University of Alberta

Mountain pine beetle outbreak and ectomycorrhizal feedback: the ecology of recovery in beetle killed forests

by

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DEDICATION

I dedicate this thesis to my family: Corinne & William, Katrina, & Phil. You were always there for me on the other line—to console me during the cold seasons when the heart grows weary, and the mind doubtful of the merit of a graduate pursuit. Your unmoving support of my aspirations can never be repaid. I thank my brother Phil, who, in firmly following his passion for astrophysics, has inspired me to look out widely across the sky of life, and aim *far*—20 or so light-years beyond the Milky Way!

ABSTRACT

The expansion of mountain pine beetle (*Dendroctonus ponderosae*; MPB) into naïve host ecosystems has been met by gaps in two key areas of research: (A) affects on the chemistry of forest soils, and (B) impacts on the regeneration of tree seedlings. To investigate linkages between both, we paired observational field and experimental greenhouse studies. In the field study, we used a natural continuum of recent (0-3 yrs) MPB-caused tree mortality (0-84%) in naïve lodgepole pine-dominated (*Pinus contorta*) forests in northwestern Alberta, to quantify impacts of MPB outbreak on: (1) input rates of pine needle-derived nutrients; (2) supply rates of plant-available nutrients; (3) concentrations of soil phenols. We found positive associations between tree mortality and pine needle nutrient concentrations (of nitrogen, phosphorus, potassium, calcium, and magnesium) and inputs (of nitrogen and phosphorus). The supply rates for plant available nitrate, and concentrations of soil phenols were altered by MPB disturbance. In the greenhouse study, we tested the main effects and interactions of (1) light intensity, (2) pine needle litter addition, and (3) soil inoculation on first-year growth of seedlings of lodgepole pine and white spruce (*Picea glauca*). Soil inoculation had the greatest impact on accumulations of biomass, and sugar and starch reserves. Seedlings of lodgepole pine grown with inocula originating from uninfested stands had enhanced biomass accumulation relative to controls; accumulation was reduced when seedlings were inoculated with soils from MPB-infested stands. Changes in soil microbial communities may limit pine regeneration following outbreak, but field studies are needed confirm this effect.

PREFACE

This document presents two ecological studies addressing two broad objectives outlined in an NSERC Strategic grant. The ideas and questions of the awarded proposal were conceived in 2010. Justine D. Karst (University of Alberta; U of A) was the project lead author, and subsequently led and participated in multiple both while serving as Killiam Postdoctoral Fellow. The proposal was awarded to principle investigators, James F. Cahill Jr. (U of A), Nadir Erbilgin (U of A), Suzanne W. Simard (University of British Columbia), and Janice E. Cooke (U of A).

The findings highlighted in this document are intended for publication and integration with additional studies (associated with the parent NSERC Strategic award) in an effort to provide an accessible synthesis to scientists and managers of the potential implications of insect disturbance on forest recovery as mediated through ecotomycorrhizal facilitation.

This thesis presents one field and one greenhouse study providing evidence needed to answer an overarching question: By what mechanisms does mountain pine beetle outbreak (MPB) impact the establishment of tree seedlings in naïve lodgepole pine-dominated forests? The findings from Chapter One—field study—highlight effects of MPB disturbance on the chemistry of forest soils in western Alberta, Canada. The findings from Chapter Two—greenhouse study—highlight the effects of altered litter, light, and ectomycorrhizal associations on conifer seedling establishment and growth.

This document was written by Paul W. Cigan. Alex Sywenky collected and processed root biomass data (Chp One). Gregory J. Pec assisted in the set-up of the greenhouse experiment and processing of seedling tissue (Chp Two). P.W. Cigan collected and processed all additional data sets, performed all statistics, and generated all the figures and tables (Chps One—Two). J.D. Karst, N. Erbilgin, and J.F. Cahill provided statistics and editing assistance. N. Erbilgin was the academic primary supervisor to P.W. Cigan, J.F. Cahill his academic co-supervisor, and J.D. Karst a research mentor to him.

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MPB	Mountain pine beetle
NSC	Non-structural carbohydrate
ECM	Ectomycorrhizal
TSB	Time since beetle

CHAPTER ONE

Thesis Introduction

1. Introduction

1.1.1 Ecological disturbances and global change

Insect disturbances are important phenomena that impact nearly all forest ecosystems because they can influence ecosystem functioning and species diversity (Connell 1978; Denslow 1985). An expanding body of knowledge gained through ecological research during the last decade has documented increasing amplitude (i.e., intensity and severity) among many types of insect disturbances (e.g., bark beetles; Raffa et al. 2008). The result of increasing disturbance amplitude may be the breaching of ecological amplitude (i.e., adaptive tolerance) for native tree species and whole forest ecosystems (Dunster and Dunster 1996), potentially causing major ecological re-organizations (e.g., change in community and trophic structure) and extinction events at biome-scales (Logan and Powell 2001; Erbilgin et al. 2014). Moreover, interactions and feedbacks between insect disturbance and global climate change—though generally difficult to quantify—are predicted to exacerbate the strain placed on ecological systems by disturbances (Dale et al. 2001; Erbilgin et al. 2014). As a result, efforts to manage ecosystems and renewable resources will face unprecedented challenges in the 21st century as changing disturbances regimes—insect & pathogen epidemics, climate-induced forest dieback, fire regime intensification—potentially stymie the range, quality, and quantity of ecological, economic, and social values exploited from nature (Raffa et al. 2008; Allen et al. 2010; van Mantgem et al. 2013). Nevertheless, basic science aimed to quantify the impact of current disturbances—while predicting those of the future—on all levels of ecological diversity and function will be of increasing importance relative to developing and applying more effective knowledge and management capacities to meet forthcoming challenges. In North America, for instance, great effort has been placed on quantifying current impacts and the future dynamics of the most severe biotic disturbance facing the continent—bark beetle epidemics.

