Indirect impacts of a non-native ungulate browser on soil ecosystem function is variable across soil horizons in the boreal forests of Newfoundland, Canada

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A thesis submitted to the School of Graduate Studies

in partial fulfilment of the requirements for the degree of

Master of Science

Department of Biology

Memorial University of Newfoundland

October 2021

St. John's Newfoundland and Labrador

ABSTRACT

Herbivores are key players in ecosystem function and connect nutrient cycling across animal and plant trophic levels. Herbivore impacts on ecosystems can be direct or indirect and it is necessary to study both paths to understand herbivore impacts on above-ground and below-ground ecosystem functioning. We conducted an experiment to test the hypothesis that non-native moose on the island of Newfoundland have negative impacts on plant communities, nutrient cycling, soil composition, and soil organism communities. We collected data on plant and invertebrate communities, climate, and soils in 11 paired exclosure-control plots in eastern and central Newfoundland that provide insight into 22-25 years of moose herbivory. Structural equations models revealed that moose had direct negative impacts on palatable tree height and abundance and an indirect negative impact on soil microbial C:N ratios. We found that moose had a direct negative impact on soil horizon depth and plant material and a positive impact on soil temperature and moisture, particularly in the first soil horizon. We detected no significant impact of moose on soil total C and N, net nitrogen mineralization, or macro-invertebrate communities. Overall, we unearthed evidence of indirect cascading impacts of moose on soil functions although these impacts are relatively weak.

DEDICATION

I dedicate my master's thesis to my family, Greg, Holly, and Megan Swain, who instilled me with a curiosity and love for nature, and whose tireless encouragement made the distance feel small and kept me going through every obstacle.

ACKNOWLEDGEMENTS

First and foremost, I want to extend my most sincere gratitude and appreciation to my supervisor, Shawn Leroux, for his never-ending support, encouragement, guidance, and expertise throughout my thesis. I want to thank Carissa Brown and Susan Ziegler for their support and advice throughout the course of my thesis. I want to thank Terra Nova National Park (TNNP) and Laura Siegwart Collier for their knowledge and allowing me to use some of their exclosure sites. I want to thank Rob Buchkowski for his constant expertise and advice throughout the course of my thesis. I would also like to thank my amazing field and lab assistants, Johanna Bosh, Tim Hong, Adam Meyer, and Jake Prosser. Their positive attitude made even the hardest days enjoyable, and I am so grateful for all their hard work that made collecting the data for this project possible.

I want to thank Luise Hermanutz (MUN) and Brian McLaren (Lakehead University) for their critical role in designing and supporting the creation of the moose exclosure sites. I also would like to thank Randy Power, John Gosse and Kirby Tulk (formerly of Parks Canada), Shawn Avery (NL Provincial Forestry Department), Janet Feltham (Parks Canada), and the students and technicians with the TNNP Resource Conservation Section, and Memorial University for their support over the many years of data collection.

I would also like to thank the Ecosystem Ecology Lab, especially Kelly MacDonald. Their suggestions inspired me and shaped my project, and their humor which encouraged me throughout ever twist and turn. Lastly, I would like to thank the amazing friends I made while living in Newfoundland for listening to me when I was stressed, going on long adventures to explore Newfoundland, and making this experience truly one I will never forget.

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CHAPTER 1: General Introduction

1.1. Background to ecological nutrient cycling

Terrestrial ecosystems are reliant on the quantity of available nutrients in the soil, to maintain ecosystem function. The molar ratios of elements (specifically C, N, and P) in organisms are typically thought to be relatively constant (Redfield 1934; 1958). However, their abundance within an organism, particularly autotrophs, may vary due to their availability in the environment (Elser et al. 2000). Changes in nutrient availability, especially in plants, can impact food web dynamics and stability, and ecosystem function (Elser et al. 2000). Ecosystem function is quantified in the abiotic and biotic processes and interactions that make up and maintain the ecosystem. Nutrients travel along ecosystem trophic levels, typically from soils or sediments to above-ground food webs and nutrient pools (e.g., trees, animals; Moore et al. 2004; Wardle et al. 2004; Bardgett and Wardle 2010). Soils and sediments are the primary store of available nutrients for plants to uptake and create above-ground biomass that is consumed by herbivores (Wardle et al. 2004; Peschel et al. 2015). As animals and plants senesce, they become an organic food source for detritivores and decomposers which ultimately transform organic nutrients to inorganic forms in soils and sediments (see review of nutrient cycling in Lavelle et al. 2005). Interactions among organisms and components of their environment regulate overall nutrient return and availability (Redfield 1934; 1958; Elser et al. 2000; Loreau 2010; DeAngelis 2012). Productivity and ecosystem type also impact the rate and pathways available nutrients travel. For example, the higher primary production of tropical forest ecosystems is more favorable for microbial communities, which promote faster N mineralization rates resulting in lower nitrogen limitation when compared to boreal forest ecosystems with lower primary productivity (Foster and Bhatti 2005). Consistent uptake and return of nutrients to the soil maintains ecosystem functioning along above-ground and below-ground ecosystem aspects.

Human activities, such as agriculture and forest harvesting, alter an ecosystem's nutrient cycling through removal of plant biomass (Mälkönen 1976; Lassaletta et al. 2014). Agriculture is essential for human survival through the rapid production of food. However, large-scale growth and harvesting of plants strip nutrients from the soil causing a deficit in nutrients that are essential for plant growth (Foster and Bhatti 2005). Similarly, trees require substantial quantities of nutrients for growth, and harvesting of full or partial trees removes nutrients from an ecosystem’s nutrient cycle in the form of plant biomass and redistributes that biomass elsewhere (Blanco et al. 2005). For example, first-generation whole tree harvesting in North Wales was found to decrease basial diameter of second-generation trees by 10% due to the removal of mass quantities of N, P and K from the ecosystem (Walmsley et al. 2009). Thus, human removal of plant biomass disrupts local nutrient cycling with the potential for impacts at regional or landscape extents.

The biogeochemical cycles of elements are coupled and are dependent on each other, so a disruption in the cycling of one element alters the cycles of many other elements (Schlesinger et al. 2011). Nitrogen is vital for plant growth and protein synthesis (Sterner and Elser 2002) and can often be a limiting nutrient (Foster and Bhatti 2005). Nitrogen in the soil occurs in inorganic (e.g., ammonia (NH_4), nitrite (NO_2), and nitrate (NO_3)) and organic (e.g., nitrogen that originated from living material) forms. Although plants can access both forms of nitrogen (see review in Schimel and Bennett 2004), inorganic nitrogen is the most accessible form for plants to uptake. Organic nitrogen must undergo mineralization to be transformed into the more accessible inorganic nitrogen (but see Schimel and Bennett 2004). For example, a study observing the thawing of permafrost in sub arctic peatlands found plant biomass production increased when the rate of nitrogen mineralization increased (Keuper et al. 2017). Nitrogen mineralization is one of the most important stages of the nitrogen cycle. Aspects such as climate warming (Keuper et al. 2017), increases in soil temperature and moisture (Knoepp and Swank 2002), and decreases in plant species richness (Meier and Bowman 2008) cause an increase in the rate of nitrogen mineralization. Arguably the most important factor in influencing the rate of nitrogen mineralization is the microbial community that mediates this stage below-ground (Li et al. 2019).

1.2. Soil as an essential component of ecosystem function

When walking in the woods or park, one readily notices the visual aspects of the area, such as plants and animals. While these aspects are crucial to terrestrial ecosystem function, the soil below your feet is the foundation of ecosystem functioning, yet due to its inconspicuous nature, it is easy to overlook. Like an above-ground ecosystem, soil composition is not homogeneous and varies at local, regional, and global extents. An ecosystem's soil is comprised of horizons with mineral and organic material composition that have unique characteristics based on surrounding abiotic and biotic factors (Soil Classification Working Group 1998; Wardle et al. 2004; Bardgett and Wardle 2010). There are generally five main soil horizons (O, A, E, B, and C) each with a different general composition and role. Organic horizons (O horizon; i.e., first soil horizon) tend to have higher relative C content, while mineral horizons (A,E,B, and C horizon; i.e., second and consecutive soil horizon) have lower relative C content (Soil Classification Working Group 1998; Pennock et al. 2015). However, an ecosystem's soil is not always comprised of all five horizons. The uppermost soil horizon in an ecosystem is the organic horizon which has the highest rate of nutrient turnover and the highest microbial diversity of any horizon (Fierer, Schimel, and Holden 2003). For example, Fierer, Schimel, and Holden (2003) used soil trenches to expose soil to a depth of 2m and found that deeper soils had an increase in two phospholipid fatty acid ratios (cyclopropyl/ monoenoic precursors, and total saturated/total monounsaturated) that are indicative of microbial nutritional stress and suggest that these microbial communities are carbon limited at depth. Soil composition and conditions have impacts on both above-ground and below-ground processes. The relationship between soils, plants, microbes, and animals is not unidirectional but rather subject to feedbacks which create the cycling of organic materials into inorganic forms. These interactions provide context on an ecosystem's interconnected nature that helps it support ecosystem functions and services.

Plants are rooted in the soil for stability and access to some of the 25 elements that are required for life and plant growth (Sterner and Elser 2002; Keuper et al. 2017). A decline in nutrient availability within the soil can decrease above-ground productivity, but the recycling of plant material (e.g., plant leaf senescence) is essential for nutrient cycling and to shade and protect soils. A reduction in the return of plant biomass to the soil (e.g., via forest harvesting) can reduces resources for microbial communities and lowers the rate of nutrient cycling (Mikola and Setälä 1998; Siemann et al. 1998; Knops et al. 1999). In addition, the opening of canopies created by disturbances such as forest fire, windthrow or insect outbreaks, can create hotter and wetter soil conditions that impact the soil organisms that can live within it (Carreiro and Koske 1992) increasing certain aspects of nutrient cycling, such as nitrogen mineralization (Knoepp and Swank 2002). For example, Matson and Vitousek (1981) found that clear-cutting in Hoosier National Forest, Indiana, USA, decreased uptake of nitrogen by plants which increased nitrifying bacteria population and the rate of nitrogen mineralization. Overall, changes in soil conditions are often inextricably linked to changes in the above-ground plant community.

Many insects and microbial communities inhabit the soil and within these communities, there is a broad diversity of organisms that perform specific functions. Soil organism diversity is composed of a multitude of insect and microbial communities that have a wide range of functions that include aspects such as nutrient turnover, degradation of natural polymers, and consumption of plants and other organisms (Meyer 1994; Iancu, Sahlean, and Purcarea 2016; Li et al. 2019). For example, some insect and bacterial communities, like blow fly and protobacteria, are essential for carrion decomposition (Pechal et al. 2013; Iancu, Sahlean, and Purcarea 2016) and some fungal communities, like mycorrhizal fungi, form a symbiosis with plants through their roots to exchange nutrients for carbon (Bennett 2010). For example, Hestrin et al. 2019 found that plants with mycorrhizal connections uptake two times more nitrogen from organic matter than plants grown with no mycorrhizal connections. Bacteria are the most active and common microbial community within the soil compared to fungi that are less active but often have a higher biomass within the soil due to their larger size (Meyer 1994). Ecosystem type has an impact on the ratio of fungi to bacteria present, with higher ratios being recorded in forests when compared to grasslands (Fierer et al. 2009). Insect and microbial communities are also sensitive to changes in above-ground and below-ground conditions. Above-ground abiotic disturbances such as fires and forestry, significantly decrease insect abundance (Knops et al. 1999) and soil microbial biomass (Holden and Treseder 2013). Like other ecosystem components, elevated soil organism community composition and diversity is typically found at lower latitudes due to increase in plant productivity and more favorable soil biotic conditions (Lu et al. 2018).

1.3. Above-ground and below-ground connections: animals, plants, and microbes

Ecosystems are comprised of multitudes of shared abiotic and biotic interactions. The direct and indirect interactions between abiotic conditions and biotic diversity of an ecosystem are crucial in maintaining ecosystem succession and nutrient cycling (Holden and Treseder 2013; Barrio et al. 2016; Andriuzzi and Wall 2017; Ellis and Leroux 2017). A direct impact is when an organism interacts specifically with another part of an ecosystem (biotic or abiotic), while an indirect impact occurs when an organism interacts with a part of an ecosystem (biotic or abiotic) via a shared biotic or abiotic component. Each interaction creates a chain of both direct and indirect impacts that can alter ecosystem functions. Abiotic factors, such as climate warming, can impact the persistence of biotic components of ecosystems (Holden and Treseder 2013; Keuper et al. 2017) while biotic factors, such as a removal or introduction of species, can in turn, impact the food web and nutrient cycling within ecosystems (Schmitz and Leroux 2020). Consequently, we must consider the feedbacks between abiotic and biotic factors in order to predict changes to ecosystem dynamics.

Inclusion of animal-specific abiotic and biotic interactions and impacts is not often represented in most current global change modeling (Dangal et al. 2017; Schmitz and Leroux 2020). However, there is growing evidence that animals impact many facets of their ecosystem, including nutrient cycling (see recent reviews in Schmitz et al. 2014; Bernes et al. 2018; Forbes et al. 2019; Schmitz and Leroux 2020). For example, Subalusky et al. (2017) demonstrated that the mass drownings of wildebeest during their annual migration in the Serengeti had both short (e.g., soft tissue decomposition) and long (e.g., bone decomposition) term influences on nutrient cycling and food webs from carcass decomposition. Zoogeochemistry (sensu Schmitz et al. 2018) is the emerging study of how animals directly and indirectly impact nutrient cycling.

Animals can move throughout ecosystems and such movement makes it challenging to pinpoint the extent of animal impacts. Mammalian herbivory is an important disturbance type in many ecosystems and as such mammalian herbivores can act as an agent of change linking above-ground and below-ground systems. Mammalian herbivores most directly impact their ecosystem through trampling and the consumption and defecation of plant material. Trampling compacts soil and decreases vegetation cover and soil microbial communities (Tuomi et al. 2021). The process of herbivory introduces nutrients into animal food webs while removing plant nutrients that may otherwise senesce and return to the soil (Wardle et al. 2001). In contrast to consumption and trampling, the defecation of digested plant material creates an influx of nutrients to the soil (Leslie et al. 1989) that can promote plant growth on a local scale (Steinauer and Collins 1995). Herbivores are linked to their ecosystems nutrient cycling through the removal and reallocation of nutrients through their many mechanisms. Over time removal of plant material reduces plant litter return to the soil (Pastor et al. 1988; Dewey et al. 1993; Hobbs 1996; Liu et al. 2015; Peschel et al. 2015; Ellis and Leroux 2017) which reduces carbon and nitrogen availability within the soil (Dewey et al. 1993; Frank 2008; Gass and Binkley 2011). Herbivores also interact with other animals within their ecosystem and these interactions are essential to understanding zoogeochemistry.

Predator-prey relationships are a key component in an ecosystem’s food web and mediate trophic cascades (see reviews in Shurin et al. 2002; Borer et al. 2005). There are many predator assemblages within an ecosystem that have different interactions and prey preferences based on trophic composition (Finke and Denno 2005). Herbivores are often the primary prey to the predators in many ecosystems and therefore predator effects are mediated through herbivores (Schmitz 2010). Human exploitation of large animals, particularly top predators (Estes et al.

2011), through harvesting and introductions indirectly changes nutrient cycling within an ecosystem (Wardle et al. 2011; Schmitz et al. 2018). The absence of predators reduces pressure on herbivores allowing their abundance and impacts to surge (Beschta and Ripple 2009). For example, Ellis and Leroux (2017) observed strong impacts of abundant moose on decreasing the height of palatable plants in a boreal ecosystem without natural predators. Decreases in soil nutrient availability then decreases the nutrient content of plant material, requiring large herbivores to consume higher quantities of plant biomass to achieve their nutrient requirements (Olff, Ritchie, and Prins 2002). The abiotic and biotic interactions of animals reviewed above create a complex web of interactions that tie into above-ground and below-ground functions.

