31 (0.76)	1.68 (0.99)	1.33 (0.33)	1.51 (1.04)	<0.01 (NA)	2.44 (0.97)	<0.01 (<0.01)	1.88 (0.34)	90
Russula favrei	1.77 (0.75)	0.39 (0.13)	0.59 (0.59)	3.93 (2.26)	0.27 (0.24)	0.04 (0.04)	0.03 (NA)	3.76 (0.84)	0.44 (0.40)	1.30 (0.34)	22
Craterellus tubaeformis	0.23 (0.23)	<0.01 (<0.01)	2.13 (0.94)	0.20 (0.12)	1.96 (0.45)	2.87 (1.25)	<0.01 (NA)	0.23 (0.11)	<0.01 (<0.01)	1.21 (0.26)	47
Cenococcum geophilum	1.12 (0.23)	0.21 (0.14)	2.28 (0.63)	<0.01 (<0.01)	1.51 (0.18)	1.94 (0.65)	<0.01 (NA)	0.65 (0.22)	0.07 (0.07)	1.16 (0.15)	90
Rhizidium phycophilum	0.26 (0.03)	0.31 (0.10)	4.38 (1.01)	0.61 (0.18)	0.71 (0.13)	2.66 (0.57)	0.58 (NA)	0.14 (0.02)	0.65 (0.46)	1.15 (0.23)	47
Mortierella pulchella	0.83 (0.17)	0.24 (0.08)	1.37 (0.18)	0.59 (0.10)	1.47 (0.21)	1.82 (0.46)	0.24 (NA)	0.55 (0.14)	0.15 (0.03)	1.05 (0.11)	20
Oidiodendron pilicola	1.00 (0.16)	0.57 (0.12)	1.04 (0.44)	2.25 (0.87)	0.71 (0.36)	1.14 (0.58)	0.21 (NA)	0.79 (0.26)	2.17 (1.29)	1.04 (0.17)	100
Russula sapinea	1.39 (0.36)	0.04 (0.04)	0.89 (0.51)	0.32 (0.20)	0.35 (0.14)	0.41 (0.18)	<0.01 (NA)	2.27 (0.72)	<0.01 (<0.01)	0.75 (0.15)	90
Cortinarius vanduzerensis	0.76 (0.40)	<0.01 (<0.01)	2.18 (1.29)	<0.01 (<0.01)	1.24 (0.26)	0.40 (0.15)	<0.01 (NA)	0.09 (0.05)	<0.01 (<0.01)	0.75 (0.18)	90
Solicoccozyma terricola	0.24 (0.10)	0.73 (0.41)	1.00 (0.45)	0.23 (0.21)	0.53 (0.19)	0.51 (0.22)	3.19 (NA)	0.22 (0.10)	1.39 (0.63)	0.57 (0.11)	100
Elaphomyces asperulus	0.08 (0.07)	<0.01 (<0.01)	0.22 (0.12)	<0.01 (<0.01)	1.80 (0.52)	0.04 (0.04)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.55 (0.19)	24
Russula veternosa	0.33 (0.3)	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)	0.09 (0.08)	3.78 (2.18)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.54 (0.31)	49