1.1.2 Mountain pine beetle disturbance

Currently, the conifer-dominated forests of western North America are experiencing the most severe and widespread epidemics of mountain pine beetle (*Dendroctonus ponderosae* Hopkins; MPB hereafter) in recorded history (Safranyik et al. 2010; Man 2012); outbreaks now impact approximately 28 M hectares of lodgepole pine (*Pinus contorta* var. *latifolia* Engelm**)** and lodgepole x jack pine hybrid (*Pinus banksiana* Lamb.) forest (Safranyik et al. 2010; Alberta Sustainable Resource Development 2012a; British Columbia Ministry of Forests, Lands and Natural Resource operations 2012). The outbreaks exceed the natural range of variability for all biotic disturbance agents of western North America in scale and severity and suggest an impending onset of severe consequences for the ecological stability of lodgepole pine-dominated forests throughout western Canada. Despite initiatives by Canadian federal and provincial governments and the forest industry to abate the spread of MPB outbreaks (Ono et al. 2003), epidemic populations of MPB from BC have expanded into Alberta's lodgepole pine and jack pine hybrid zone (Cullingham et al. 2011); this is a naïve host ecosystem where both the impacts of beetle disturbance on ecosystem health and the probability of further success and expansion of MPB remain uncertain. Forest composed of naïve hosts are potential 'stepping stones' for further beetle expansion across the entire boreal forest of North America (Lusebrink et al. 2013; Erbilgin et al. 2014). Consequently, host-range expansion poses a transcontinental threat to Canadian forests and incites major questions about the long-term health of its forests at the biome scale.

Ecologists continue to document a significant cumulative impact of beetle epidemics within affected landscapes; this includes changes in productivity (Kurz et al. 2008), successional trajectories (Caldwell et al. 2013), and planning horizons for ecosystem management (Alberta Sustainable Resource Development 2012b). Furthermore, evidence from recent studies demonstrates that MPB epidemics may reduce biodiversity (Martin et al. 2006), accelerate nutrient cycling (Griffin et. al. 2011), simplify forest structure (Klutsch et al. 2009), and increase the risk of wildfire (Page et al. 2012). As such, integrative research that investigates the impacts of MPB attack on forest structure, soil chemistry, and ecological interactions is imperative to understand long-term impacts on forest resilience.

1.1.3 Research gaps for MPB disturbance

The ecological resilience of beetle-killed forests (i.e., capacity to recover), as well as the rapid advance of the MPB leading-edge zone together underscore two areas of critical, yet lacking research that might help quantify environmental impacts associated with MPB outbreaks. The first and perhaps most critical area of research need is to investigate the impacts of MPB outbreak on the chemistry of forest soils. Soils are not only a critical ecosystem storage pool from which plants acquire essential resources, but they are also habitat for a taxonomically and functionally diverse suite of organisms whose health, abundance, and function are the foundation for many ecological services derived from forests (e.g., nutrient cycling). Substantial mortality of overstory trees can potentially reduce in soils nutrient and water uptake while simultaneously increasing nutrient input (Morehouse et al. 2008; Griffin et al. 2011). The effects of changes to soil nutrient pools and moisture availability—and nutrient cycling overall—may affect key aspects of vegetative dynamics (e.g., productivity rates) that may influence future microbial and vegetative composition.

The second major research need involves a comprehensive investigation of ecological mechanisms underlying the forest recovery processes after severe MPB outbreak. This is particularly important in western Canada where efforts to reduce MPB spread through removal of infested trees has shown limited success. Additionally, addressing forest recovery is essential to respond to the recent attacks in western North America with greater immediacy, and to preliminarily assess the efficacy of novel recovery-based management interventions for forests predicted to become infested in the near term. In addition, a paucity of descriptive ecological studies on the impact of MPB outbreaks on community-level processes has resulted in an information gap concerning the role of interspecific interactions in shaping patterns of vegetative response. The changing nature of such interactions over a shifting base of resources is among the most critical, yet understudied, areas of investigation needed to better understand the mechanisms behind vegetative dynamics as they will ultimately drive natural forest ecosystem recovery after MPB outbreaks.

1.1.4 Thesis aim

The overarching goal of this thesis was to clarify key mechanisms of natural forest recovery in stands of MPB-killed lodgepole pine stands in northwest Alberta. It was aimed to better understand the biotic and abiotic aspects of forest recovery by linking knowledge of disturbance-induced change on environmental conditions to their impacts on physioecological mechanisms influencing the regeneration of tree seedlings. I have paired observational studies from the field with experimental studies from the greenhouse to characterize the linkages between understory light, biomass and nutrient chemistry of pine needle biomass, soil chemistry, ectomycorrhizal mutualisms, and conifer seedling regeneration.

In Chapter Two, “Above- and below-ground impacts of MPB outbreak in a naïve host ecosystem”, I ask: how does MPB outbreak impact (1) input rates of pine needle biomass and nutrients; (2) supply rates of plant-available nutrient; (3) concentrations of total mineral soil phenols; and (4) root biomass? In Chapter Three, “Disrupted mutualisms limit seedling establishment following mountain pine beetle outbreak”, I ask: how are the initial establishment and following-year flush potential for seedlings of lodgepole pine and white spruce (*Picea glauca* (Moench) Voss) impacted by MPB-altered (1) light intensity; (2) pine needle litter presence and quality; and (3) ECM fungi colonization.