1.4. Moose as a non-native agent of change on the island of Newfoundland, Canada

Newfoundland is a Canadian island off the east coast of North America. Newfoundland's ecosystem is comprised of southern, middle, and northern boreal zones (Bell 2002). Only half of the twenty-six mammalian species that inhabit the island are native (Strong and Leroux 2014). Woodland caribou (*Rangifer tarandus*) are the only native large herbivore; however, they are not the most prevalent ungulate. Originally two moose were brought to Newfoundland in 1878 but it wasn't until four moose were brought from New Brunswick in 1904 that the moose population was established (Pimlott 1953). The moose population increased rapidly as there was little competition with other large herbivores and the main predator, wolves (*Canus lupus*) was extirpated on the island ~ 1930 (McLaren et al. 2004). Moose densities have exceeded 4 moose/km^2 (>1,000kg/km^2) in many ecoregions around the island (McLaren et al. 2004) which is some of the highest densities of moose across their circumboreal distribution. The rate at which moose have flourished on Newfoundland has led to drastic changes to Newfoundland’s forest ecosystems.

The interconnected nature of ecosystems makes it crucial to understand the extent of impacts caused by non-native species. Moose are large ungulate browsers who prefer nitrogen rich woody shrubs and shoots. Classical studies of moose on Isle Royale, USA (Pastor et al. 1988; Pastor and Naiman 1992), and Sweden (Danell, Edenius, and Lundberg 1991; Persson et al. 2005) show moose significantly impact their native ecosystems. For example, long term exclosures experiments of moose on Isle Royale, USA, have found that moose shift boreal forest ecosystems to open forests and moose savannas (McInnes et al. 1992; Rotter and Rebertus 2015). The non-native status and abundance of moose on the island of Newfoundland makes Newfoundland an ideal place to understand moose impacts. By foraging on preferred, low C:N ratio foliage over time moose can cause a shift in plant community to taller and more abundant non-preferred plants with foliage that has a relatively high C:N ratio. A larger proportion of a plant’s biomass is located below-ground in low productive ecosystems (e.g., boreal forests, arctic tundra) making a shift in plant community composition due to herbivory slower than higher productive ecosystems (Milchunas and Lauenroth 1993; Osem, Perevolotsky, and Kigel 2002). Low productive ecosystems may be more resilient to a shift in plant community composition; however, the lower nutrient availability and primary productivity of boreal forests strengthen the effects of large herbivores on above-ground community height and resilience (Pastor and Naiman 1992). The apparent nature of moose effects above-ground are known; however, it is unclear the extent to which these above-ground effects trickle below-ground impacting soil composition and conditions.

Previous studies of moose impacts in Newfoundland (McLaren et al. 2004; McLaren, Taylor, and Luke 2009; Gosse et al. 2011; Ellis and Leroux 2017) have primarily focused on above-ground changes. Recently, the importance of looking beyond visual, above-ground changes to understand the breadth of large ungulate browsers impacts on ecosystems has emerged. Plant litter that is easily decomposable (i.e., low C:N ratio) is often the most nutrient rich and is most heavily consumed by moose (Pastor and Naiman 1992). Decrease in plant litter quality returning to the soil impacts nutrient return and creation of soil organic material. Many studies have linked moose directly to changing soil nutrient availability (Pastor et al. 1988; Dewey et al. 1993), composition (Ellis and Leroux 2017; Kolstad et al. 2018), and microbial communities (Pastor et al. 1988). However, it is important to understand that in most cases moose are not directly interacting with below-ground aspects. It is also important to include disturbance history of an ecosystem (e.g., insect outbreak, forest disturbance) when studying the extent of ungulate impacts on forests (MacSween, Leroux, and Oakes 2019; Leroux, Wiersma, and Vander Wal 2020). Moose effects are mediated by disturbance type and intensity. Over a five year period, Nuttle et al. (2013) found that the positive impact of fire and canopy gaps on promoting understory plant community composition was no longer apparent due to herbivore browsing. Redirecting our way of identifying moose impacts below-ground must include above-ground below-ground connections.

1.5. Thesis overview

The aim of my thesis is to explore and understand moose impacts on boreal ecosystems both above-ground and below-ground. The island of Newfoundland provides a unique opportunity to study the vast impacts of non-native herbivores in the absence of predation. By comprehensively looking at above-ground and below-ground components, we aimed to understand moose impacts through both direct and indirect pathways. In Chapter 2, we report on a field study using eleven 22-25 year old paired exclosure (i.e., fenced) and control plots in eastern Newfoundland, Canada. In this field study we tested the general hypothesis that moose have both direct and indirect impacts on above-ground and below-ground functioning (see Chapter 2 for specific predictions). We measured above-ground components such as plant community height, species, and cover. We also measured below-ground components such as soil horizon depth, temperature, moisture, percent plant material, and organismal community composition. We found that moose had very prevalent impacts on above-ground plant communities and cascading impacts on microbial C:N ratio in the first soil horizon (i.e., organic horizon), but we observed no cascading effects on the second soil horizon. We conclude that although moose impacts in Newfoundland can reach below-ground and affect soil composition and organismal communities in surficial soils, these effects are still weak.

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CHAPTER 2: Indirect impacts of a non-native ungulate browser on soil ecosystem function is variable across soil horizons in the boreal forests of Newfoundland, Canada

2.1. Introduction

Ecosystems are a collection of organisms interacting with each other and their abiotic environment (Tansley 1935). Interactions among organisms and their environment often have cascading effects on ecosystem functions, such as elemental cycling (Forbes et al. 2019). Studies of consumer organisms (herbivores, predators, omnivores, detritivores) across biomes demonstrate that organisms at higher trophic levels play crucial roles in ecosystems (Schmitz et al. 2018). Consumption of plant material by herbivores serves as a connection between below-ground nutrients and animal food webs through the uptake of nutrients from soils by plants which then reallocate them into above and below-ground biomass (Wardle et al. 2004; Peschel et al. 2015). Mammalian herbivory in particular has been studied in a variety of ecosystem types such as grasslands (Thrift, Mosley, and Mosley 2013; Cline et al. 2017), savannas (McNaughton 1988; Frank 2008), and forests (Dewey et al. 1993; Ellis and Leroux 2017). Often mammalian herbivore interactions are characterized based on visual changes to the ecosystem, such as vegetation height (Liu et al. 2015) or shifts in plant species composition (Frank et al. 2011; Ellis and Leroux 2017). However, herbivore effects that may be conspicuous above-ground can be linked to a suite of effects below-ground, and such effects can be largely “non-visual” or difficult to decipher. By identifying the below-ground effects of above-ground herbivores, we can get a better idea of how herbivore-plant interactions can influence ecosystem function.

There are two main types of mammalian herbivores: grazers and browsers. Grazers tend to consume low laying vegetation, such as grasses (*Poa sp.*), whereas browsers prefer the shoots, and leaves of woody tree and shrub species such as birch (*Betula sp.*) and aspen (*Populus sp.*). Grazers, such as elk (*Cervus canadensis*), wildebeest (*Connochaetes sp.*), and reindeer (*Rangifer tarandus*), tend to live in ecosystems with abundant grass to consume such as savanna or tundra ecosystems (Pastor, Cohen, and Hobbs 2006; J. Sitters et al. 2019). Browsers such as giraffes (*Giraffa camelopardalis*), moose (*Alces alces),* and deer (*Odocoileus sp.*) tend to be distributed in forest ecosystems such as the tropical, temperate, and boreal forests (Pastor, Cohen, and Hobbs 2006). The interaction between grazers or browsers and ecosystem functions have been widely studied (Andriuzzi and Wall 2017; Bernes et al. 2018; Forbes et al. 2019; Zhou et al. 2019). Elk grazing and trampling, for example, can increase bulk density in soils (Gass and Binkley 2011; Thrift, Mosley, and Mosley 2013), which, may contribute to an observable decrease in soil microbial activity (Peschel et al. 2015; Cline et al. 2017). Likewise, preferential browsing of palatable, high-quality forage (i.e., low C:N ratio) by moose can shift plant species composition in forests to plants with low-quality foliage and litter (i.e., high C:N ratio; Pastor et al. 1988; Dewey et al. 1993; Ellis and Leroux 2017). However, such cascading effects of grazers and browsers are not universal. There is great interest in identifying key predictors of the strength of indirect effects of grazers and browsers in diverse ecosystems (Tuomi et al. 2021; Leroux, Wiersma, and Vander Wal 2020).

Grazer and browser impacts on ecosystem functions all stem from three main direct impacts; trampling, foraging, and defecation (Dewey et al. 1993; Judith Sitters and Venterink 2015; Ellis and Leroux 2017; Forbes et al. 2019). Direct interactions are where an organism interacts specifically with another part of an ecosystem (biotic or abiotic). However, indirect interactions occur when an organism interacts with a part of an ecosystem (biotic or abiotic) via a shared biotic or abiotic component. The indirect impacts of herbivores can cause a chain reaction that crosses the above-ground and below-ground ecotone and therefore be difficult to decipher through visual inspection (Figure 2.1). Changes to above-ground plant community composition or cover can contribute substantial changes to soil horizon composition, nutrient turnover, and the rate of microbial processes (Quideau et al. 2001; Vancampenhout et al. 2009). For example, Peschel et al. (2015) observed that elk grazing in the sagebrush steppe of Wyoming, USA, had a direct negative effect on shrub biomass and this direct effect was correlated to an indirect decrease in soil microbial respiration and community richness. Below-ground systems are not only sensitive to above-ground changes, but also changes within the below-ground system itself. For example, decreases in soil C:N ratio are associated with lower soil depth (Natelhoffer and Fry 1988), lower fungi: bacteria ratios (Grosso, Bååth, and De Nicola 2016; M. N. Högberg, Högberg, and Myrold 2007), and increased net N mineralization rates (Booth, Stark, and Rastetter 2005). Indeed, recent studies have documented changes in soil composition and processes after herbivore exclusion in a variety of habitat types and among browsers and grazers (Table 2.1). Evidence from these studies suggests that herbivores can have positive, negative, or neutral effects on soil features such as soil nutrients, microbial activity, and soil moisture (Table 2.1).

While herbivore type (grazer or browser) is an important predictor of these effects, even within one herbivore type there is substantial variability in the strength of indirect cascading effects of herbivores on ecosystem function (Table 2.1). For example, classic studies of moose effects on boreal ecosystems observed an increase in soil C and N concentrations mediated by moose exclusion (Dewey et al. 1993), but more recent research at other boreal sites observed no effect of moose on soil N availability and soil C:N ratio (Ellis and Leroux 2017; Kolstad et al. 2018). A reason for this disparity may be that most ungulate-ecosystem studies do not have universal soil standards and often process soil samples as one unit (but see Kolstad et al. 2018), including all horizons, so the results may depend on the diversity of soil sampling procedures. In addition, soil samples often are collected at varying depths and can create another reason for discrepancies within soil studies. An ecosystem's soil is not homogeneous, instead it's made up of layers called horizons that each have a unique composition that is dependent on aspects such as depth and above-ground plant community composition (Quideau et al. 2001; Fierer, Schimel, and Holden 2003). Each horizon is primarily composed of one of two soil types, organic and mineral, and each horizon differs in color, type, and depth that can alter the level at which impacts are detectable (Soil Classification Working Group 1998). Due to the unique composition of each soil horizon, when exposed, horizons are often visually distinct and run in layers parallel to the soil surface. The first and uppermost soil horizon is mainly an organic horizon and the rate of soil processes are most heavily impacted by the above-ground conditions and plant community (Vanhala et al. 2008; Bhattarai, Bhattarai, and Pandey 2015). The second horizon and those below are typically mineral horizons and with above-ground disturbances taking longer to impact these deeper horizons. The differences between organic and mineral horizons are not only compositional but also functional as the organic horizon typically has a higher percent carbon content (>17% total C) and has faster C and N mineralization rates (Laganière et al. 2015; Liu et al. 2015). For example, a study done on moose impacts on the boreal forests of Norway by Kolstad et al. (2018) found soil C and N concentrations decreased as soils descend from organic to mineral horizons. We surmise that studying changes in mineralization rate, nutrient content, and microbial community composition along soil horizons may help reconcile context-dependent results across studies of ungulate effects on ecosystems.

We examined the direct and indirect effects of moose on both above-ground and below-ground boreal ecosystem functions. Using long-term (22 to 25 years) paired moose exclosure (i.e., fenced) and control plots to test a suite of predictions on the ecosystem influences of moose browsing. Specifically, we predict that areas where moose are excluded will: **(i)** have taller and more abundant tree communities and lower shrub cover (Ellis and Leroux 2017; Bernes et al. 2018), **(ii)** have a lower soil temperature, soil moisture, and soil pH (Frank et al. 2011; Gass and Binkley 2011; Cline et al. 2017; Ellis and Leroux 2017; Kolstad et al. 2018), **(iii)** have deeper first soil horizon with more non-decomposed plant material (Kolstad et al. 2018), **(iv)** have higher soil N (Pastor et al. 1988; Dewey et al. 1993; Gass and Binkley 2011; Ellis and Leroux 2017), and C (Dewey et al. 1993; Frank et al. 2011; Gass and Binkley 2011; Peschel et al. 2015; Ellis and Leroux 2017) concentrations, **(v)** higher microbial and macro-organism community diversity (Peschel et al. 2015; Cline et al. 2017), and **(vi)** higher net N mineralization rates (Frank 2008), than areas with moose present (Table 2.1). Overall, we hypothesize that moose impacts on soil function will be stronger within the first soil horizon (i.e., organic horizon) compared to the next visually distinct second soil horizon (i.e., mineral horizon).

2.2. Methods

Study Area

We conducted this study in the central-east coast maritime boreal forests of Newfoundland with 9 sites in Terra Nova National Park (TNNP), and 2 sites east of TNNP (i.e., Bunyan's Cove and Fox Marsh; Figure 2.2). TNNP and Bunyan's Cove are part of the middle boreal ecoregions with black spruce (*Picea mariana*), balsam fir (*Abies balsamea*), white birch (*Betula papyrifera*), and trembling aspen (*Populus tremuloides*) dominated forests (Bell 2002). Fox Marsh is part of the southern boreal ecoregions with balsam fir, and white birch dominated forests (Bell 2002). Although moose are native to most boreal forests, Newfoundland's moose population stems from the introduction of two moose in 1878 and four moose in 1904 (Pimlott 1953). Thereafter, the moose population increased rapidly as there was little competition with other large herbivores and no natural predators on the island after the extirpation of wolves (*Canus lupus*) in ~ 1930s (McLaren et al. 2004). Moose prefer nitrogen-rich shoots, leaves, and shrubs with individuals consuming roughly 3000-5000kg of dry matter each year (Dewey et al. 1993). Woodland caribou (*Rangifer tarandus*) are the only other ungulate on the island of Newfoundland, but they are rarely observed in the proximity of the experimental sites. Sites with paired exclosure and control plots were established in TNNP in 1998 and in Bunyan's Cove and Fox Marsh in 1995. Sites have four different disturbance histories; no recent disturbance (n = 2), spruce budworm outbreak in 1970-80s (n = 2), spruce budworm outbreak in 1990s (n = 5), and clear-cut (n = 2). Exclosures measure 35m x 35m in TNNP and Bunyan's Cove and 15m x 15m in Fox Marsh. Exclosures are tall enough (~3m) to exclude all large animals, with the lower portion open to allow small animals (e.g., snowshoe hare (*Lepus americanus*)) to access the area. We did not sample within 5m of the inside border of each exclosure (Figure 2.3). Control plots were located within ~10-20 meters of their paired exclosure and had the same dimensions. We established four sub-plots in each exclosure and control (Figure 2.3) and conducted field data collection from July 20th to September 2nd 2020. We ensured that data was collected in a standardized way; for example, we aimed to have the same number of days between sampling times and lab processing for each site (Table A.1, Table A.2). For example, if site X was sampled first for component Y, then we would return to site X first for component Z. If return time was 30 days for site X, then we also aimed for a return time of ~ 30 days for all other sites (see below).

Plant Community

To test prediction (i) that exclosures will have higher tree height and abundance and lower shrub cover than controls, we surveyed the plant community between July 20 and August 2. Specifically, we surveyed each sub-plot to identify trees, shrubs, and ground cover. Trees above 3 meters were counted and categorized by coniferous or deciduous and by species for the whole sub-plot (4m x 4m). Similarly, we identified all tree saplings (i.e., < 3m) by coniferous or deciduous, species, and measured each individual's height for the whole sub-plot. We identified all shrubs to species and then measured the percent cover of shrubs for the whole sub-plot.