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK	Relative Abundance (%)	Frequency (%)
Cortinarius pseudocandelaris	<0.01 (<0.01)	0.22 (0.20)	0.12 (0.12)	0.11 (0.09)	0.66 (0.62)	<0.01 (<0.01)	<0.01 (NA)	1.77 (1.63)	1.53 (1.15)	0.50 (0.27)	49
Russula emetica	1.34 (0.87)	0.08 (0.07)	0.36 (0.09)	0.23 (0.20)	0.64 (0.20)	0.22 (0.13)	<0.01 (NA)	0.13 (0.06)	<0.01 (<0.01)	0.48 (0.14)	90
Cortinarius acutus	0.31 (0.11)	0.04 (0.03)	0.05 (0.03)	0.03 (0.01)	1.09 (0.85)	<0.01 (<0.01)	0.04 (NA)	0.56 (0.48)	<0.01 (<0.01)	0.44 (0.25)	92
Russula xerampelina	0.31 (0.20)	<0.01 (<0.01)	1.11 (0.53)	0.09 (0.06)	0.47 (0.13)	0.74 (0.44)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.39 (0.10)	27
Sphaerographium nyssicola	2.04 (2.02)	<0.01 (<0.01)	0.68 (0.64)	<0.01 (<0.01)	<0.01 (<0.01)	0.19 (0.11)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.39 (0.30)	45
Penicillium penicillioides	0.62 (0.15)	0.53 (0.12)	0.18 (0.04)	0.25 (0.02)	0.33 (0.10)	0.20 (0.06)	0.32 (NA)	0.55 (0.12)	0.37 (<0.01)	0.37 (0.04)	100
Pseudotomentella humicola	0.39 (0.15)	1.20 (0.61)	0.23 (0.08)	0.24 (0.07)	0.25 (0.09)	0.33 (0.12)	<0.01 (NA)	0.15 (0.11)	0.85 (0.02)	0.34 (0.06)	98
Inocybe olympiana	<0.01 (<0.01)	1.96 (1.46)	<0.01 (<0.01)	1.33 (1.33)	0.14 (0.11)	<0.01 (<0.01)	<0.01 (NA)	0.38 (0.27)	<0.01 (<0.01)	0.34 (0.17)	45
Oidiodendron maius	0.65 (0.15)	0.17 (0.06)	0.11 (0.03)	0.59 (0.19)	0.20 (0.09)	0.18 (0.04)	0.05 (NA)	0.54 (0.22)	0.26 (0.03)	0.33 (0.05)	39
Amanita flavoconia	0.94 (0.37)	0.99 (0.76)	0.19 (0.14)	0.09 (0.07)	0.04 (0.02)	<0.01 (<0.01)	<0.01 (NA)	0.27 (0.13)	<0.01 (<0.01)	0.27 (0.08)	100
Russula brevipes	<0.01 (<0.01)	0.19 (0.19)	<0.01 (<0.01)	0.02 (0.02)	0.08 (0.05)	1.14 (1.13)	<0.01 (NA)	0.72 (0.27)	0.07 (0.07)	0.27 (0.14)	45
Cistella acuum	0.02 (<0.01)	1.56 (1.36)	<0.01 (<0.01)	1.44 (0.86)	<0.01 (<0.01)	0.01 (<0.01)	0.02 (NA)	0.08 (0.05)	0.10 (0.08)	0.26 (0.13)	35
Cortinarius laetus	0.21 (0.20)	<0.01 (<0.01)	0.13 (0.05)	<0.01 (<0.01)	0.67 (0.37)	0.01 (<0.01)	<0.01 (NA)	0.11 (0.1)	<0.01 (<0.01)	0.25 (0.11)	67
Russula sanguinea	<0.01 (<0.01)	0.22 (0.12)	<0.01 (<0.01)	0.32 (0.19)	0.06 (0.05)	<0.01 (<0.01)	<0.01 (NA)	1.29 (1.09)	0.51 (0.42)	0.24 (0.14)	33
Clavulina cinerea	0.55 (0.46)	<0.01 (<0.01)	0.10 (0.06)	0.31 (0.27)	0.30 (0.10)	0.22 (0.12)	<0.01 (NA)	0.05 (0.02)	<0.01 (<0.01)	0.24 (0.08)	82
Mortierella humilis	0.18 (0.07)	0.58 (0.26)	0.06 (0.02)	0.37 (0.24)	0.16 (0.11)	0.20 (0.13)	1.30 (NA)	0.12 (0.05)	0.36 (0.14)	0.23 (0.05)	100
Meliniomyces variabilis	0.15 (0.04)	0.09 (0.02)	0.47 (0.09)	0.39 (0.05)	0.13 (0.02)	0.26 (0.12)	0.18 (NA)	0.11 (0.04)	0.07 (0.04)	0.20 (0.03)	100