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CHAPTER TWO

Above- and belowground impacts of mountain pine beetle outbreak in a naïve host ecosystem

2. Introduction

2.1.1 Mountain pine beetle outbreak and soil nutrient dynamics

Bark beetles (Coloeptera: Curculinionidae, Scolytinae) are the most destructive biotic agents of conifers throughout the northern hemisphere (Furniss and Caroline 1977). Some species among this taxon display episodic and severe population eruptions on decadal to millennial time scales driven by host-tree condition and abundance, and more recently climate warming (Raffa et al. 2008; Bentz et al. 2010; Safranyik et al. 2010). The mountain pine beetle (*Dendroctonus ponderosae* Hopkins; hereafter MPB), is an aggressive, coniferophagous species that is currently undergoing continent-scale range and host expansion throughout conifer forests of western North America. Outbreak populations now span unprecedented gradients of latitude, elevation, and forest composition, and have killed conifers on over 28 million forested hectares (Alberta Sustainable Resource Development 2012; British Columbia Ministry of Forests, Lands and Natural Resource Operations 2012; Man 2012). They have also expanded into the lodgepole (*Pinus contorta* var. *latifolia* Engelm**)** x jack pine hybrid (*Pinus banksiana* Lamb.) forest, thus threatening most of the Canadian boreal forest ecosystem. However, the state of basic scientific understanding remains sparse regarding the population dynamics, long-term success, and ecological effects of MPB infestation in both lodgepole and jack pine stands that have heretofore lacked an historic analog for severe bark beetle disturbance—"novel forests."

In contrast to other biotic attack agents, such as defoliators, which rapidly devour large proportions of tree foliage, beetles feed on long-lived cambial tissues with low feeding efficiency (Johnson and Myanishi 2007), effectively killing the tree, but leaving foliage and the forest over- and understory intact (Simard et al. 2012). Thus, while physical disturbance to the canopy, understory, and soil as a result of MPB infestation is neither direct or acute, chronic stand-level decline, (Bowler et al. 2012) coupled with accelerated rates of unconsumed litter inputs (Morehouse et al. 2008), can result in a cascade of interlinked effects, including changes to primary productivity, as well as above- and belowground nutrient inputs and dynamics (Romme et al. 1986; Morehouse et al. 2008; Griffin et al. 2011).

Beetle-driven tree mortality in the historical range may directly influence soil nutrient supply dynamics by altering the production (Griffin et al. 2011), chemical composition (Page et al. 2012), and turnover rates of above- and belowground inputs of plant litter and secondary compounds (Fahey 1983; Vogt et al. 1986; Berg 2000; Prescott 2005). Earlier work shows that soil ammonium (NH_4^+), for example, may rise 0-3 years post-beetle outbreak and eventually fall between 4 and 30 years according to a relative balance between N inputs, immobilization, and hydrological export (Huber 2005; Clow et al. 2011; Griffin et al. 2011). In addition, plant-derived phenolic secondary metabolites (e.g., phenols and tannins), entering the rhizosphere as leachates from living (e.g., fine roots) and senesced (e.g., pine litter) plant tissues, may also influence the dynamics of soil nutrients, including N (Hattenschwiler and Vitousek 2000). In fact, the abundance of soil phenols can control several key aspects of N cycling including rates of mineralization, nitrification, and leaching, as well the dominant plant-available forms of nitrogen (Northup et al. 1995; 1998). Beetle disturbance, by potentially causing a short-term decline in living tree fine root biomass and associated production of C-rich phenolic exudates, as well as a concomitant pulse of phenol-containing above- and belowground litter input (Morehouse et al. 2008; Karolewski et al. 2011), may alter soil-phenol fluxes via these two key pathways (McClaugherty 1983; Meier et al. 2008). Thus, a likely—yet unknown—effect of MPB-driven tree mortality could be alterations in the abundance of phenolic acids, with consequences for the dynamics of soil N that build upon ecological explanations posited in earlier studies (e.g., Griffin et al. 2011; Edburg et al. 2012). However, in the context of early post-MPB disturbance (1-3 yrs), no studies have addressed questions related to plant-litter-soil dynamics specifically, or temporal dynamics of above and below ground macronutrient in general. Yet, addressing this critical knowledge gap may yield information that could improve evaluations of the ecological viability of various management practices (e.g., reforestation). In addition, it may expand our understanding of edpaphic conditions present during early post-beetle stages that may potentially influence the response of residual and regenerating vegetation throughout stand recovery.

2.1.2 Study objective

The goals of this research were to determine the role of MPB disturbance—(1) severity, and (2) time since beetle outbreak (TSB)—on responses among above- and below-ground biomass and nutrient properties, including: (1) input rates of pine needle biomass and nutrients, (2) supply rates of plant-available nutrient; (3) concentrations of total mineral soil phenols; and (4) root biomass.

2.2 Methods

2.2.1 Study area

The study was conducted in lodgepole pine-dominated stands of fire origin (circa. 1900) within the lower foothills natural sub-region of northwestern Alberta (118° 59'W; 54°39'N; 1027 m) located roughly 60 km southwest of the city of Grande Prairie (Fig. 2.1). The regional climate is humid-continental with long cold winters and short cool summers in which most precipitation occurs in June (76.5 mm), with a mean annual precipitation of 446.6 mm. Mean annual temperature is 1.9° C, with mean daily minimum values of -20.5° C in January and mean daily maximum values of 22.1° C in July (Environment Canada).

The topography of the study area consists of gently undulating to rolling till-covered hills and plateaus at elevations between 950 m and 1100 m above sea level. Soils are Orthic Gray Luvisols derived from weakly to moderately calcareous, medium- to fine-textured glacial tills that are well to imperfectly drained, and support the most diverse forest tree species communities in Alberta (Natural Regions Committee 2006). Species mixtures comprising the secondary species structure within many lodgepole pine-dominated sites within the region, include black spruce (*Picea mariana* Mill.), white spruce (*Picea glauca* (Moench) Voss.), balsam fir (*Abies balsamea* (L.) Mill.), trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marshall), and green alder (*Alnus viridis* (Chaix) DC). The understory vegetation includes mixures of devil's club (*Oplopanax horridus* (Sm.) Miq.), fire weed (*Chamerion angustifolium* (L.)), Holub, wild sarsaparilla (*Aralia nudicaulis* L.), and Canadian mayflower (*Maianthemum canadense* Desf).