Soil Temperature and Moisture

To test the soil temperature and moisture portion of prediction (ii) that exclosures will have lower soil temperature and moisture than controls, we placed one Meter Environment Teros 11 temperature/moisture probe in each of our exclosure and control plot (i.e., two probes per site) between July 20 and August 2. The probes at each site were linked to one Meter Environment ZL6 data logger. We did not place probes in each site's sub-plot and at 2 of our sites because of cost restrictions (Ocher Hill and Bread Cove). At each plot, the probes were placed in an area which had a plant community that was representative of the whole plot. Specifically, we dug a hole the length of the probe and submerged the prongs horizontally into the first soil horizon, and then we filled the hole with soil. Probes collected soil temperature (˚C) and water content (m^3/m^3) data every 6hr for approximately one month (see Table A.1). Soil gravimetric moisture was also calculated by drying wet prepared samples to constant mass at 60˚C (~48hr; see below).

Soil percent moisture was calculated by subtracting the before and after weights then dividing by the before weight and lastly multiplying it by 100 (((before-after)/before) *100))).

Macro-organisms

To test the macro-invertebrate portion of prediction (v) that exclosures will have higher macro-organism community diversity than controls, we placed a pitfall trap near a location with representative plant community and terrain in the center of each sub-plot between July 20 and August 2. Pitfall traps have a selection bias that favors the collection of insects that live on and travel across the soil surface. To ensure continuity between plots, pitfall traps were all established in a consistent manner. We dug a small hole and placed a 16oz plastic collection cup with a rim diameter of 8.9cm to fit snugly with the rim slightly elevated from the ground. The cup was filled 1/3 of the way with anti-freeze to preserve and kill macro-organisms and covered with a plastic "roof" to protect it from rain and debris. After 29-35 days (see Table A.1), we carefully poured out excess anti-freeze into a waste container and placed all specimens in a 20ml vial filled with 70% ethanol. We replaced the ethanol in each vial four times to clean and remove small debris. We processed sub-plot vials one at a time in the lab to identify, clean, separate, and count specimens. Initially, we poured each vial into a small tray. Slugs and worms were measured for length (mm) and replaced into the original vial and covered in 90% ethanol to preserve. All remaining specimens in the small tray were placed on a larger tray and grouped by morphospecies and dried for 24hr. After drying, specimens greater than 3mm were weighed and all similar specimens were grouped together. Specimens less than 3mm were viewed under a dissecting microscope and split between flies and others. All others less than 3mm were counted and grouped with similar specimens. Flies were counted, and the number of different morphospecies was counted. All flies were placed in a vial filled with 70% ethanol and labeled with their sub-plot label. Like specimens between all sub-plots were grouped together and were placed in community vials filled with 70% ethanol. After all sub-plot vials were separate, morphospecies were identified to order.

Soil Cores

To test portions of predictions (ii) - (vi), we collected two soil cores at each corner of each sub-plot (n = 8 soil cores per sub-plot; Figure 2.3) between July 20 and August 2. The initial core was collected to be taken back to the lab (hereto referred to as the initial core). The second core was taken adjacent to the initial core and was placed in a Ziploc bag, buried, and left to measure net nitrogen mineralization (hereto referred to as the secondary core, see below). We used a 25.4cm x 6.4cm soil corer to take a 25cm core or until we hit rock. To test the horizon depth portion of prediction (iii) that exclosures will have deeper first soil horizon than controls, we measured each soil horizon present in the initial core and recorded the color of the second soil horizon. We then separated the initial core on-site at the lines of soil horizon break. Only the first and second visually distinct soil horizons were separated from the core and placed in separate sealed bags (n=2 soil horizons, n=4 bags per soil horizon per sub-plot, hereto known as corner samples). We visually classified a soil horizon by color, composition, and lines of horizon break (Soil Classification Working Group 1998). In our boreal ecosystem the first and second soil horizons are generally organic and mineral material horizons, respectively, and are typically visually distinct. Hereafter, we refer to the uppermost and mostly organic horizon as the first soil horizon and the horizon below that which typically has a higher mineral makeup as the second soil horizon. Percent carbon is a common way to differentiate organic and mineral horizons in boreal podzols with organic horizons having > 17% C and mineral horizons having < 17% C composition (Soil Classification Working Group 1998; Pennock et al. 2015). We classified our soil horizons as the first and second soil horizon because despite being the first two visually distinct horizons, not all samples met their corresponding total percent C requirements to be classified as organic and mineral horizons. In cases where our visual assessments did not agree with lab-based measures of horizons (i.e., using the 17% C threshold), we ran our structural equation models (see below) on the full data and a subset of the data that met the %C criterion. We processed all initial cores in the lab after all sites were visited and cores collected. We processed them in the order of collection to ensure they were treated similarly (i.e., they spent the same amount of time in the fridge at 4°C; Table A.2). For each sub-plot sample (n=4 corner samples per horizon), we sieved each corner sample through 2 different sized sieves (no.5 and no.10). Additionally, to test the horizon percent plant material portion of prediction (iii) that exclosures will have a higher first soil horizon percent plant material than controls, we weighed each corner sample for the first soil horizon before and after sieving to identify percent non-decomposed material. Using a scale, we combined equal amounts of each corner sample to create 75g-100g of soil to homogenize sub-plot samples for future measures (n=4 combined to n=1 per sub-plot). Soil needed for lab processes was weighed to the amount needed and separated by placing in either 1.5ml, 15ml, or 50ml tubes (hereafter referred to as “prepared samples”). Soil used to create prepared samples for DNA extraction (0.5g) and Chloroform fumigation (20g dry weight, split into two 10g) was kept fresh and refrigerated (4°C). Soil used to create prepared samples for net nitrogen mineralization (~40g) and pH (~5g) was frozen. Lastly, soil used to create prepared samples for total C and N (~5-20g) was dried at 60°C until constant mass.

Soil pH

To test the soil pH portion of prediction (ii) that exclosures will have lower soil pH than controls, we transferred the ~ 5g prepared sample (see above) to a refrigerator to thaw 24hr before measuring pH. We made the prepared soil sample into a slurry using 10ml (2-6ml in cases with low soil content) distilled water. The slurry was left to sit for 30 minutes to let it acclimate to the air. After acclimating, we submerged a calibrated electronic pH sensor (Acumet AE150 Benchtop pH Meter) and took a pH reading once the reading was stable for 10 seconds.

Nitrogen and Carbon Content

To test prediction (iv) that exclosures will have higher total soil N and C than controls, we dried wet prepared samples to constant mass at 60˚C (~48hr). Once dried, the prepared samples were weighed, and that weight was subtracted from the initial weight to quantify the gravimetric moisture of the sample (data on moisture will be used for soil percent moisture and chloroform fumigation as well). Approximately 5g of the dried soil was transferred to a 20ml glass vial and kept in a sealed bag with silica packets. Vials were sent to the Agriculture & Food Laboratory at the University of Guelph. There each dried sample was tested for percent C and N content using a Leco CN828 instrument. This instrument uses catalytic combustion (950˚C) to separate C and N from foreign gases and analyzes them using thermal conductivity detection and infra-red detection.

Microbial community biomass and diversity

To test the soil microbial component of prediction (v) that exclosures will have higher microbial community diversity than controls, we used chloroform fumigation and DNA extraction to measure aspects of microbial communities directly within the soil, such as microbial biomass and species presence and abundance, respectively. Chloroform fumigation was done on two prepared samples containing wet soil whose weight was relative to 10g dry (using standard methods; Brookes et al. 1985; Beck et al. 1997). Chloroform fumigation is a common method used to lyse microbial cells in order to measure microbial biomass (Fierer, Schimel, and Holden 2003). The first sample (fumigated sample) underwent chloroform fumigation and K_2SO_4 extraction, while the second (un-fumigated sample) only had K_2SO_4 extraction. We fumigated the first prepared samples (n=16) for each site together in order of site collection (Table A.2). The first prepared samples were placed in 50ml beakers and placed in a glass vacuum desiccator. A 50ml beaker containing 30ml chloroform was placed in the center of the desiccator with six boiling chips. A vacuum was made until chloroform boiled three times, with the vacuum being released and boiling chips replaced for the first two times. For the third time, we sealed the desiccator, covered it with a black bag to help keep chloroform from breaking down, and left it to sit for three days (72hr). After three days, the samples were removed from the desiccator. The fumigated and non-fumigated samples both underwent K_2SO_4 extraction on the same day. For K_2SO_4 extraction, we placed each soil sample into a 300ml shaker bottle and added 50ml K_2SO_4. We placed the samples on a shaker table for 1hr at a speed of 200. Samples were then filtered through Whatman No. 1 paper to remove soil. We then placed the liquid extract into 15ml or 50ml vials depending on volume and froze them at -20˚C. The non-fumigated sample acted as our “before” containing only dissolved C and N from the soil, where the fumigated sample acted as our “after” containing both soil and microbial C and N. We then sent the extracts to the Laboratoire de chimie organique et inorganique of the Ministère des Forêts, Quebec. A Shimadzu TOC-V CPN Total Organic Analyzer equipped with a TNM-1 Total Nitrogen unit was used to analyze samples for TC and TN. Standards for this analyzer is 3 mg/L N (as NO_3-) in HCl 0.05% from a multi-element commercial standard (AccuSPEC, SCP Science). Results were given in units of mg/L and were converted to mg/g soil ((mg/L * L K_2SO_4)/g soil) and then divided by a standard to account for typically extraction error (C: 0.45 (Beck et al., 1997) and N: 0.54 (Brookes er al., 1985)). To find the C and N attributed to microbial biomass we subtracted before and after concentrations for each sample.

We used FastDNATM Soil Spin Kits (MP Biomedicals) to isolate DNA from a .5g prepared soil sample following manufacturer's instructions. We processed prepared samples in the order that the sites were visited, and soil cores were obtained, but individual samples (i.e., sub-plot) within each site were chosen randomly (Table A.1). We stored the extracted DNA at -80C to preserve it. DNA was tested in a spectrometer to test quality and quantity to assist with PCR. DNA was sent to the Agriculture & Food Laboratory at the University of Guelph for PCR and sequencing. PCR was competed to target the V3 region of the 16s rRNA gene (primers: Eub338F and Eub518R; Fierer et al. 2005) for bacteria and ITS region (primers: ITS1f and 5.8S; Gardes and Bruns 1993; Vilgalys and Hester 1990) for fungi. Sequencing was completed using a MiSeq sequencer with a MiSeq v2 reagent kit (illumina) and 2x250 paired-end cycles. Taxonomic assessments were made using the Greengenes (DeSantis et al. 2006) and UNITE (Kõljalg et al. 2013) databases for bacteria and fungi, respectively.

Net Nitrogen Mineralization

To test prediction (vi) that exclosures will have higher net N mineralization rates than controls, we compared DIN content from the initial cores to the secondary core (retrieved between August 19th and September 2nd). Secondary cores from each corner were kept intact and placed in large freezer bags (26.8x27.3 cm) and replaced into the hole they came from, so each soil horizon was returned to its initial placement. We incubated the soil cores in situ for ~ 30 days (Table A.1). When retrieved, we separated the cores on-site at the line of soil horizon break, taking only the first and second visibly distinct soil horizon, the same as the initial core. The first and second soil horizons were placed in separate sealed bags (n=4 bags per soil horizon, n=2 per sub-plot, n=8 bags total per plot). Soil processing was completed the same as the initial soil samples. Prepared samples of approximately 20-40g were made from both the initial core and incubated secondary core and placed in 50ml vials. Prepared samples were sent to the Agriculture & Food Laboratory at the University of Guelph, where Ammonium (NH4-N) and Nitrate (NO3-N) concentrations were calculated. They extracted each soil sample with 2M KCL and analyzed this extract using the Sea; AQ2 discrete analyzer. The initial core acted as our “before”, and the secondary core acted as our “after” Ammonium and Nitrate contents. We used the following equation to find the amount of nitrogen potentially mineralized over a ~ 30-day field incubation period: $N_{\text{Net N Mineralized}} =[(\text{Nitrate}_{\text{After}} + \text{Ammonium}_{\text{After}})-(\text{Nitrate}_{\text{Before}} + \text{Ammonium}_{\text{Before}})]/\text{Days Incubated}$ (Robertson et al. 1999).

Statistical Analysis

Structural equation model of cascading impacts of moose in maritime boreal forests

To test our predictions on the direct and indirect impacts of disturbance and moose on above-ground and below-ground maritime boreal forest ecosystems, we fit two piecewise structural equation models (SEMs): one for the first soil horizon (i.e., O horizon) and one for the second soil horizon (i.e., mineral horizon; Shipley 2000; 2009; Lefcheck 2016). We did not fit SEMs to all our data but rather to the subset of key *a priori* pathways that capture the main effects while not resulting in overfit models (Figure 2.4). To this end, we incorporated our predictions into the SEMs as response variables (predictions i, v, vi) and as co-variates (predictions ii, iii, iv, v). The SEMs were created using one generalized linear mixed model with a Poisson error structure and a logit link with adult tree count as response, and four linear mixed models with height of palatable saplings, shrub percent cover, soil microbial C:N ratio, and soil net N mineralization as response variables. We also included five co-variates for some of our response variables. Specifically, we included soil percent moisture, mean depth of the first soil horizon, and first soil horizon percent plant material as co-variates in the understory plant models (i.e., height of palatable saplings and shrub percent cover) and microbial C:N ratio model, and we included soil total percent C and N as covariates in the soil net N mineralization model. Disturbance and moose exclusion are measured at the site and plot level, respectively, while all other variables are measured on the sub-plot level (Table 2.2). Moose exclusion (categorical variable; exclosure and control) is the main predictor variable in our SEMs. We included disturbance as a secondary predictor variable because recent evidence in boreal (see review in (Leroux, Wiersma, and Vander Wal 2020) and other ecosystems (e.g., temperate forest; Nuttle et al. 2013) demonstrates that large ungulate effects can be mediated by disturbance history. Empirical evidence in our study system (e.g., Charron and Hermanutz 2017; Leroux et al. 2021) suggests that canopy opening size created by disturbance is a good measure of disturbance effects. Consequently, we model disturbance as a categorical variable with three levels: open, partial, and closed. Sites are classified as a random intercept in each model to account for the paired design and spatial autocorrelation between exclosure and control plots at each site. While the above-ground variables are consistent in our two SEMs, the below-ground variables (soil microbial C:N ratio, soil net N mineralization, soil percent moisture, and soil total percent C and N) were collected for the first and second soil horizons and used in the first soil horizon and second soil horizon SEM, respectively. We removed data for 13 first horizon and 16 second horizon sub-plots that were impacted by animals (e.g., rodent digging up soil bag) or where chemical analysis was not successful. Due to differences in sub-plots used for the first and second horizons SEMs above-ground SEM coefficients vary slightly.

We evaluated conditional independence among nodes in our SEMs with Shipley's test of directed separation (i.e., d-sep test) to ensure that there are no missing relationships without paths in our SEMs. This test showed that the SEMs did not meet the assumption of conditional independence with eight missing relationships for the first soil horizon and five for the second soil horizon model. Two paths that appeared highly related were moose exclusion on total adult tree count, and height of palatable saplings on shrub percent cover. We added these two paths to both SEMs as they capture key ecological relationships. Specifically, after 22-25 years of moose exclusion, we might expect a direct impact of moose on adult trees to be emerging (McLaren, Taylor, and Luke 2009; Gosse et al. 2011) and we expect a negative effect of saplings on shrub percent cover because of competition and shading (Sadanandan Nambiar and Sands 1993; Kneeshaw and Bergeron 1999). Comparing the Fisher C value against the chi-squared distribution showed that the addition of these two pathways allowed both horizons to pass the Fisher C threshold (p-value > 0.05). However, the d-sep test showed that first soil horizon SEM and second soil horizon SEM were still missing two and three relationships, respectively. Consequently, we added paths between height of palatable saplings and soil total percent carbon, and soil net N mineralization and percent soil moisture, to the first soil horizon SEM, and between adult tree count and soil total percent carbon, soil net N mineralization and soil percent moisture, and soil net N mineralization and soil first horizon depth, to the second soil horizon SEM as correlated errors (Lefcheck 2016). We conducted visual assessment of the residual plots for each model included in the SEM and we report the marginal and conditional R^2 and standardized coefficient estimates for each model (Figure 2.5, Table 2.3, Table 2.4). We used *R* v.4.0.3 (R Core Team, 2020) to complete analyses along with the packages *lme4* (Bates et al. 2021) for mixed models, and *psem* (Lefcheck 2016) for SEMs.