Species	AGG	LOS	LOU	LOW	RAM	REE	SSK	TAR	WSK	Relative Abundance (%)	Frequency (%)
Lecanicillium flavidum	0.60 (0.12)	0.09 (0.04)	0.05 (<0.01)	0.05 (0.01)	0.16 (0.02)	0.04 (<0.01)	0.01 (NA)	0.39 (0.11)	0.03 (<0.01)	0.20 (0.04)	71
Inocybe nitidiuscula	<0.01 (<0.01)	<0.01 (<0.01)	0.05 (0.04)	<0.01 (<0.01)	0.59 (0.43)	<0.01 (<0.01)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.17 (0.13)	31
Oidiodendron echinulatum	0.44 (0.18)	0.53 (0.45)	0.06 (0.04)	0.06 (0.04)	0.07 (0.02)	0.04 (<0.01)	0.89 (NA)	0.14 (0.11)	0.04 (0.03)	0.17 (0.05)	96
Tricholoma inamoenum	0.03 (0.03)	<0.01 (<0.01)	<0.01 (<0.01)	1.02 (0.78)	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (NA)	0.38 (0.17)	<0.01 (<0.01)	0.15 (0.09)	14
Russula aeruginea	<0.01 (<0.01)	<0.01 (<0.01)	0.27 (0.26)	<0.01 (<0.01)	0.14 (0.06)	0.34 (0.30)	<0.01 (NA)	0.37 (0.24)	<0.01 (<0.01)	0.15 (0.06)	43
Elaphomyces muricatus	0.23 (0.22)	<0.01 (<0.01)	0.17 (0.10)	<0.01 (<0.01)	0.31 (0.12)	0.13 (0.10)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.15 (0.05)	57
Lactarius pseudomucidus	0.11 (0.06)	<0.01 (<0.01)	0.23 (0.11)	0.11 (0.08)	0.26 (0.07)	0.06 (0.03)	0.14 (NA)	0.07 (0.05)	0.08 (<0.01)	0.15 (0.03)	86
Russula decolorans	0.04 (0.04)	<0.01 (<0.01)	0.19 (0.19)	<0.01 (<0.01)	0.24 (0.17)	0.44 (0.43)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.15 (0.07)	35
Cortinarius pseudoduracinus	0.28 (0.17)	0.23 (0.17)	0.02 (<0.01)	0.05 (0.04)	0.12 (0.06)	<0.01 (<0.01)	0.18 (NA)	0.23 (0.16)	0.38 (0.04)	0.14 (0.04)	90
Clavulina castaneipes	0.01 (0.01)	<0.01 (<0.01)	0.12 (0.06)	<0.01 (<0.01)	0.36 (0.13)	0.05 (0.04)	<0.01 (NA)	0.14 (0.06)	<0.01 (<0.01)	0.14 (0.04)	59
Mycena amicta	0.09 (0.07)	0.04 (0.02)	0.03 (0.02)	0.63 (0.41)	0.11 (0.07)	0.01 (0.01)	<0.01 (NA)	0.10 (0.02)	0.06 (0.04)	0.13 (0.05)	84
Amphinema byssoides	0.08 (0.04)	0.46 (0.29)	0.06 (0.05)	0.40 (0.12)	0.06 (0.04)	0.01 (<0.01)	<0.01 (NA)	0.12 (0.05)	0.06 (0.05)	0.12 (0.03)	82
Tricholoma sejunctum	<0.01 (<0.01)	<0.01 (<0.01)	0.02 (0.02)	<0.01 (<0.01)	0.38 (0.21)	<0.01 (<0.01)	<0.01 (NA)	<0.01 (<0.01)	<0.01 (<0.01)	0.11 (0.06)	12

Appendix C

Table C.1: Summary of the analysis of variance (ANOVA) model of chlorophyll fluorescence (F_v/F_m) in *Thuja plicata*, using factors of treatment (non-invaded vs deer-invaded), region (Gwaii Haanas) and soil (living vs sterilized). Data were analyzed using a squared power transformation. Significant effects indicated in bold ($p < 0.05$).

Factor	Df	SS	Mean SS	F	p
Treatment	1	0.0567	0.05674	9.531	**0.00283**
Region	1	0.0028	0.00285	0.478	0.49129
Soil	1	0.0576	0.0576	9.677	**0.00264**
Treatment × Region	1	0.018	0.01796	3.017	0.08649
Treatment × Soil	1	0.0127	0.01273	2.139	0.14777
Region × Soil	1	<0.0001	0.00003	0.005	0.9449
Treatment × Region × Soil	1	0.0091	0.00911	1.531	0.21983
Residuals	75	0.4465	0.00595		

Degrees of freedom (Df), Sum of squares (SS)