In 2006 and 2009, long-distance in-flights of MPB from British Columbia initiated a regional outbreak that spread into stands of mature overstory-pine at record-breaking densities—killing over 1 million trees—with new attacks as recent as 2012 (Albert Sustainable Resource Development, 2012). During such outbreak population phases, adult MPB beetles use an aggregation strategy to selectively mass attack, colonize, mate in, and ultimately kill large diameter trees. Successful attackers feed on host cambial tissues causing mechanical injury (i.e., stem girdling) and physiological dysfunction (i.e., occlusion of vascular tissues by phytopathogenic fungi) which reduce transpiration and photosynthetic carbon (C) gain (Raffa et al. 2005; McDowell et al. 2011; Hubbard et al.

2013). Trees die within weeks to months and advance through three stages of crown color in the following sequence and general time frames: (1) *green-attack*—dead trees with full crowns of fading needles, occurring 0-1 year post attack; 2) *red-attack*—dead trees with the majority of orange-colored needles remaining, occurring 1-3 years post attack; 3) *gray-attack*—dead tree with no needles remaining; occurring 3 or more years post attack (Wulder et al. 2006; Safranyik et al. 2010).

2.2.2. Site selection and characterization of overstory structure

I used AESRD Aerial Detection Survey Maps (AESRD, 2010) and field inspections to select eleven sites dominated by overstories of even-aged lodgepole pine with varying levels of MPB-caused mortality. In May 2011, I installed one 30 m X 30 m survey plot at each site, within which four subplots (6 m X 6 m) were positioned at each corner to sample forest structure and MPB infestation (Fig. 2.2). I measured the total height and diameter at breast height (DBH; cm) of all live and dead standing trees (woody stems ≥ 12 cm at DBH) and saplings (woody stems < 12 cm at DBH and total height ≥ 1.4 m). All trees were recorded for species, condition (live or dead), stage of crown color (Wulder et al. 2006), estimated number of years dead (Keen 1955; Klutsch et al. 2009), and proximate cause of death (e.g., MPB; disease; unknown). Mean overstory age was determined by accessing AESRD records for each of the adjacent AESRD-administered Permanent Sampling Plots.

Beetle attacks were confirmed by the presence of adult emergence holes, pitch tubes, boring dust (only for the current year of attacks), and subcortical life stages (Safranyik and Carroll 2007). To estimate year of attack on all MPB-killed trees, I crossed checked records for stages of crown color (green-, red-, and gray-attack) and degradation classes (1 – ≥ 6 yrs dead) with physical signs of beetle attack. From these estimates, I determined the number of years elapsed between initial infestations of MPB and the start of my field sampling (hereafter, time since beetle; TSB). In both June and September 2012, I re-visited all previously sampled trees to document levels of current-year MPB-caused tree mortality and update crown color stages and degradation classes. From these data, I calculated density and basal area by stem type, species, and of beetle kill. Severity of beetle disturbance was calculated as the proportion of pre-MPB (i.e., prior to outbreak) live tree basal area that was killed by MPB, multiplied by 100 (hereafter, percent tree mortality).

2.2.3 Sampling protocols

In each site, I established a systematic grid (4 m X 7 m) originating 1 m from the base of the plot's southwest-most tree to locate ten points for sampling rates of needlefall and soil nutrient supply, and total root biomass and mineral soil phenols (Fig. 2.2). To determine rates of needlefall, litter traps (22 cm X 50 cm) were positioned at each point, from which litter was collected monthly from June to September in 2011 and 2012, and once in May 2012 following winter (i.e., October-May). Non-needle litter, including twigs and non-pine foliage was not included in the subsequent analysis. Collected samples were shipped to the laboratory at the U of A, oven-dried at 60 ^{O}C for 48 h, and weighed. I analyzed macro- and micro-nutrient chemistry by compositing July-collected samples by site. Total inorganic N concentrations were analyzed by the Dumas Combustion Method using a Costech 4010 Elemental Analyzer System (Costech Analytical Technologies Inc., Valencia, CA, USA). Metal cations were analyzed using atomic absorption spectrophotometry (Baker and Suhr 1982) (U of A-NRAL 2013).

In June 2011 and 2012, I sampled supply rates of macro- and micro-nutrient using Plant Root Simulator (PRS™) probes (Western Ag Innovations, Inc., SK, Canada; Qian and Schoenau, 2002). Probes employ an ion-exchange resin membrane embedded in a plastic stake to achieve a cumulative (i.e., residence term) measure of bioavailable mineral-ions in soils. Probes were inserted into mineral soils of the A-horizon at a distance of 20 - 50 cm from each litter trap at a sampling density of four individual cation and anion probe pairs; these were composited to comprise 1 observation per litter trap. Probes where extracted in August of each year, shipped to the laboratory for cleaning, and then re-shipped to the Laboratories of Western Ag Innovations for analysis of nutrient supply rates. Soil volumetric moisture content was measured in August 2011 and 2012 at a distanced of 25 cm from each pair of PRS-probes using a portable ML2 ThetaProbe Soil Moisture Sensor (Dynamax Inc., Houston TX, USA.). Soil pH and particle size distribution was determined by collecting and pooling 25 locally dispersed soil sub-samples per site in July 2011, promptly air-drying samples, and then shipping them to the lab for analysis (Bouyoucos 1962; McLean 1982).

In May 2012, temperature and humidity sensors (HOBO U23 Pro v2 Temperature/Relative, Onset Computer Corp., Bourne, MA.) housed in PVC pipe (5 cm diameter X 12 cm long) were mounted to the forest floor at a distance of 0.5 m from each of 7 litter traps per site; sensors logged readings for temperature and humidity at 30 min intervals until their removal in September 2012.