Linear mixed models of moose impacts on maritime boreal forests and soils

We fit additional linear mixed models to test predictions related to moose effects on soil conditions, composition, nutrient content, and organism communities that were not included as response variables in our SEMs (predictions ii, iii, iv, v). Specifically, we fit 13 linear mixed models with soil temperature and water content over one month, soil pH, soil first horizon depth, soil first horizon percent plant material, soil total percent C and N, fungi: bacteria abundance ratio, and insect diversity as response variables and moose exclusion as the explanatory variable. Soil temperature and water content over one month and insect diversity are measured at the plot and sub-plot level, respectively, while all other variables are measured on the sub-plot level for specific soil horizons (Table 2.2). Sites were classified as a random intercept in each model to account for the paired exclosure and control design at each site. We conducted visual assessment of the residual plots and computed marginal and conditional R^2 for each linear mixed model (Table 2.5). Additionally, microbial phyla distribution was estimated using the median percent abundance of each bacterial and fungal phyla identified from soil DNA extraction and analysis. We report median percent abundance of each bacterial and fungal phyla for each plot type. We also report which plot had the highest median percent abundance for each bacterial and fungal phyla and how many times larger that median percent abundance was is in comparison to its paired plot for both soil horizons (Table A.5, Table A.6).

2.3. Results

Structural equation model of cascading impacts of moose in maritime boreal forests

The SEM models for the first (i.e., organic horizon) and second (i.e., mineral horizon) soil horizons provided a good fit to the data (first horizon: Fisher's $C=38.41_{df=44}$, p=0.709; second horizon: Fisher's $C=36.58_{df=42}$, p=0.707). There were nine and eight statistically significant (p<

0.05) pathways for the first and second soil horizon, respectively (Figure. 2.5). We found strong direct relationships between disturbance and moose exclusion and the above-ground components of the two models (Figure 2.6). Above-ground coefficients for the first and second horizon SEMs varied slightly due to differences in sub-plot removal (see methods for details); however, the relationships were consistent between horizons. Sites with open and partially open canopies from disturbance on average had more adult trees than closed canopy sites (Table 2.3, Table 2.4; mean number adult trees per sub-plot in open: 4.28, partial: 4.18, closed:1.75). There were also on average more adult trees in moose exclosure (i.e., moose not present) plots compared to control (i.e., moose present) plots (Table 2.3, Table 2.4; mean number adult trees in exclosure: 6.12, control: 1.39). Moose exclusion had a strong positive effect on the mean height of palatable saplings and a negative effect on shrub percent cover (Table 2.3, Table 2.4; mean height of palatable saplings in exclosure: 86.30cm, control: 60.60cm; mean shrub percent cover in exclosure: 46.5%, control: 70.4%). Contrary to our predictions, adult tree count had no significant effect on the mean height of palatable saplings but did have a negative effect on shrub percent cover (standardized regression coefficient β= first horizon: -0.29, second horizon: -0.25). Height of palatable saplings also had a negative effect on shrub percent cover (β= first horizon: -0.22, second horizon: -0.27). The last consistent relationship seen in both soil horizon SEMs was a positive effect of the first horizon percent plant material on the height of palatable saplings (β= first horizon: 0.32, second horizon: 0.29).

There were three significant relationships that were not consistent between the first and second soil horizon SEMs and primarily deal with below-ground connections (Figure 2.7). The SEM for the first soil horizon showed a positive relationship between palatable saplings height (β= 0.27) on soil microbial C:N ratio. The first soil horizon SEM also showed a negative relationship between soil percent moisture (β= -0.44) on microbial C:N ratio. The SEM for the second soil horizon showed a negative relationship between soil percent moisture and shrub percent cover (β=-0.38) but no relationships linking above-ground plants to soil microbial C:N ratio. Finally, both SEMs did not reveal any cascading impacts of moose on net N mineralization rates (Tables 2.3, Table 2.4). A few of our visually classified first and second soil horizons did not fit the classic definition of a organic and mineral horizon based off percent carbon content (organic: >17%, mineral:<17%; Soil Classification Working Group 1998; Pennock et al. 2015). Consequently, we compared our initial SEM results to a second set of SEMs where we removed 12 and 15 sub-plots where the first soil horizon had less than 17% C and second soil horizon had more than 17% C, respectively (Figure A.1). The SEM fit to this revised data set showed qualitatively similar results for the main effects in the first horizon with the addition of a positive relationship between first horizon percent plant material and microbial C:N ratio emerging in the SEM for the revised first horizon data (Figure A.1, Table A.5). The SEM fit to the revised data set for the second horizon showed qualitatively similar results for the main effects except no relationship between total adult tree count and shrub percent cover (Figure A. 1, Table A.6). Also, two covariates that were significant in the SEM for the full second horizon dataset were not significant in the SEM fit for the revised second horizon data (i.e., no relationships between first horizon percent plant material on height of palatable saplings and second horizon percent moisture on shrub percent cover; Figure A. 1, Table A.6). Given the majority of our results are similar for the original and revised data sets, we focus the discussion on the results for the full or original data set.

Linear mixed models of moose impacts on maritime boreal forests and soils

We fit 13 linear mixed models to evaluate our predictions that went beyond our SEM (Table 2.5, Table A.7).

Prediction ii – soil temperature, moisture, and pH

We found very little evidence of moose impacts on soil temperature, water content, moisture, and pH (marginal R^2 range from <0.001 to 0.021 across models) but large variation in these soil features across sites (conditional R^2 range from 0.185 to 0.754 across models; Table 2.5, Table A.7). The median soil water content over one month was 1.1x larger in the exclosure than in the control plots.

Prediction iii- first soil horizon depth and percent plant material

We found weak evidence of moose impacts on the first soil horizon depth and percent plant material (marginal R^2 range from 0.049 to 0.056 across models) but moderate variation in these soil features across sites (conditional R^2 range from 0.351 to 0.376 across models; Figure 2.8, Table 2.5, Table A.7). The first soil horizon depth and percent plant material in the exclosure were 1.2x and 1.05x higher than in the control, respectively.

Prediction iv- soil total C and N

We found no evidence of moose impacts on soil total percent C and N (marginal R^2 range from <0.001 to 0.012 across models) but large variation in these soil features across sites (conditional R^2 range from 0.553 to 0.667 across models; Table 2.5, Table A.7). Soil nitrogen was 1.14x higher in the exclosure first soil horizon and the control plot second soil horizon when compared to their respective horizon and plot. The first soil horizon had 1.77x and 1.69x more nitrogen than the second soil horizon in the exclosure and control plot, respectively. The first soil horizon had 1.81x more carbon than the second soil horizon in both plot types.

Prediction v- microbial and insect communities

We found very little evidence of moose impacts on microbial fungi: bacteria ratio and insect diversity (marginal R^2 range from <0.001 to 0.026 across models) but some variation in these soil features across sites (conditional R^2 range from 0.047 to 0.119 across models; Table 2.5, Table A.7). There were 12 orders of macro-organisms identified from pitfall traps (Table A.8). There were 28 phyla identified for both bacteria and fungi (bacteria: Table A.3, fungi: Table A.4). Out of the 28 phyla, there were eight and three predominant bacteria and fungi phyla, respectively (>1% median abundance). Three of the predominant bacterial phyla's median abundances were highest in the exclosure plots when compared to control plots, Proteobacteria (first horizon: 1.02x, second horizon: 1.09x), Bacteriodetes (first horizon: 1.15x, second horizon: 1.06x), and Thermotogae (first horizon: 1.32x, second horizon: 1.57x), for the first and second soil horizon. Two of the predominant bacterial phyla median abundances were highest in the control plots when compared to exclosure plots, Actinobacteria (first horizon: 1.13x, second horizon: 1.04x), and Acidobacteria (first horizon: 1.06x, second horizon: 1.09x), for the first and second soil horizon. In the first soil horizon, Firmicutes (first horizon: 1.12x), Fibrobacteres (first horizon: 1.08x), and Plantctomycetes (first horizon: 1.04x) median abundance were higher in the control plots, but in the second soil horizon (second horizon: 1.03x, 1.16x, and 1.06x, respectively) their median abundance was higher the exclosure plots than control plots. For the first and second soil horizon one predominant fungal phyla median abundance, Basidiomycota (first horizon: 1.14x, second horizon: 1.14x), was highest in the exclosure plots, and one, Ascomycota (first horizon: 1.02x, second horizon: 1.21x), was highest in control plots when compared to exclosure plots. In the first soil horizon, Mortierellomycota (first horizon: 1.05x), median abundance was higher the control plots, but for the second soil horizon (second horizon: 1.27x) their median abundance was higher the exclosure plots than control plots.

2.4. Discussion

Classic studies of iconic large ungulate systems (see review in Wardle et al. 2004) suggest the prevalence of cascading indirect impacts of large ungulates on ecosystem function (Dewey et al. 1993; Pastor et al. 1988). Recent work has called into question the generality of these earlier findings (Ellis and Leroux 2017; Kolstad et al. 2018) and attempts to explain wide context-dependency in findings across ecosystems (Daskin and Pringle 2016; Andriuzzi and Wall 2017). The importance of looking beyond visual changes and descending below-ground to understand the extent of large ungulate impacts on ecosystem functioning is emerging. We surmise that part of the context dependency of this body of research may be driven by how soils are sampled and linked to ungulate impacts. Combining horizons that have different compositions and functions can greatly reduce the resolution at which we can identify ungulate impacts on ecosystems. In our 22-25 year experiment we measured above-ground and below-ground effects of moose on two soil horizons. We found that moose have a significant impact on above-ground plant communities and below-ground soil conditions (soil percent moisture, first soil horizon depth, and first soil horizon percent plant material). As we expected, our structural equation models (SEMs) identified some evidence of cascading moose effect on the first soil horizon (i.e., organic horizon) but little evidence of such effects in the second soil horizon (i.e., mineral horizon). Our path analysis and partitioning of potential moose impacts across two soil horizons provides a holistic perspective on complex large ungulate-ecosystem feedbacks.

The three direct interactions moose have with their surroundings, foraging, trampling, and defecating, first impact above-ground functions leading to strong and consistent above-ground effects observed across study systems (Dewey et al. 1993; Ellis and Leroux 2017; Forbes et al. 2019). Moose prefer foraging on nitrogen-rich shoots from tree and shrub, making it harder for nitrogen-rich palatable species to grow to canopy-level heights. As expected, through our SEMs, we found evidence that moose exclusion (i.e., moose absent) has a positive impact on the total abundance of adult trees and on the height of palatable sapling communities (Figure 2.7). Above-ground effects of large ungulate herbivores on palatable saplings is ubiquitous (Côté et al. 2014; Bernes et al. 2018) and evident across ecosystem types such as boreal (Rotter and Rebertus 2015; Kolstad et al. 2018) and temperate (Wardle et al. 2001; Nuttle et al. 2013) forests. However, after 22-25 years of exclusion, and counter to other earlier research in our study system (Gosse et al. 2011; Ellis and Leroux 2017; Leroux et al. 2021), we identified a direct impact of moose on the total count of adult trees. We also observed that not only did moose exclusion have a negative effect on shrub cover, but so did the count of adult trees and the height of palatable saplings (Figure 2.5, Figure 2.6). Such tree community impacts may be explained by adult and sapling trees creating shade and competition of resources for shrubs (Sadanandan Nambiar and Sands 1993; Kneeshaw and Bergeron 1999). By primarily foraging on preferred plant species, browsers alter competition between species making it easier for non-preferred species to flourish. Almost all of our control (i.e., moose present) plots had more non-preferred shrub species compared to preferred species. When moose are present, fewer and shorter trees are present, allowing non-preferred shrubs to prosper and have more extensive ground coverage. Our model supports this finding via a negative effect of moose exclusion on shrub percent cover. We also found that areas with higher disturbance (open and partial) had more adult trees. Increase in adult tree count can be attributed to the opening of canopy by disturbance allowing for more sapling trees to grow and reach adult height (McLaren, Taylor, and Luke 2009; Gosse et al. 2011). Significant relationships between moose exclusion and above-ground response variables are our first clues of moose indirect impacts on their surroundings.

Changes in above-ground plant height and community composition can greatly alter soil conditions and composition (Wardle et al. 2001; Bardgett and Wardle 2010). For example, a study in the boreal forest of Alaska, USA and western Canada found that deciduous trees took up over 25 times more available water than coniferous trees during snowmelt (Young-Robertson et al. 2016). Above-ground shading also determines the amount of sunlight and rain that reaches exposed soil. As predicted, we found moose exclusion led to a decrease in soil temperature and moisture (Figure 2.8; opposite effect on soil water content) and this finding is consistent with a recent moose-boreal forest study in Norway (Kolstad et al. 2019). More specifically, we would expect to record a greater impact of moose on the first soil horizon as it has the most direct interaction with above-ground aspects over the time frame of our experiment and is usually recognized as having a higher percentage of organic material when compared to deeper mineral horizons (Soil Classification Working Group 1998; Pennock et al. 2015). Soil organic matter's chemistry and decomposition rate can be affected by changes to plant litter composition (Meier and Bowman 2008; Kohl et al. 2018). There is strong evidence that moose exclusion causes an increase of nutrient-rich plant litter that is returned to the soil (Pastor et al. 1988; Dewey et al. 1993; Hobbs 1996; Liu et al. 2015; Peschel et al. 2015; Ellis and Leroux 2017). More litter being returned to the soil increases plant material buildup in boreal forests with slow decomposition rates, leading to a quicker formation of the first horizon (Jenny 1941). The composition of organic horizons in cold climates, such as the boreal forest, closely mirror the composition of litter being returned to the soil (Vancampenhout et al. 2009). In support of our predictions, we observed that moose exclusion was correlated with a deeper first horizon and greater amount of plant material within the first soil horizon (Figure 2.7). Changes to soil composition and conditions due to moose can not only impact plant growth and nutrient turnover, but also affect soil organism community composition and abundance.

Changes in above-ground and below-ground biotic and abiotic conditions will impact soil organisms such as microbial (Carreiro and Koske 1992; Meier and Bowman 2008) and insect (Knops et al. 1999; Bennett 2010; Oliver et al. 2016) communities. As expected, our SEM for the first soil horizon showed the height of palatable saplings and soil percent moisture had a positive and negative impact on soil microbial C:N ratio, respectively (Figure 2.5, Figure 2.7). Importantly, this effect is mediated via an indirect path linking moose to height of palatable saplings and shrub percent cover to microbial C:N ratio. Moose herbivory causes a decrease in tree and shrub cover, which creates wetter and cooler soils (Kolstad et al. 2018; 2019), which can make it difficult for some microbial communities to prosper in the area. In boreal ecosystems, microbial C:N and fungi: bacteria ratios are usually positively related (Sterner and Elser 2002). A lower microbial C:N ratio would suggest a higher bacterial community, which is often positively correlated with N mineralization and turnover (M. N. Högberg, Chen, and Högberg 2007). Counter to our expectation; however, we did not find an indirect or direct path linking moose to fungi: bacteria ratio or net N mineralization in our study system in either soil horizons (Figure 2.5, Figure 2.7). This suggests that indirect cascading impacts by moose might be relatively limited in our study system. In addition, we also found insect abundance and diversity to be impacted by soil conditions but did not find any evidence of moose effects on these features (Table 2.5). Insects often live within the soil so features like soil moisture and available plant material for herbivore insects to consume are important to their survival (Oliver et al. 2016).

Understanding how different compositional soil horizons respond to impacts by agents of change is crucial for disentangling the potential indirect effects large ungulates have on ecosystem function (Kolstad et al. 2018). Some of the context dependencies in studies looking at ungulate impact on soils could be related to the differences in horizons and if they are accounted for within the collection methods. By splitting the first and second soil horizons, we were able to identify compositional differences that are associated with above-ground conditions and better capture the extent of indirect impacts of moose in our study system. Our SEM found cascading impacts of moose on microbial C:N ratios only in the first soil horizon. This confirms that moose are not only having cascading impacts on below-ground functioning, but also more strongly impacting the first or organic soil horizon. In comparison, our SEM for the second or mineral soil horizon did not show any evidence of cascading effects of moose on soil functioning. This finding is supported by the fact that the first soil horizon is typically composed of organic material and has higher microbial activity (Bhattarai, Bhattarai, and Pandey 2015). Moose effects on soils are fairly limited meaning observed variations in soils across sites may be attributed to differences in soil types and composition across sites that may be linked to other factors such as topography, plant, and organismal communities (Jenny 1941). We found that the first horizon had significantly higher C and N content than the second horizon which is supported by the fact that the organic horizon typically contains at least 17% C whereas mineral horizons typically contain less than 17% C (Soil Classification Working Group 1998; Pennock et al. 2015). There are many different ways of collecting soils, such as soil cores (Frank 2008; Cline et al. 2017; Kolstad et al. 2019) and stencil cut horizons (Laganière et al. 2015). Soil studies that do not take soil horizons into account usually collect a sample to a standard depth without considering the proportion of each soil horizon in the sample. The lack of standards between studies may explain the variation between results in studies looking at herbivore impact on soils. We recommend a) explicit documentation and investigation of the impacts of soil sampling methods, and b) standardized approaches to soil sampling in future work on large ungulate-soil functioning.

The exclosures used in our study were established 22 to 25 years before we collected our data. The age of exclosures of this study is both longer (Gass and Binkley 2011; Ellis and Leroux 2017; Kolstad et al. 2019) and shorter (Pastor et al. 1993, Cline et al. 2017) when compared to the length of exclusion seen in related studies. Forbes et al. (2019) reported that the median length of wild large ungulate exclosure studies is 6 years, making an experiment of our length rarely observed. However, moose were established in Newfoundland around 90 years before the exclosures were built (Pimlott 1953) and became abundant in and around our study area in the 1980's (McLaren et al. 2004). The history of disturbance and large ungulate herbivory prior to exclosures being erected is rarely reported in large ungulate-ecosystem studies (Leroux et al. 2021). We surmise, however, that site legacy could be critical to predicting large ungulate effects on ecosystems (Leroux, Wiersma, and Vander Wal 2020). Specifically, soils, particularly mineral horizons, are slow to form as they are very dependent on many environmental factors such as climate, organismal communities, and topography (Jenny 1941) so they could contain the legacy of moose interaction. As age of exclosures increase, we recommend that future work track the legacy and impacts of moose below-ground. Continuing to track below-ground impacts over time will allow us to understand how the duration of moose presence changes an ecosystem over the time frame of forest community regeneration and soil formation.

Moose are not native to the island of Newfoundland and have become an agent of change since their introduction over 100 years ago. Our study was able to capture the impacts of moose on both above-ground and below-ground systems. By including interactions and levels of ecosystem function, we can better understand how moose impact their surroundings. We found that they had a direct effect on above-ground plant community as well as below-ground soil conditions and composition. Separating soil horizons allowed us to tease apart and better identify moose indirect impacts on soil and microbial communities. Moose had a cascading indirect impact on microbial communities in the first soil horizon through soil moisture and sapling palatable height. We suggest looking beyond what is visually changing to truly understand the extent of impacts happening within an ecosystem.

2.5. References

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Table 2.1: Literature summary of evidence in support (or not) for each concept that shows diverse qualitative effects (+: Positive, -: Negative, 0: None) of ungulate herbivores on soil ecosystems. Studies are from exclosure experiments. Red text shows studies on browsers (moose specifically), and Blue text shows studies on grazer (a variety including elk and cows). We based our predictions on this body of evidence.

Concept	**Supporting Articles**	**Opposing articles**
Soil moisture decreases in exclosure	(-) Kolstad et al., 2018; Frank et al., 2011; Gass and Binkley 2011; Cline et al., 2017	(+/0) Ellis and Leroux 2017
Soil temperature decreases in exclosure	(-) Kolstad et al., 2018	
Soil microbial activity (fungal and bacteria richness) increases in exclosure	(+) Peschel et al., 2015; Cline et al., 2017	(-/0) Kolstad et al., 2018
Net Nitrogen mineralization decreases in exclosure	(-) Frank 2008	(+/0) Dewey et al., 1993; Peschel et al., 2015
Soil carbon increases in exclosure	(+) Dewey et al., 1993; Ellis and Leroux 2017; Frank et al., 2011; Gass and Binkley 2011; Peschel et al., 2015	(-/0) Kolstad et al., 2018; Thrift et al., 2013
Soil nitrogen increases in exclosure	(+) Pastor et al., 1988; Dewey et al., 1993; Ellis and Leroux 2017; Gass and Binkley 2011	(-/0) Kolstad et al., 2018; Thrift et al., 2013; Peschel et al., 2015
Soil pH decreases in exclosure	(-) Ellis and Leroux 2017	(+/0) Peschel et al., 2015
Soil litter increases in exclosure	(+) Pastor et al., 1988; Dewey et al., 1993; Ellis and Leroux 2017; Hobbs 1996; Liu et al., 2015; Peschel et al., 2015	
Organic horizon depth increases in exclosure	(+) Kolstad et al., 2018	
Bulk density decreased in exclosure	(-) Kolstad et al., 2018; Gass and Binkley 2011; Thrift et al., 2013	

Table 2.2: Data collected from each method and the level at which they were collected for each site.

Type of Data and Number of Points per Site	**Data Sets**
Site (n=1)	-Disturbance
Plot (n=2)	-Moose Exclusion
Plot Multiple (many points taken over time)	-Soil Temperature and Moisture (every 6hr for ~30 days)
Sub-Plot(n=8)	-Insect data >Count, Order Diversity, and Biomass -Plant Data >Adult Tree Count (>3m) >Percent Canopy Cover >Tree Sapling Count and Height (<3m) >Shrub Count and Percent Cover >Ground Cover
Sub-Plot split, two Horizons(n=16)	-Total Soil N -Total Soil C -Soil Moisture % -pH -Net N Mineralization -Microbial DNA -Microbial Biomass
Sub-Plot One Horizons at each corner(n=16)	-First Horizon Depth -First Horizon Percent Plant Material

Table 2.3: Piecewise structural equation model results for the first (i.e., organic) soil horizon. Results of the piecewise structural equation examining the direct and indirect impact of disturbance and moose exclusion on above-ground and below-ground function in maritime boreal forests. Disturbance is a categorical variable measured as canopy openness (i.e., open, partial, closed) created by budworm or forest harvesting. Site was included as a random intercept in each model. Standardized Estimate: standardized coefficient estimate, Raw Estimate: non-standardized coefficient estimate, SE: standard error of coefficient estimate, and p: p-value with statistical significance shown as *** <0.001, ** <0.01, and * <.05.

Response	Predictor	Standardized Estimate	Raw Estimate	SE	P
Adult Tree Count	Budworm disturbance = Open		1.25	0.149	<0.001 ***
	Budworm disturbance = Partial		1.11	0.139	<0.001 ***
	Budworm disturbance = Closed		0.232	0.264	0.379
	Moose Exclusion = Exclosure		1.66	0.111	<0.001 ***
	Moose Exclusion = Control		0.066	0.167	0.695
Mean Height of Palatable Saplings	Moose Exclusion = Exclosure		79.5	9.88	<0.001 ***
	Moose Exclusion = Control		70.6	9.69	<0.001 ***
	Adult Tree Count	0.009	0.088	1.61	0.958
	First Horizon Percent Moisture	-0.014	-0.010	0.099	0.926
	First Horizon Depth	0.092	7.13	10.6	0.528
	First Horizon Percent Plant Material	0.323	1.65	0.659	0.024 *
Shrub Percent Coverage	Moose Exclusion = Exclosure		0.512	0.049	<0.001 ***
	Moose Exclusion = Control		0.668	0.048	<0.001 ***
	Mean Height of Palatable Saplings	-0.221	-0.001	0.0005	0.026 *
	Adult Tree Count	-0.292	-0.016	0.006	0.013 *
	First Horizon Percent Moisture	-0.128	-0.0004	0.0004	0.357
	First Horizon Depth	-0.017	-0.006	0.042	0.884
	First Horizon Percent Plant Material	0.140	0.003	0.003	0.230
Microbial C:N Ratio	Mean Height of Palatable Saplings	0.265	0.006	0.003	0.019 *
	Shrub Percent Coverage	0.190	0.967	0.558	0.093
	First Horizon Percent Moisture	-0.440	-0.008	0.002	0.003**
	First Horizon Depth	0.238	0.445	0.225	0.057
	First Horizon Percent Plant Material	-0.213	-0.026	0.015	0.081

Net N Mineralization	Microbial C:N Ratio	0.032	0.073	0.288	0.807
	First Horizon %C	-0.145	-0.036	0.062	0.583
	First Horizon %N	0.193	1.90	2.33	0.445

Table 2.4: Piecewise structural equation model results for the second (i.e., mineral) soil horizon. Results of the piecewise structural equation examining the direct and indirect impact of disturbance and moose exclusion on above-ground and below-ground function in maritime boreal forests. Disturbance is a categorical variable measured as canopy openness (i.e., open, partial, closed) created by budworm or forest harvesting. Site was included as a random intercept in each model. Standardized Estimate: standardized coefficient estimate, Raw Estimate: non-standardized coefficient estimate, SE: standard error of coefficient estimate, and p: p-value with statistical significance shown as *** <0.001, ** <0.01, and * <.05.

Response	Predictor	Standardized Estimate	Raw Estimate	SE	p
Adult Tree Count	Budworm disturbance = Open		1.18	0.184	<0.001 ***
	Budworm disturbance = Partial		1.13	0.166	<0.001 ***
	Budworm disturbance = Closed		0.575	0.294	0.050
	Moose Exclusion = Exclosure		1.70	0.130	<0.001 ***
	Moose Exclusion = Control		0.224	0.176	0.205
Mean Height of Palatable Saplings	Moose Exclusion = Exclosure		82.9	9.62	<0.001 ***
	Moose Exclusion = Control		66.7	9.61	<0.001 ***
	Adult Tree Count	0.001	0.013	1.57	0.994
	Second Horizon Percent Moisture	-0.219	-0.216	0.123	0.122
	First Horizon Depth	0.071	5.529	9.25	0.579
	First Horizon Percent Plant Material	0.292	1.448	0.597	0.026 *
Shrub Percent Coverage	Moose Exclusion = Exclosure		0.481	0.049	<0.001 ***
	Moose Exclusion = Control		0.650	0.048	<0.001 ***
	Mean Height of Palatable Saplings	-0.277	-0.001	0.001	0.010 *
	Adult Tree Count	-0.249	-0.014	0.007	0.037 *
	Second Horizon Percent Moisture	-0.378	-0.002	0.001	0.010 **
	First Horizon Depth	0.033	-0.013	0.041	0.765
	First Horizon Percent Plant Material	0.172	0.004	0.003	0.126
Microbial C:N Ratio	Mean Height of Palatable Saplings	0.003	0.0004	0.018	0.984
	Shrub Percent Coverage	-0.099	-2.77	3.39	0.423
	Second Horizon Percent Moisture	-0.133	-0.018	0.022	0.436

	First Horizon Depth	-0.038	-0.417	1.35	0.765
	First Horizon Percent Plant Material	-0.052	-0.036	0.087	0.694
Net N Mineralization	Microbial C:N Ratio	0.0004	0	0.262	0.999
	Second Horizon %C	0.145	0.025	0.049	0.630
	Second Horizon %N	0.377	2.46	1.82	0.212

Table 2.5: Linear mixed model results for the first (i.e., organic) and second (i.e., mineral) soil horizon. Results of the linear mixed models examining the direct impact of moose exclusion on below-ground function in maritime boreal forests. Site was included as a random intercept in each model. Standardized Estimate: standardized coefficient estimate, Standard Error: standard error of coefficient estimate, and Marginal and Conditional R^2: persentage of data attributed to explanitory variable and both explanitory varible and site.

Response	**Prediction Number**	**Standardized Estimate**	**Standard Error**	**Marginal and Conditional R^2**
Soil pH- First Horizon	ii	-0.054	0.065	0.002, 0.702
Second Horizon		0.073	0.064	0.004, 0.754
Soil Temperature over one month	ii	-0.550	0.080	0.021, 0.185
Soil Moisture over one month	ii	0.003	0.003	<0.001, 0.469
First Soil Horizon Depth	iii	0.294	0.112	0.049, 0.376
First Soil Horizon Percent Plant Material	iii	0.304	0.113	0.056, 0.351
Soil Total Percent C- First Horizon	iv	-0.131	1.53	<0.001, 0.617
Second Horizon		-2.178	1.22	0.012, 0.667
Soil Total Percent N- First Horizon	iv	-0.001	0.044	<0.001, 0.553
Second Horizon		-0.055	0.038	0.009, 0.641
Insect Diversity	v	-0.095	0.065	0.026, 0.047
Fungi: Bacteria Ratio- First Horizon	v	0.010	0.127	<0.001, 0.056
Second Horizon		-0.118	0.076	0.025, 0.119

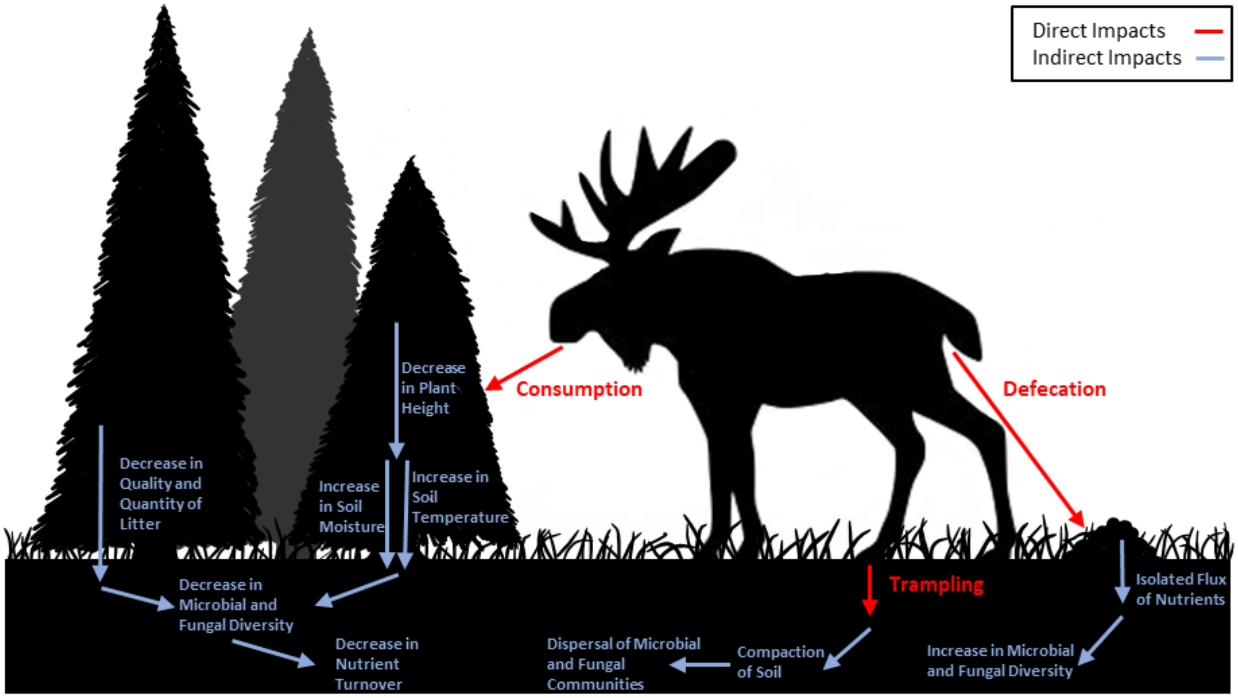

Figure 2.1: Conceptual diagram depicting of how the three main direct impacts by a browser can have far-reaching indirect impacts. Predicted direct impacts of a large ungulate browser are shown in red and indirect impacts are shown in blue (see main text for details).