In July 2012, I sampled for mineral soil total phenols using a course quantitative assay for phenolic acids and compounds (Tel and Covert 1992). Soil samples were collected 1 m from each of 7 litter traps per site. Four soil cores (2 cm width) were extracted from the top of the A-horizon to a depth of 6 cm, composited by litter trap location, placed on ice, and shipped to the laboratory for 48 hrs of air-drying. I added the Folin-Ciocalteau phenol reagent (Sigma-Aldrich, Oakville, ON, Canada) to prepared soil water extracts and performed spectroscopy (Enesys 10S UV-Vis Spectrophotometer, Thermo, Fisher Scientific, Madison, WI, USA) to determine absorbance (750 nm).

In August 2012, I sampled total root biomass using a coarse and relative measure to determine if general patterns of root biomass are associated with MPB disturbance. At one random location within 2 meters of each litter trap, I removed the horizon of organic soil and extracted a soil core (20 cm depth x 5 cm width) and measured its length (± 5 mm). Samples were kept frozen until processed, which involved washing roots over a 2mm sieve and collecting approximately 95% of visible roots for analysis. Fine (< 2 mm diameter) and woody (≥ 2 mm diameter) roots were separated, dried at 60°C for 48 hrs, and weighed. I standardized estimates of root biomass by dividing the biomass of each root sample by the volume of its soil core.

2.2.4 Statistical analysis

To test the respective roles played by MPB disturbance—(1) percent tree mortality, and (2) time since beetle outbreak (TSB)—on the responses among above- and belowground biomass and chemistry, I used both linear mixed effects (LME) and generalized linear (GLM) models in R (R development core team, 2013). Results from exploratory analyses using Pearson product-moment (i.e., correlation) tests revealed an absence of any significant (and thus confounding) relationships between above- and belowground site characteristics (i.e., topography, forest structure, soil texture) and the explanatory variables used for MPB disturbance. However, the explanatory variables for disturbance, percent tree mortality and TSB, were significantly correlated to one another ($P < 0.001$; $r = 0.64$). Therefore, to disentangle the unique from shared contribution of each of these explanatory variables on my response variables, I performed sequential regression (Graham 2003). I regressed TSB values against those of percent tree mortality and then used the residual error values (i.e., residuals) in place of the raw TSB values in all subsequent LME and GLM models. I averaged the respective sets of observations for each of my response variables by site and sampling year, such that only site-level means were used for all analyses. Data were available from two consecutive growing seasons (2011 and 2012) for most, but not all, response variables (total mineral soil phenolics, root biomass, canopy openness, and soil surface temperature; measured in 2012 only); LME was used to test the significance of main effects and interactions for all multi-year variables. Fixed factors included: percent tree mortality, TSB residuals, year, and interactions between and among each fixed factor; site was the random factor ($N = 22$; 11 sites x 2 yrs). While year and TSB residuals are both discrete time-based factors, I chose to include both in all LME models. This allowed us to account for the variation attributable to environmental stochasticity between sampling years (e.g. mean summer precipitation), while simultaneously testing the impacts of initial stages of beetle disturbance and recover on ecosystem properties that may respond in a time-sensitive manner. GLM was used to analyse total mineral soil phenolics and root biomass. Fixed factors were percent tree mortality (2012) and TSB residuals (2012; $P < 0.002$; $r = 0.68$); pre MPB live tree basal was also included as a fixed factor to account for differences in total root biomass attributable to variation in tree density across sites ($N = 11$; 11 sites x 1 yr). To reduce my probability of making type 2 errors, inherently inflated by using statistical models parameterized from relatively small sample sizes, and which used full factorial interactions terms, I determined statistical significance for LME and GLM tests at $\alpha < 0.1$. For my LME and GLM, I tested for the presence of normality, homoscedasticity, and spatial autocorrelation on the residuals of the regression models (using Shapiro-Wilk tests, Bartlett's test, and correlograms, respectively); assumptions of the parametric tests were satisfied, and no spatial autocorrelation was detected.

To decipher the presence of broad patterns in the response of nutrient supply rates to beetle disturbance, I created a synthetic response variable (hereafter, "soil fertility") using the axis scores generated from a Principle Component Analysis (PCA) test (Ref) parameterized with my soil nutrient data. I performed a PCA test using log transformed, site-level means of nutrients supply rates of all available nutrients (except total nitrogen), and used the PC 1 axis scores as a response variable (soil fertility) in the linear mixed effects models.

2.3 Results

2.3.1 Site characteristics and MPB infestation

Across 11 sites, pre-MPB live basal area ranged between 36.4 and 70.75 $m^2 ha^{-1}$ (mean = 51.5 ± 3.03 $m^2 ha^{-1}$), of which *P. contorta* comprised between 60 and 100% (mean = 93.3 ± 3.8%; Table 2.1). The percentage of tree basal area killed by MPB (i.e., percent tree mortality) ranged from 0 – 84%, and was not related to pre-MPB live tree basal area, or any pre-MPB topographic or edaphic site characteristics (Table 2.2). In 2011 and 2012, TSB ranged from 0 – 2 yrs, and 0 – 3 yrs, respectively (Table 2.1), and also showed no relationship to any pre-MPB topographic or edaphic site characteristic (Table 2.2). Average age of overstory trees was similar (121 ± 0.4 yrs) across study sites, as were ecosite classes (Beckingham et al. 1996) (Table 2.1).

2.3.2 Soil nutrient supply rates, total mineral soil phenols, and total root biomass

The supply rates of both NO_3^- and total nitrogen increased in magnitude (i.e., slope) between sampling years across the gradient of tree mortality (Table 2.3; Figs. 2.3, 2.4). Supply rates of P increased both between years and across the gradient of tree mortality (Table 2.3). Supply rates of K declined over the gradient of tree mortality and showed a reduced magnitude of decrease in associated with TSB residuals between years (Table 2.3; Fig. 2.5). Supply rates of Mg increased between sampling years, while those of Fe showed a three-way interaction amongst sampling year, percent tree morality, and TSB beetle residuals.