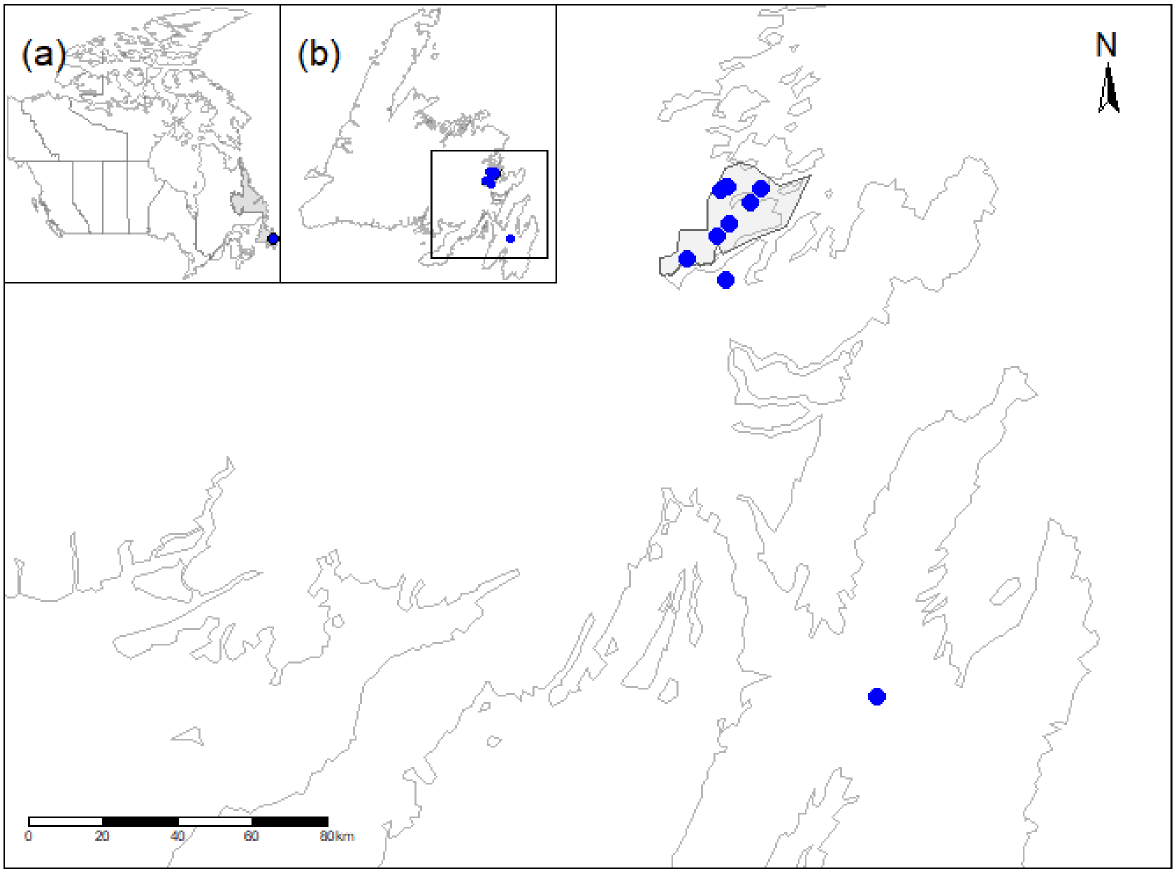

Figure 2.2: Locations of the 11 paired exclosure/control plots used in our study. Located in the eastern portion of Newfoundland, Canada, nine are in Terra Nova National Park, one in Clarenville, and one in Fox Marsh. Due to close proximity, individual dots for Blue Hill West and Blue Hill East as well as Outport Trail and Minchen's sites are overlapped and not clear at this resolution.

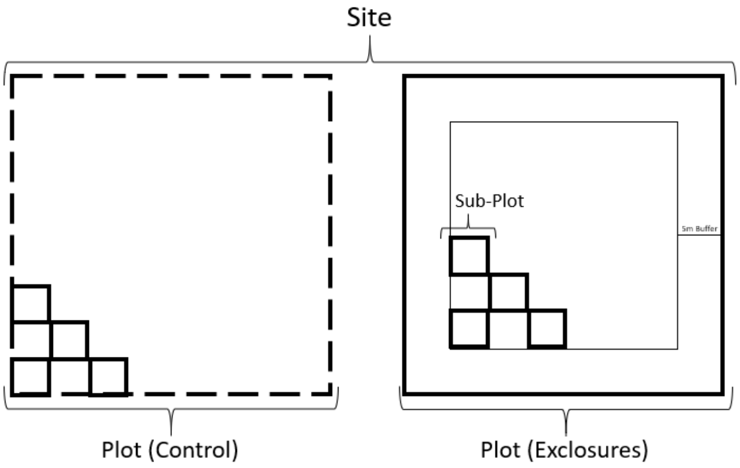

Figure 2.3: Exclosures in TNNP is 35mx35m (outside TNNP are a bit smaller). We left a 5m buffer around the edge of the fence to make sure there has been no moose impact within sub-plots. Four sampling sub-plots were established in the southwest corner of each plot. Soil cores were collected from each corner of the sub-plot. Note that the particular location of sub-plots (here shown in the lower diagonal of the plot) was not consistent at sites where moose had recently breached the site to ensure we sampled the corner with the lowest moose impact (Ocher Hill and Bread Cove in TNNP).

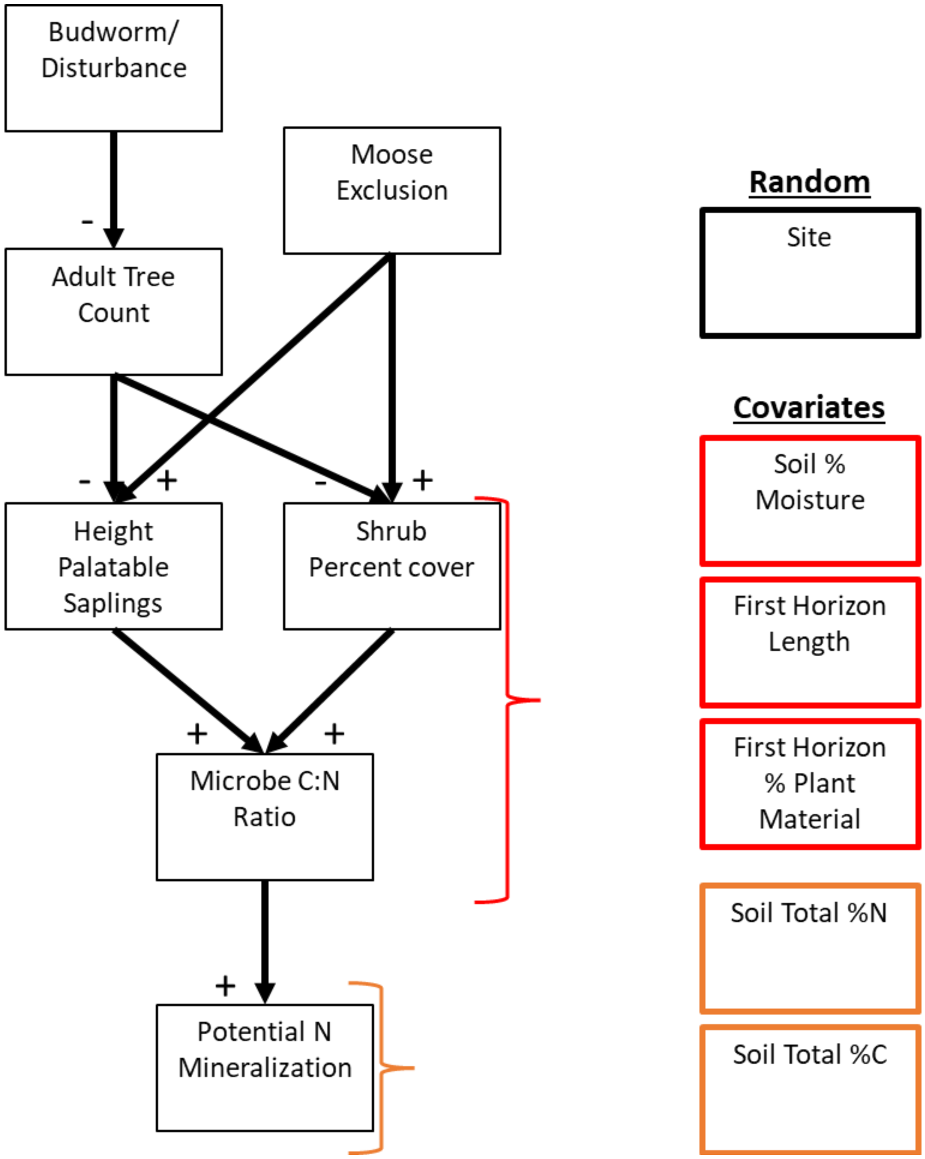

Figure 2.4: Conceptual diagram showing predicted effects of disturbance and moose exclusion on components of maritime boreal forests above-ground and below-ground. The direction of predicted effect are denoted with + (positive) or – (negative) where the arrows join the boxes. Co-variates are incorporated to include relationships that are important but not modelled as responses. This diagram acts as the blueprint for a piecewise structural equation model for both the first (i.e., organic) and second (i.e., mineral) soil horizon. See methods for further details on model structure.

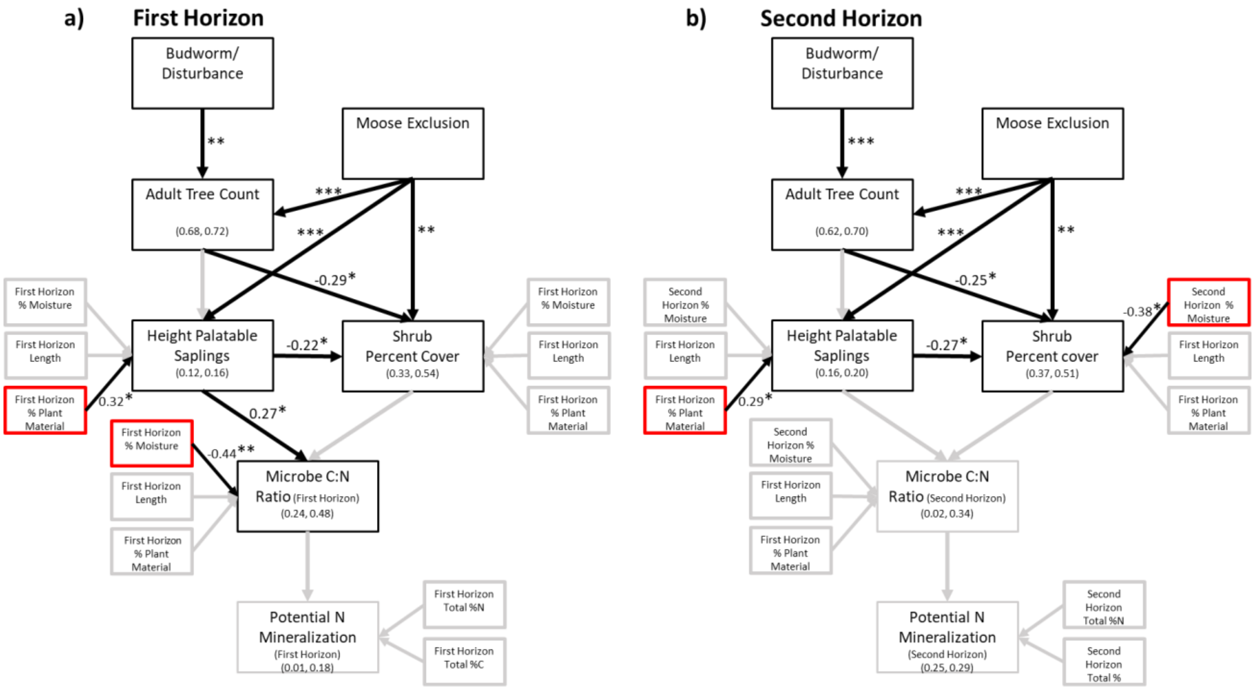

Figure 2.5: Results of piecewise structural equation models examining the impact of disturbance and moose exclusion on above and below-ground components of maritime boreal forest on the a) first (i.e., organic) and b) second (i.e., mineral) soil horizon. Black arrows and boxes denote statistically significant relationships, and gray arrows and boxes denote non-significant relationships. Numbers on black errors are the standardized coefficient estimates with *** <0.001, ** <0.01, and * < .05. Red boxes show covariates with coefficients that had a significant relationship with a main dependent varible in the model. Values at the bottom of each main box are the marginal and conditional R^2 for each model response variable.

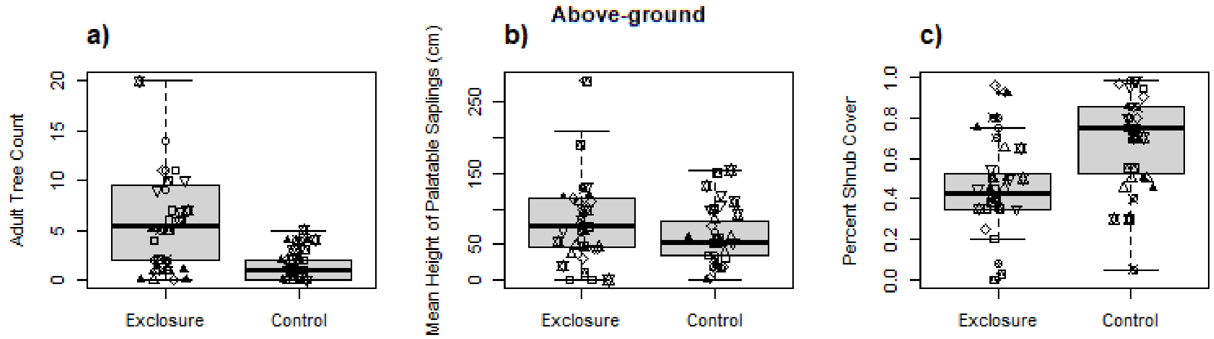

Figure 2.6: a) Adult tree count, b) mean height of palatable saplings, c) percent shrub cover in 11 paired exclosure and control sites in Newfoundland, Canada. Symbols are defined as follows: ◦=Platters Cove, ▫=Blue Hill East, ◇=Blue Hill West, △=Outport Trail, ▽=Bunyan's Cove, ✡=Blue Hill Center, ▲=Minchen's, ⊠=Halls Beach, •=Ocher Hill, ▪=Bread Cove, and ⊡=Fox Marsh.

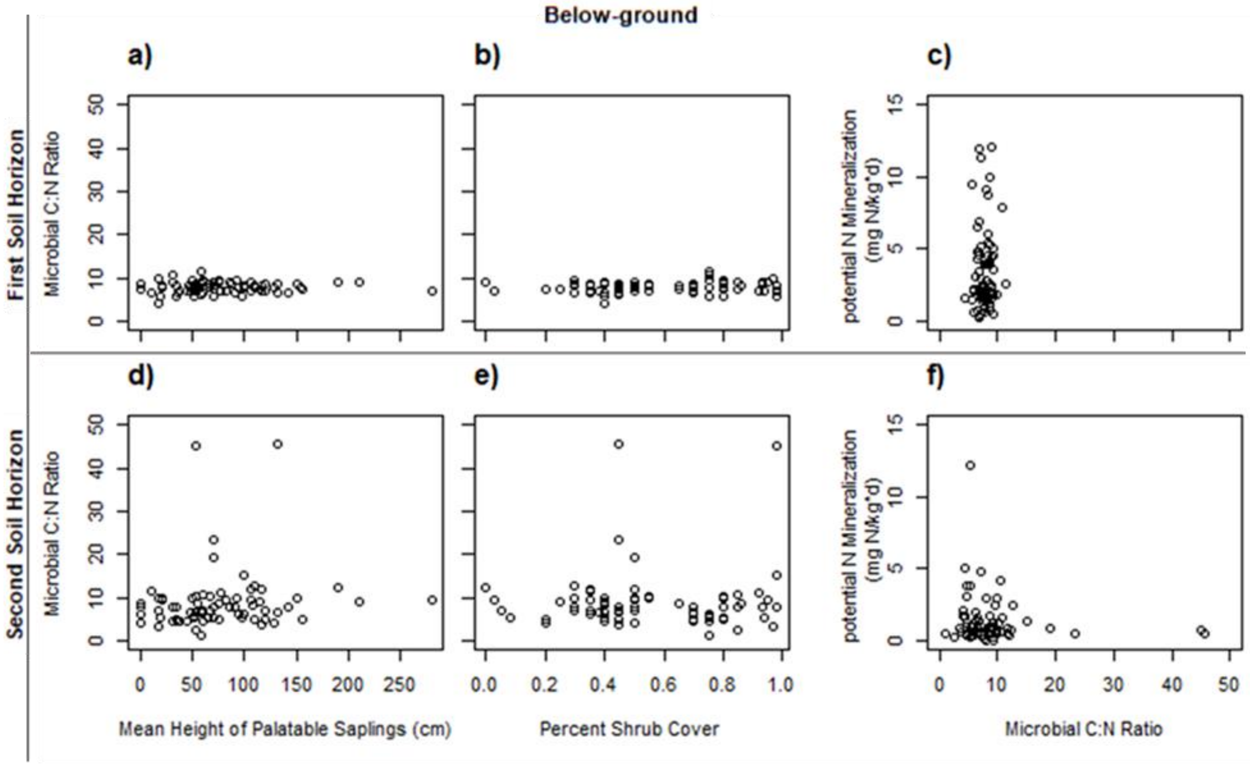

Figure 2.7: Microbial C:N ratio in the a) first soil horizon, d) second soil horizon compared to the mean height of palatable saplings. Microbial C:N ratio in the b) first soil horizon, e) second soil horizon compared to percent shrub cover. Net N mineralization in the c) first soil horizon, f) second soil horizon compared microbial C:N ratio.

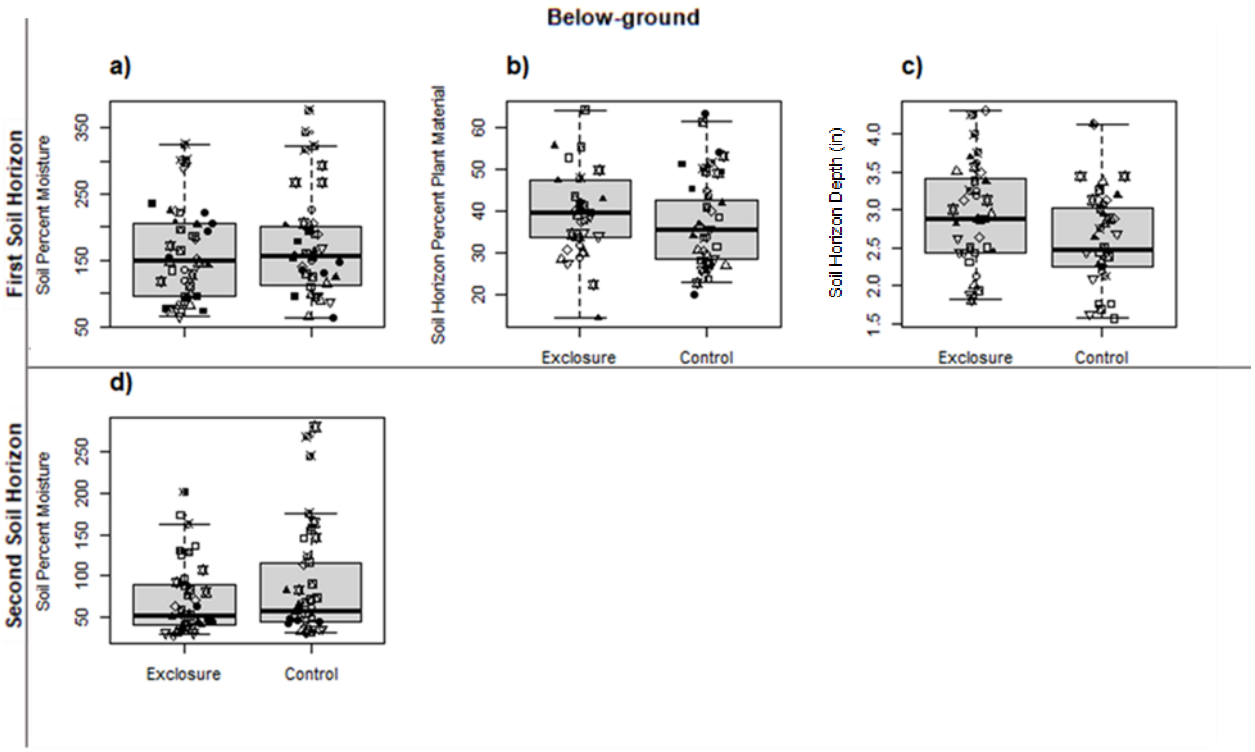

Figure 2.8: a) First soil horizon percent moisture content, b) first soil horizon percent plant material, c) first soil horizon depth, d) second soil horizon percent moisture in 11 paired exclosure and control sites in Newfoundland, Canada. Symbols are defined as follows: ○=Platters Cove, □=Blue Hill East, ◇=Blue Hill West, △=Outport Trail, ▽=Bunyan's Cove, ✡=Blue Hill Center, ▲=Minchen's, ⊠=Halls Beach, ●=Ocher Hill, ■=Bread Cove, and ⊠=Fox Marsh.

CHAPTER 3: Summary and conclusion

3.1. Impacts of moose on above-ground and below-ground systems

Large herbivore browsers, such as moose, play an active role in ecosystem functioning through the coupling of above-ground and below-ground systems through direct (e.g., trampling, foraging, and defecating) and indirect (e.g., a shift in plant community, increase in soil temperature and moisture) impacts. Plant species with high relative N and P content experience higher levels of herbivory as large herbivores selectively browse them because they require these nutrients for growth and reproduction (Olff, Ritchie, and Prins 2002). Selectively browsing of N rich woody plants by moose (Dewey et al. 1993) indirectly shifts plant community composition towards non-preferred species with a higher C:N ratio. Studies in Isle Royal, USA (Dewey et al. 1993), Newfoundland, Canada (Ellis and Leroux 2017), and Trøndelag County, Norway (Kolstad et al. 2019), found that selective browsing by moose results in decreased nutrient content and return of plant litter, which subsequently decreases the return and abundance of nutrients within the soil. Research demonstrates that the browsing of palatable plant species not only decreases above-ground abundance and nutrient content of preferred plants, but in turn, limits nutrient and organic material returned to the soil (Dewey et al. 1993; Ellis and Leroux 2017; Kolstad et al. 2018).

Despite the evidence that large herbivores impact above-ground systems, there are inconsistent results across studies and ecosystem types reporting strong, weak, or insignificant impacts of moose on below-ground systems. To better understand the reach of moose impacts below-ground, the inclusion of soil horizons and above-ground below-ground connections may help in the identification of moose impacts. Understanding the reach of moose impacts on both above-ground and below-ground systems is even more pertinent in ecosystems where moose are not native. Changes in an ecosystem's animal species composition through human mediated introduction (e.g., development of land and invasion) and removal (e.g., hunting and habitat loss) of animal species also has profound impacts that drive local and global ecosystem change (Wardle et al. 2011). When considering native and non-native moose impacts, connecting above-ground and below-ground systems allows us to better understand the role large herbivore browsers play in ecosystem function.

We aimed to identify the extent of moose impact on both above-ground and below-ground systems in the maritime boreal forests of Newfoundland, Canada. Using 11 paired exclosure and control plots, we tested a series of hypotheses based on previous studies that identify large herbivore impacts above-ground and below-ground. The following results were in support of our hypotheses:

- Moose presence had a negative effect on palatable sapling height and adult tree count and a positive effect on shrub cover
- Moose presence had a negative effect on the depth and percent plant material content of the first soil horizon
- Moose presence had a positive effect on soil temperature and moisture
- Moose presence had an indirect negative impact on soil microbial C:N ratio and this effect was only observed in the first soil horizon (i.e., organic horizon)

The above results are consistent with previous research on large herbivore impacts on boreal forests and soils. Ellis and Leroux (2017) found that Newfoundland's moose population decreased the overall height of both palatable and non-palatable plant species and decreased first soil horizon depth. A study of moose browsing over a six year period in the Gaspésie Peninsula in Quebec by De Vriendt et al. (2021) found that moose browsing decreased tree sapling density and rate of saplings reaching canopy height. Also, an eight year exclusion study in Norway by (Kolstad et al. 2018) found that moose presence increased soil temperature and moisture and decreased first soil horizon depth. Our results align with previous studies that reveal moose have impacts on above-ground plant communities and some cascading impacts below-ground on soil composition, conditions, and organismal communities.

We found moose had a direct impact on plant communities, and soil composition and conditions and an indirect impact on microbial C:N ratio; however, the following results did not support our hypotheses:

- Moose presence had no effect on potential nitrogen mineralization rates
- Moose presence had no effect on soil percent C and N
- Moose presence had no effect on soil pH
- Moose presence had no effect on insect abundance and diversity

The above results are contradictory to previous research on large herbivore impacts. For example, Niwa et al. (2008) found that when herbivore browsing intensity increased, N mineralization rates decreased. Classic studies of moose impacts on Isle Royale, USA, ecosystems demonstrated that moose had a negative effect on soil nutrient availability (Pastor et al. 1988; Dewey et al. 1993). However, more recent research in other boreal ecosystems, report limited impacts of moose on soil nutrient availability (Ellis and Leroux 2017; Kolstad et al. 2018). We note that important methodological differences in sampling and what was measured among studies could attribute to the differences in findings between classical and more resent research on herbivore impacts on soils. In addition, previous studies have found that large herbivore presence increased soil pH (Ellis and Leroux 2017) and decreased insect diversity (Côté et al. 2014).

Moose above-ground impacts are more apparent due to the heavy browsing of palatable saplings decreasing overall sapling height and reducing the amount of saplings that mature to adult height. Research across boreal study systems find variable indirect effects of moose on soil ecosystems (Dewey et al. 1993; Ellis and Leroux 2017; Kolstad et al. 2018; 2019). We found moose impacts below-ground were only observed in the microbial C:N ratios in the first soil horizon. By separating soils by lines of horizon break, we surmise that we were able to more accurately isolate moose impacts on soils. An eight year exclusion study by Kolstad et al. (2018) also separated soil horizons but found limited evidence that direct moose impacts had cascading indirect impacts below-ground. We were able to identify indirect impacts to microbial communities which may be the first sign of moose impacts on soil functioning and nutrient turnover. We surmise that the longer exclusion length at our sites (22-25 years) is approaching a more consistent timeframe to capture the turnover of nutrients in the first soil layer (O’brien and Stout 1978). Formation and turnover of soil horizons can take decades to centuries (Perrin, Willis, and Hodge 1964; O’brien and Stout 1978) and is dictated by outside abiotic and biotic factors (Jenny 1941). Consequently, the length of our study gives us better inference to identify potential below-ground moose impacts when compared to studies over shorter time frames. We found that moose have strong direct impacts on above-ground systems and have weak but measurable indirect impacts on below-ground systems in Newfoundland over a 22-25 year period. Our findings provide further context to how the introduction of moose to Newfoundland over 100 years ago is altering Newfoundland’s forest ecosystems.

3.2. Limitations and Implications

Globally, the direct and indirect impacts large herbivores are documented on both above-ground and below-ground systems (see reviews in Wardle et al. 2004; Andriuzzi and Wall 2017; Forbes et al. 2019). Contrary to our findings, there is strong support of large herbivore presence decreasing soil C (Dewey et al. 1993; Frank et al. 2011; Gass and Binkley 2011; Peschel et al. 2015) and N (Pastor et al. 1988; Dewey et al. 1993; Gass and Binkley 2011) content and potential N mineralization rates (Peschel et al. 2015). Our evidence of weak to no apparent effect of Newfoundland's moose population on below-ground components of nutrient cycling could be attributed to forest primary productivity and moose exclusion time. The maritime boreal forests of Newfoundland and boreal forests more generally, have lower primary productivity than most temperate and tropical forest ecosystems. In addition, the low productivity and growth rate of plants in Newfoundland may require a longer time to reveal moose impacts below-ground (Osem, Perevolotsky, and Kigel 2002; Leroux, Wiersma, and Vander Wal 2020). For example, a 40 year exclusion study by Dewey et al. (1993) in Isle Royale, Michigan, USA, revealed a negative effect of moose browsing on net soil N mineralization rates. Consequently, it will be important to continue to monitor the long-term exclosure-control sites we have measured to track moose effects throughout forest regeneration.

It is important to note that few large herbivore-ecosystem studies report the disturbance history of their sites. Changes in an ecosystem can reroute normal ecosystem function; however, changes take time to become detectible. Moose were introduced and established to the island of Newfoundland in 1904 (Pimlott 1953) and now moose densities on the islands are among the highest reported across their circumboreal distribution (McLaren et al. 2004). Exclosure sites used in our study were established between 22 and 25 years prior to this study's data collection.

Exclosures in Newfoundland were erected approximately 90 years after the introduction of moose (Pimlott 1953) and approximately 10 years after the most recent population peak so they could still contain some legacy effects of moose interactions (McLaren et al. 2004; Gosse et al. 2011; Leroux et al. 2021). Future studies should report the disturbance history and length of exclusion to ensure these aspects are included when considering the strength of impacts reported. By understanding the potential for a legacy of moose impacts, we can track the rate at which the legacy of moose impacts diminish over time after exclusion.

Findings from my study give us an understanding of the extent of moose impacts on soil functioning in Newfoundland. Knowing the current extent of moose impacts can help with future conservation and protection of Newfoundland’s native forest ecosystems. Evidence of moose significantly altering the natural boreal forest of Newfoundland supports the importance of preserving and restoring Newfoundland’s natural habitats (Gosse et al. 2011). Many suggest that a key to reestablishing natural boreal forests includes the mass planting of heavily browsed sapling species (Charron and Hermanutz 2017; Noonan, Leroux, and Hermanutz 2021). To ensure the success of saplings, one must ensure soil conditions and soil nutrient composition will support sapling growth (Kardol et al. 2014). We recommend that restoration strategies integrate soil and vegetation reintroduction in order to increase the chances of successful boreal forest restoration (Holden and Treseder 2013; Pec et al. 2019). Microbial communities are integral for maintaining and promoting many aspects of nutrient cycling and plant growth (Högberg et al. 2017; Hestrin et al. 2019; Li et al. 2019; Pec et al. 2019). Additionally, we recommend that further research be done to catalog and understand the extent of moose impact on crucial microbial species and the resulting potential impacts on ecosystem function.

Plants in boreal forests have higher percent biomass in belowground roots when compared to other forest types (e.g., temperate and tropical), with 80%-90% of roots within the top 30cm of soil (Jackson et al. 1996). The low productivity nature of boreal forests also requires plants to have higher proportions of biomass below-ground (Osem, Perevolotsky, and Kigel 2002). Areas with low productivity and a higher proportion of plant biomass below-ground may be more resistant to shifts in plant community due to herbivores primarily impacting a plant's above-ground biomass (Milchunas and Lauenroth 1993). Large herbivores are known to have an impact on a plant's below-ground biomass. For example, a study by Bagchi and Ritchie (2010) found that large herbivore grazing in the Trans-Himalayan shrub-steppes decreased shrub below-ground biomass by 35%. Higher below-ground plant biomass in turn maintains a higher microbial biomass (Fierer et al. 2009). A future direction for research on moose impacts in Newfoundland could study the impacts of moose presence and absence on plant below-ground biomass. Understanding how moose impacts plant below-ground biomass may help explain why we see slower shifts in plant community and soil composition and conditions in Newfoundland's maritime boreal forests.

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APPENDIX

Table A.1: Dates of initial and secondary visit for each site. Initial visit included plant survey, soil temperature and moisture probe placement, pitfall trap placement, initial and secondary core collection, secondary core burial. Secondary visit included collection of soil temperature and moisture probe, pitfall traps, and secondary core.

Site	Initial Visit	Secondary Visit
Platters Cove	7/20/2020	8/20/2020
Blue Hill East	7/21/2020	8/20/2020
Blue Hill West	7/22/2020	8/20/2020
Outport Trail	7/23/2020	8/21/2020
Bunyan’s Cove Center	7/25/2020	8/24/2020
Blue Hill Center	7/26/2020	8/25/2020
Minchin’s trail	7/28/2020	8/28/2020
Halls Beach	7/29/2020	9/2/2020
Ocher Hill	7/30/2020	8/28/2020
Bread Cove	7/31/2020	8/28/2020
Fox Marsh	8/2/2020	9/1/2020

Table A.2: Dates soil processing and time sensitive lab procedures were completed for each site.