Results from my Principle Component Analysis test showed that principle component axis one (PC1) accounted for 36% of the overall variation in the supply rates for all nutrient elements measured. Overall, negative relationships were observed between PC1 and (in order of greatest to least strength) Zn, Mn, Al, NH_4^+, P, NO_3^-, Fe, K, and S, with all elements except K and S showing strong to moderately strong relationships ($r \geq 0.40$). Contrastingly, positive relationships were observed for the supply of Mg ($r = 0.52$) and Ca ($r = 0.11$) (Fig. 2.6). Soil fertility, an aggregate measure of soil nutrient supply created from the PC1 scores, was negatively associated with TSB residuals when tested via my linear mixed effects model (Table 2.3).

Soil pH decreased between sampling years and in relation to TSB residuals.

Concentrations of total mineral soil phenols were negatively related to percent tree mortality (2012) and supply rates of NO_3^- (2012) (Table 2.4).

The biomass of fine versus woody and total roots responded differently to beetle disturbance, though all root fractions were positively related to pre MPB live basal area (Table 2.4). Fine root biomass declined as a function of tree mortality (2012) (Fig. 2.7) and TSB residuals (2012) (Fig. 2.8), while woody root biomass showed no relationship to disturbance, and total root biomass showed a positive interaction between tree mortality and TSB residuals (Table 2.4).

2.3.3 Pine needle biomass and nutrient input

Rates of pine needlefall were negatively related to TSB residuals, and had a relationship to percent tree mortality that shifted directions between years, from positive to negative (Table 2.3).

Needle N concentrations declined between sampling years and across the gradient of tree mortality, but increased in relation to TSB residuals (Table 2.3). Needle P concentrations increased over the gradient of tree mortality (Table 2.3). Needle K concentrations declined in relation to TSB residuals, and had a relationship with tree mortality which changed directions between years, from negative to positive (Table 2.3; Fig. 2.9). Needle Mg concentrations increased in relation to TSB residuals and between years (Table 2.3). Needle Ca concentrations had a relationship with tree mortality that shifted directions between years, from negative to positive (Table 2.3; Fig. 2.10).

Inputs of N, P, and K from needle biomass were all impacted by MPB disturbance but differed in the strength, direction, and magnitude of their responses (Table 2.3). Inputs of N and P showed similar patterns in which both increased in relation to TSB residuals and percent mortality, but declined in magnitude between years in relation to tree mortality (Table 2.3; Figs. 2.8, 2.9). Inputs of K showed a three-way interaction amongst year, tree morality, and TSB beetle residuals.

2.4 Discussion

My study demonstrates that both the severity of, and number of years following, tree mortality caused by MPB in a novel habitat are significant factors in the redistribution of needle biomass and nutrients from canopy to soils, supply rates of plant-available nutrients, concentrations of total mineral soil phenols, and the total biomass of plant roots. Mountain pine beetle disturbance, by causing selective mortality of mature lodgepole pine trees, appears to alter key pathways through which nutrients cycle—input, immobilization, nutrient-chemical interactions, and export—thereby directly and indirectly affecting their flux and availability above- and belowground. Below, I discuss the relative importance of, and possible linkages between, nutrient cycling through these pathways in lodgepole pine forests within MPB's newly expanded range.

2.4.1 Rates of soil nutrient supply and needle nutrient input

Supply rates of all soil macronutrients (N, P, and K) were associated with MPB disturbance to varying strengths and magnitudes, but differed in the specific characteristic of the disturbance (i.e., severity, or time) to which they were associated. For example, tree mortality was significantly related to the supply rates of K, and marginally related to those of P, while TSB residuals, in contrast, was related to supply rates of K and NO_3^-, but not P (Table 2.3). Thus, the specific pathways of nutrient cycling that are influenced by these two characteristics of disturbance may differ by nutrient in both relative sensitivity, in the case of tree mortality, and in length of time required to produce an effect, in the case of TSB residuals. Nevertheless, in spite of my ability to detect micro-scale patterns between the supply rates of specific nutrients and one or more of my independent variables, a macro-scale pattern indicating overall soil fertility was apparent in my study. Soil fertility was positively related to TSB residuals indicated a general increase in most nutrients (except Mg and Ca) as time since beetle elapsed.

Changes in the rates of nutrient uptake due to death of canopy trees is a likely factor underlying these patterns. Mass attack of trees reduces their associated rates of hydraulic conductance and evapo-transpirative moisture loss (Hubbard et al. 2013), thereby reducing rates of nutrient and water uptake from soils. As the proportion of MPB-killed trees accumulates within stands during the progression of beetle infestations, concentrated over mostly 1 - 2 years in the current study, a cumulative decline in nutrient and water demand is expected to increase nutrient availability, as I observed for total N, NO_3^-, and P. Supply rate of these nutrients were associated purely with levels of tree morality, whereas those of K were negatively associated with tree mortality and positively associated with TSB residuals, but exhibited declining magnitude of effect in relation to this factor between years. Although the physiological impacts of mass attack on nutrient translocation in trees occur within days to weeks at the scale of individuals (Hubbard et al. 2013), the impacts of vascular dysfunction for rates of nutrient uptake and hence nutrient supply at the stand scale may require multiple growing seasons after initial beetle infestation before they are detectable. While a lag effect of reduced rates of uptake may help explain the trends for supply rates of K, it is inconsistent with observed trends for the supply rates of total N, NO_3^-, and P, which were not time, but instead mortality, sensitive. However, this doesn't necessarily indicate lesser relative importance of potentially reduced rates of uptake for the later three nutrients, but may instead reflect differential rates of demand for given nutrients by surviving trees and hence differential time thresholds for detection (Griffin et al. 2011). Additionally, other time-dependent pathways of nutrient cycling, such as nutrient inputs, may also be contributing to soil nutrient supply rates.

Mountain pine beetle disturbance augmented both background rates of needlefall and concentrations of needle nutrients, thereby compounding the effect on aboveground inputs of N and P from canopy to soil surface following infestation. Rates of needlefall were negatively related to TSB residuals and showed a significant year by tree mortality interaction, which together demonstrate that both severity of and time since disturbance drive canopy needle loss. Tree mortality was related negatively to concentrations of needle N but positively to those if needle P, while needle N was positively related to TSB residuals. Earlier studies have also documented elevated concentrations of needle N (Morehouse et al. 2008; Griffin et al. 2012) and P (Page et al. 2012) associated with MPB disturbance and attributed this effect to failed foliar nutrient resorption prior to needle abscission. Sites with relatively high proportions of MPB-killed trees likely contributed needle mass at both greater rates and needle P concentrations than did those with relatively low proportions. Nevertheless, my results show that both responses are time-dependent, for which time lags of 1-3 years between initial infestation and eventual onset of persistent needle drop likely explain the effect of time on both the quantity and quality of needles. Similarly, rates of input for needle-derived N and P showed a year by tree mortality interaction, with reduced magnitudes of increase in relation to tree mortality between years, further illustrating the interactive effects of severity and time on pulses of above-ground nutrient input in response to beetle disturbance. While severity of disturbance likely influences the amplitude of peaks in needle biomass and nutrient input, the number of growing seasons that have passed since initial infestation is likely to underlie the timing of such peaks. Based on results from my non-metric dimensional scaling tests using my individual crown decay data, such peaks occur during the red attack phase.

A pulse of N-, P-, and K-rich needle litter may have contributed to elevated supply rates of these same nutrients in soils. The rates of input for needle P and P in soils were each positively related to tree mortality, suggesting a possible causal link between these responses. In other nutrients, however, paralleled responses between rates of nutrient input and supply were either not evident or idiosyncratic, in the case of inputs of needle N and NO3. Moreover, the significant effect of time on the supply rates of K may indicate that mobilization of this nutrient from fresh needles on the soil surface to inorganic mineral forms in soil solution is constrained by time lags. Although litter quality and quantity can influence rates of litter decomposition and mineralization (Prescott et al. 2004), which in turn may increase rates of mobilization thereby decreasing such lag times, additions of fresh-fallen surface litter might initially contribute little to processes of breakdown which are most active in mineral soils (Prescott et al. 2004). Moreover, substantial C input associated with a pulse of needlefall may, despite above-background concentrations of needle N, initiate a period of temporary deficit in soil nutrient availability (e.g., N depression) driven by rapid nutrient assimilation within microbial biomass (Fahey 1983; Remsburg and Turner 2006). Under these ecological constraints, the cumulative effects of nutrient inputs on nutrient supply rates may not peak until at least five year after beetle disturbance (Huber 2005).

The insulating effects of additions of needle biomass to the litter layer—deepest during the red- and gray-attack stage—may modify soils temperature (Griffin et al., 2011) and moisture regimes (Cullings et al. 2003). Griffin et al. (2011) demonstrated that in stands disturbed by MPB, temperatures at the litter-soil interface were the strongest predictor of net N mineralization rates, with an inverse relationship, and lowest temperatures observed in the gray stage. In contrast, both the concentrations of needle N and total litter N pool size were only weakly related to net mineralization in the same study. Tree mortality in my study did not influence average ambient temperature at the soil surface, but was positively associated with minimum temperatures. I also detected a positive but insignificant trend between rates of needlefall and soil moisture, suggesting the possibility of an indirect effect of needlefall on subsurface conditions. The addition of litter may reduce evapotranspiration and increase the retention of soil moisture. Thus, recent inputs of needles, while potential drivers of sub-surface temperature and moisture—and hence temperature- and moisture-sensitive processes such N mineralization—may not be equally subject to temperature-driven changes in rates of nutrient turnover at the soil surface. I suggest that the effects of MPB disturbance on nutrient supply rates via aboveground substrate quantity and quality enhancement may be initially attenuated by time lags in nutrient turnover. Meanwhile, needlefall-induced abiotic changes to soil (e.g., temperature) might have a relatively weak, but more immediate effect on nutrient supply rates.

2.4.2 Total mineral soil phenols and root biomass

Mountain pine beetle disturbance may decrease the abundance and production of living root biomass which may in turn further inflate nutrient supply rates. Rates of uptake, for example, are driven primarily by the abundance of fine roots and the availability of nitrogen (Hari et al. 2013). As decomposition of tree roots outpaces their replacement, an expected signature of tree mortality within the rhizosphere is a decline in total (live and dead) root biomass, as confirmed by my results. The functional consequences for the rhizosphere of decline among both fine and coarse roots may include both direct impacts through sink effects (i.e., reduced nutrient uptake) as described above, and indirect source effects. Living roots produce and release an array of secondary metabolites into the rhizosphere, with some having the potential to influence nutrient cycling. For example, polyphenols, a broad class of plant secondary metabolites, are well-known to interact with plant essential nutrients in ways that may enhance or inhibit the availability of certain nutrients, particularly N (Hattenschwiler and Vitousek 2000). Interesting, my results agree in several key aspects with those of earlier investigations conducted in the beetle’s historical range demonstrating strong linkages between disturbance and altered rates of N cycling (Morehouse et al. 2008; Griffin et al. 2011), and increased availability of soil NO_3^{-} (Morehouse et al. 2008; Griffin et al. 2011; Clow et al. 2012). Beetle disturbance has been shown to impact N dynamics by altering pathways of nutrient cycling including: vegetative immobilization and long-term retention (Griffin et al. 2011; Collins et al. 2011), litter deposition and N mineralization (Morehouse et al. 2008; Griffin et al. 2011), and hydrological export (Rhoads et al. 2013). These pathways, as well as altered chemical interactions between total mineral soil phenols and N, may help explain similar patterns observed for NO_3^{-} in my study.