Site	Soil Sifting Initial Bag	Soil Sifting Secondary Bag	DNA Extraction	Chloroform Fumigation
Platters Cove	8/5/2020 and 8/6/2020	9/4/2020	8/5/2020 and 8/6/2020	9/11/2020
Blue Hill East	8/7/2020 and 8/8/2020	9/8/2020	8/7/2020 and 8/8/2020 and 8/9/2020	9/14/2020
Blue Hill West	8/9/2020 and 8/12/2020	9/9/2020	8/9/2020 and 8/12/2020 and 8/13/2020	9/18/2020
Outport Trail	8/12/2020 and 8/13/2020	9/10/2020	8/14/2020	9/21/2020
Bunyan’s Cove Center	8/14/2020 and 8/16/2020	9/14/2020	8/16/2020 and 8/18/2020	9/25/2020
Blue Hill Center	8/18/2020 and 8/19/2020	9/17/2020	8/26/2020 and 9/8/2020	10/9/2020
Minchin’s trail	8/19/2020 and 8/20/2020	9/21/2020	8/26/2020 and 8/27/2020	10/12/2020
Halls Beach	8/21/2020 and 8/24/2020	9/25/2020	8/27/2020	10/16/2020
Ocher Hill	8/25/2020	9/23/2020	8/27/2020 and 9/4/2020	10/19/2020
Bread Cove	8/26/2020	9/24/2020	9/8/2020 and 9/9/2020	10/22/2020
Fox Marsh	8/27/2020 and 9/1/2020	9/28/2020	9/9/2020 and 9/10/2020	10/26/2020

Table A.3: Mean DNA percent abundance for each bacterial phyla identified for the first (i.e., organic) and second (i.e., mineral) soil horizon.

Phyla	**Median Abundance – Exclosure First Horizon (%)**	**Median Abundance - Control First Horizon (%)**	**Median Abundance – Exclosure Second Horizon (%)**	**Median Abundance – Control Second Horizon (%)**
Proteobacteria	34.7	34.2	37.0	33.8
Actinobacteria	16.6	19.1	17.1	17.8
Acidobacteria	12.3	13.2	12.4	13.6
Bacteroidetes	7.87	6.67	4.36	4.09
Unknown	6.75	7.60	7.43	8.08
Firmicutes	3.28	3.73	4.85	4.71
Thermotogae	3.14	2.15	3.43	1.62
Fibrobacteres	2.85	3.10	5.32	4.47
Planctomycetes	2.60	2.72	3.37	3.16
Tenericutes	0.361	0.339	0.201	0.264
Thermodesulfobacteria	0.267	0.544	0.377	0.527
Nitrospirae	0.201	0.240	0.386	0.520
Verrucomicrobia	0.197	0.196	0.263	0.309
Gemmatimonadetes	0.189	0.198	0.258	0.265
Chloroflexi	0.179	0.107	0.356	0.243
Chlamydiae	0.166	0.202	0.251	0.251
Cyanobacteria	0.161	0.220	0.258	0.210
Lentisphaerae	0.128	0.099	0.115	0.088
Chlorobi	0.060	0.085	0.104	0.111
Thermi	0.058	0.054	0.040	0.047
Synergistetes	0.041	0.065	0.080	0.132
Armatimonadetes	0.031	0.039	0.026	0.032
Spirochaetes	0.026	0.025	0.055	0.037
Caldithrix	0.015	0.014	0.058	0.017
Fusobacteria	0.007	0.181	0.099	0.146
Chrysiogenetes	0.005	0.001	0.001	0.001
Deferribacteres	0.005	0.008	0.016	0.012
Elusimicrobia	0.004	0.005	0.003	0.003
Caldiserica	0.002	0.004	0.002	0.003
Euryarchaeota	0.001	0.002	0.002	0.002

Table A.4: Mean DNA percent abundance for each phyla identified from targeting the ITS region which targest primarlly fugal pylas for the first (i.e., organic) and second (i.e., mineral) soil horizon.

Phyla	**Median Abundance – Exclosure First Horizon (%)**	**Median Abundance - Control First Horizon (%)**	**Median Abundance – Exclosure Second Horizon (%)**	**Median Abundance – Control Second Horizon (%)**
Basidiomycota	55.1	47.5	53.6	46.3
Ascomycota	35.6	44.6	35.4	44.6
Mortierellomycota	2.78	2.93	4.61	3.39
Unknown	2.42	2.64	2.58	2.47
Rozellomycota	0.233	0.290	0.515	0.395
Mucoromycota	0.118	0.084	0.396	0.147
Chytridiomycota	0.081	0.122	0.141	0.121
Entomophthoromycota	0.059	0.088	0.111	0.109
Streptophycophyta	0.055	0.079	0.104	0.083
Cercozoa	0.044	0.039	0.053	0.040
Glomeromycota	0.032	0.062	0.076	0.087
unidentified	0.021	0.028	0.043	0.032
GS19	0.017	0.007	0.005	0.019
Olpidiomycota	0.016	0.015	0.018	0.022
Arthropoda	0.014	0.013	0.006	0.006
Protozoa_phy_Incertae_sedis	0.012	0.014	0.004	0.006
Zoopagomycota	0.010	0.015	0.145	0.012
Entorrhizomycota	0.008	0.008	0.010	0.005
Blastocladiomycota	0.007	0.012	0.007	0.013
Rotifera	0.007	0.008	0.007	0.007
Kickxellomycota	0.005	0.004	0.006	0.006
Monoblepharomycota	0.004	0.003	0.002	0.002
Neocallimastigomycota	0.003	0.002	0.002	0.002
Anthophyta	0.002	0.001	0.002	0.002
Chlorophyta	0.002	0.001	*NA*	*NA*
Annelida	0.002	0.007	*NA*	*NA*
Cnidaria	0.002	0.002	0.002	0.003
Calcarisporiellomycota	0.002	*NA*	0.002	0.004
Ochrophyta	*NA*	0.002	*NA*	*NA*
GS01	*NA*	*NA*	*NA*	*NA*

Table A.5: Piecewise structural equation model results for the first (i.e., organic) soil horizon when we removed sub-plots where the first horizon had < 17% C. Disturbance is a categorical variable measured as canopy openness (i.e., open, partial, closed) created by budworm or forest harvesting. Site was included as a random intercept in each model. Standardized Estimate: standardized coefficient estimate, Raw Estimate: non-standardized coefficient estimate, SE: standard error of coefficient estimate, and p: p-value with statistical significance shown as *** <0.001, ** <0.01, and * <.05.

Response	Predictor	Standardized Estimate	Raw Estimate	SE	P
Adult Tree Count	Budworm disturbance = Open		1.25	0.152	<0.001 ***
	Budworm disturbance = Partial		1.09	0.150	<0.001 ***
	Budworm disturbance = Closed		0.301	0.267	0.251
	Moose Exclusion= Exclosure		1.67	0.116	<0.001 ***
	Moose Exclusion = Control		0.092	0.174	0.594
Mean Height of Palatable Saplings	Moose Exclusion = Exclosure		80.3	12.1	<0.001 ***
	Moose Exclusion = Control		70.8	11.2	<0.001 ***
	Adult Tree Count	-0.062	-0.725	1.70	0.680
	First Horizon Percent Moisture	-0.015	-0.011	0.122	0.932
	First Horizon Depth	0.159	12.4	11.7	0.317
	First Horizon Percent Plant Material	0.378	2.02	0.779	0.015 *
Shrub Percent Coverage	Moose Exclusion = Exclosure		0.532	0.052	<0.001 ***
	Moose Exclusion = Control		0.668	0.049	<0.001 ***
	Mean Height of Palatable Saplings	-0.265	-0.001	0.0005	0.025 *
	Adult Tree Count	-0.317	-0.018	0.007	0.014 *
	First Horizon Percent Moisture	-0.182	-0.0006	0.0005	0.246
	First Horizon Depth	-0.016	-0.006	0.047	0.903
	First Horizon Percent Plant Material	0.145	0.004	0.003	0.280
Microbial C:N Ratio	Mean Height of Palatable Saplings	0.320	0.007	0.003	0.015 *
	Shrub Percent Coverage	0.147	0.710	0.578	0.235
	First Horizon Percent Moisture	-0.454	-0.008	0.003	0.007**
	First Horizon Depth	0.177	0.314	0.232	0.196
	First Horizon Percent Plant Material	-0.271	-0.033	0.016	0.048
Net N Mineralization	Microbial C:N Ratio	0.065	0.161	0.348	0.657
	First Horizon %C	-0.226	-0.067	0.067	0.355

First Horizon %N	0.211	2.31	2.49	0.387

Table A.6: Piecewise structural equation model results for the second (i.e., mineral) soil horizon when we removed sub-plots where the second horizon had > 17% C. Disturbance is a categorical variable measured as canopy openness (i.e., open, partial, closed) created by budworm or forest harvesting. Site was included as a random intercept in each model. Standardized Estimate: standardized coefficient estimate, Raw Estimate: non-standardized coefficient estimate, SE: standard error of coefficient estimate, and p: p-value with statistical significance shown as *** <0.001, ** <0.01, and * <.05.

Response	Predictor	Standardized Estimate	Raw Estimate	SE	p
Adult Tree Count	Budworm disturbance = Open		1.15	0.208	<0.001 ***
	Budworm disturbance = Partial		1.09	0.176	<0.001 ***
	Budworm disturbance = Closed		0.045	0.476	0.924
	Moose Exclusion = Exclosure		1.56	0.185	<0.001 ***
	Moose Exclusion = Control		-0.038	0.232	0.872
Mean Height of Palatable Saplings	Moose Exclusion = Exclosure		93.1	11.5	<0.001 ***
	Moose Exclusion = Control		68.7	11.2	<0.001 ***
	Adult Tree Count	-0.049	-0.538	1.76	0.771
	Second Horizon Percent Moisture	0.103	0.192	0.278	0.528
	First Horizon Depth	-0.029	-2.48	12.0	0.847
	First Horizon Percent Plant Material	0.270	1.30	0.703	0.092
Shrub Percent Coverage	Moose Exclusion = Exclosure		0.481	0.051	<0.001 ***
	Moose Exclusion = Control		0.665	0.053	<0.001 ***
	Mean Height of Palatable Saplings	-0.365	-0.002	0.0005	0.002 *
	Adult Tree Count	-0.191	-0.010	0.007	0.138
	Second Horizon Percent Moisture	-0.184	-0.002	0.001	0.197
	First Horizon Depth	0.021	-0.009	0.047	0.856
	First Horizon Percent Plant Material	0.205	0.005	0.003	0.107
Microbial C:N Ratio	Mean Height of Palatable Saplings	0.045	0.007	0.023	0.771
	Shrub Percent Coverage	-0.065	-1.96	4.53	0.676
	Second Horizon Percent Moisture	-0.103	-0.029	0.045	0.547
	First Horizon Depth	-0.046	-0.604	1.81	0.748

	First Horizon Percent Plant Material	-0.096	-0.069	0.109	0.545
Net N Mineralization	Microbial C:N Ratio	0.0215	0.002	0.013	0.889
	Second Horizon %C	-0.170	-0.036	0.063	0.603
	Second Horizon %N	0.315	1.46	1.39	0.328

Table A.7: Median for each sub analysis response varible. Plot times increase coloum reports plot type with the higest meams and how much higher it is dispayed as how many times larger.

Response	Prediction Number	Exclosure Median	Control Median	Plot Times Increase
Soil pH- First Horizon	ii	4.44	4.57	Control: 1.03x
Second Horizon		4.87	4.73	Exclosure: 1.03x
Soil Temperature over one month	ii	14.8°C	15.7°C	Control: 1.06x
Soil Moisture over one month	ii	0.239 m^3/m^3	0.214 m^3/m^3	Exclosure: 1.10x
First Soil Horizon Depth	iii	3.06 in	2.44 in	Exclosure: 1.20x
First Soil Horizon Percent Plant Material	iii	39.4%	27.6%	Exclosure: 1.05x
Soil Total Percent C- First Horizon	iv	29.3%	29.4%	Control: 1.00x
Second Horizon		5.54%	5.34%	Exclosure: 1.04x
Soil Total Percent N- First Horizon	iv	0.94%	0.81%	Exclosure: 1.14x
Second Horizon		0.215%	0.25%	Control: 1.14x
Insect Diversity	v	1.25	1.34	Control: 1.06x
Fungi: Bacteria Ratio- First Horizon	v	0.804	0.773	Exclosure: 1.04x
Second Horizon		0.810	0.789	Exclosure: 1.03x

Table A.8: Percent abundance of each macro-organism order collected using pitfall traps for both exclosure and control plots.

Order	Exclosure	Control
Stylommatophora	13.9	17.6
Coleoptera	47.4	32.4
Araneae	3.0	4.91
Diptera	13.3	8.50
Hymenoptera	16.8	28.0
Lithobiomorpha	0.618	0.756
Opisthopora	1.65	1.64
Acari	0.772	0.378
Isopoda	1.60	3.46
Amphipoda	0.721	1.20
Lepidoptera	0.309	0.315
Orthoptera	0.051	0.819

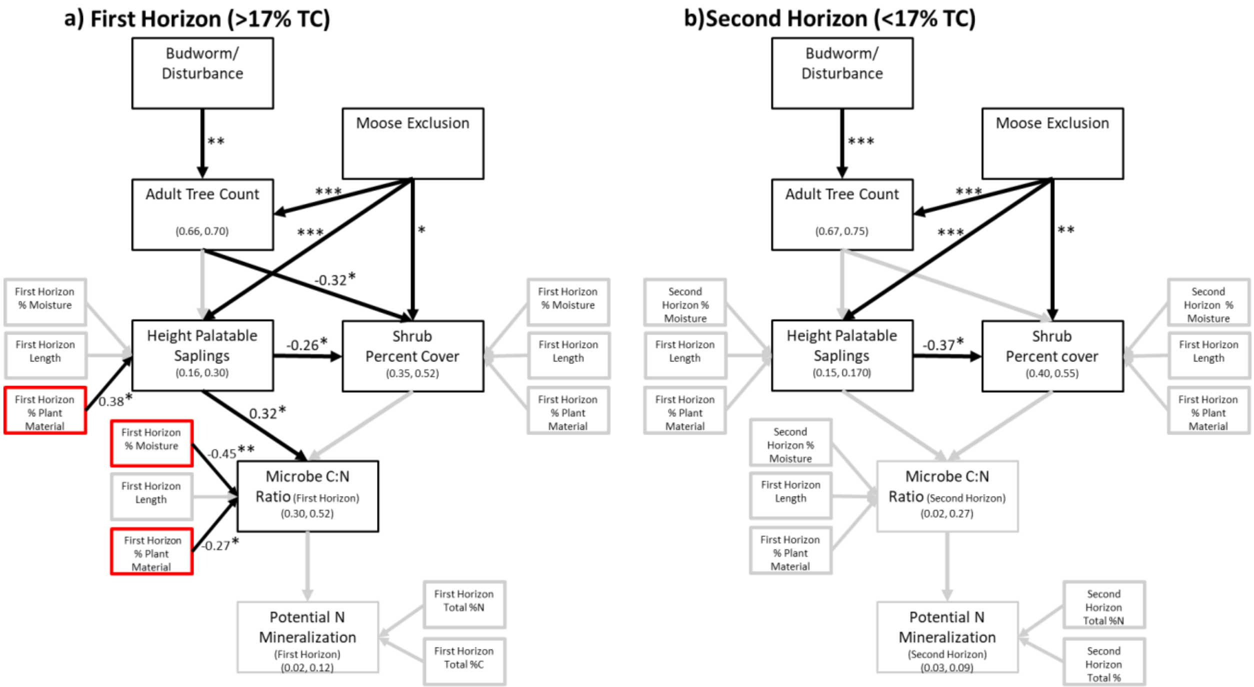

Figure A.1: Results of piecewise structural equation models examining the impact of disturbance and moose exclusion on above and below-ground components of maritime boreal forest on the a) first (i.e., organic) and b) second (i.e., mineral) soil horizon. For these models, we removed sub-plots where the first horizon had < 17% C and the second horizon had > 17% C. Black arrows and boxes denote statistically significant relationships, and gray arrows and boxes denote non-significant relationships. Numbers on black arrors are the standardized coefficient estimates with *** <0.001, ** <0.01, and * < .05. Red boxes show covariates with coefficients that had a significant relationship with a main dependent varible in the model. Values at the bottom of each main box are the marginal and conditional R^2 for each model response variable.