Concentrations of total mineral soil phenols showed a sharp decline as a function of tree mortality. Polyphenols (e.g., tannins), which enter the soil primarily as soluble leachates released from plant tissues that are both living (e.g., roots and shoots) and dead (decomposing litter), can influence the cycling of N in forest soils via allelopathic interference (Hattenschwiler and Vitousek 2000). Polyphenols may inhibit the activity of nitrifying bacteria (e.g., *Nitrosomas spp.*), in effect slowing rates at which soil NH_4^+ is converted to $NO_3^—$ (i.e., nitrification) (Northup et al. 1995). Conversely, relatively reduced concentrations of total mineral soil phenols—likely linked to reductions in live tree and root biomass and hence production in the present study—are associated with accelerated rates of N mineralization and nitrification (Hattenschwiler and Vitousek 2000). In support of this general principle, my results show a negative relationship between concentrations of total mineral soil phenols and $NO_3^—$ supply. Thus, the positive effect of MPB disturbance on the supply rates of $NO_3^—$ may be partially explained by accelerated rates of nitrification within soils of reduced phenol-mediated allopathic constraint. Moreover, such effects are consistent with earlier investigations in the beetle's historical range that documented consistently high rates of nitrification during the red-attack stage (Morehouse et al. 2008; Griffin et al. 2011).

The residence time of nutrients such as $NO_3^—$ in soils will be largely influenced by the balance between input, utilization, and export (Fahey et al. 1985; Knight et al. 1985). For example, in some undisturbed conifer forests, plant-litter-soil interactions can produce high concentrations of soil phenol which promote the retention of N in soils (Northup et al. 1995). In contrast, disturbance-induced reductions in concentrations of phenols may impair resistance to N losses by accelerating nitrification to the extent of saturation of $NO_3^—$ (Hattenschwiler and Vitousek 2000). Rates of moisture input, which also impacts the residence time of nutrients via heightening their potential for uptake and/or leaching, can be increased by canopy needle loss. As canopies become increasingly diffuse, with lower leaf area indices and greater canopy openness—a condition I observed in my study—reduced rates of canopy interception may increase water volumes entering soils (Pugh and Gordon 2012). This combination of effects could promote leaching and surface runoff of NO_3^-. If, however, soil nutrient supply rates continue to remain relatively high or even rise after outbreak, uptake by surviving vegetation may buffer against losses.

My results indicate that MPB disturbance increases the availability of soil nutrients through effects that are direct (e.g., reduced uptake) and indirect (e.g., nitrogen-phenol interactions, litter input), and that both types of effects are time-dependent. Although the pathways that underlie these effects are unique, their impacts on soil nutrient rates appear to be parallel, and likely compounding. It's conceivable, therefore, that the cumulative impacts of altered nutrient cycling pathways could yield pools of soil nutrients in excess of net annual demand among surviving plants, thereby heightening the risk for losses through leaching. Rapid immobilization of nutrients by undisturbed over- and understory vegetation may, however, mitigate this risk. Although I did not quantify the balance between immobilization and losses of nutrients, and recognize that both processes may have impacted my results, recent evidence suggests that compensatory growth responses among residual vegetation may act as an effective mechanism for N immobilization and retention. For example, Griffin et al. (2011) showed that while significant correlations existed between rates of net N mineralization and concentrations of N in current year lodgepole pine foliage—indicating a strong temporal coupling between biogeochemical processes that mobilize versus immobilize N after MPB disturbance—elevated foliar N concentrations actually persisted 30 years after MPB infestation, which demonstrates long-term N retention. In addition, findings from post-MPB regeneration studies (Collins et al. 2011), as well as observational (Clow et al. 2012) and model simulation (Rhoads et al. 2013) studies on watershed discharge of NO_3^- indicate that MPB disturbances will be met by strong compensatory responses among surviving vegetation. Despite a convergence of nutrient influx through multiple independent pathways that promote a pulse of belowground nutrient resources after outbreak, vegetative assimilation followed by long-term nutrient storage in biomass is likely to buffer against the associated heightened risk of nutrient losses.

2.5 Conclusion

The extensive scale of 21st century MPB outbreaks invokes fundamental questions about the ecological resilience, risk of spread of biotic attack agents into novel forested landscapes, and the long-term economic viability of the North American forest industry. Forest resilience to beetle-infestation will be determined by key, often interdependent and time dependent, ecosystem process and responses including post-disturbance nutrient cycling. My results which consider temporal and severity aspects of such events indicate that many changes that follow disturbance, including aboveground nutrient input and belowground nutrient mobilization will occur 1-3 year after MPB outbreak (i.e., Red-attack). Changes at different levels may continue to occur as further nutrient release from the breakdown of needles aboveground, and roots belowground, ensues. Beyond the time frame observed in this study (1-3 yrs post outbreak), however, it may remain difficult to predict the future changes, which may be influenced by the severity of the disturbance and pre-disturbance site characteristics, including the health and density of advanced regeneration, and edaphic properties.

Mountain pine beetle-driven tree mortality likely results in immediate reductions to key functions played by trees within the rhizosphere, including the production and release of secondary phenolic compounds. Consequent reductions in soils phenols may have an important influence over the supply of NO_3^- following MPB infestations. Moreover, infestation sharply increases litterfall biomass and quality, creating a time-sensitive pulse of needle nutrient inputs, which may have a relatively small impact on soil nutrient levels during early stages of overstory disintegration (1-4 yrs), but may become increasingly important as needle nutrients are mobilized through decomposition and mineralization. The mineral form, supply rate, and residence time of key macronutrients, such as N, will be a large determinant of how residual and regenerating vegetation respond to MPB disturbance. I suggest that additional lines of investigation are warranted that attempt to elucidate the roles and interactions among key soil players and processes such as phenolic compounds, N, ectomycorrhizal fungi (Treu et al. In-press), and seedling regeneration in MPB killed stands within MPB's expanded range.

Figure 2.1. Current extent of the mountain pine beetle (*Dendroctonus ponderosae*) range in western Canada. The insert depicts the relative position of our study area and individual research sites. Photo credit: Catherine I. Cullingham, University of Alberta.

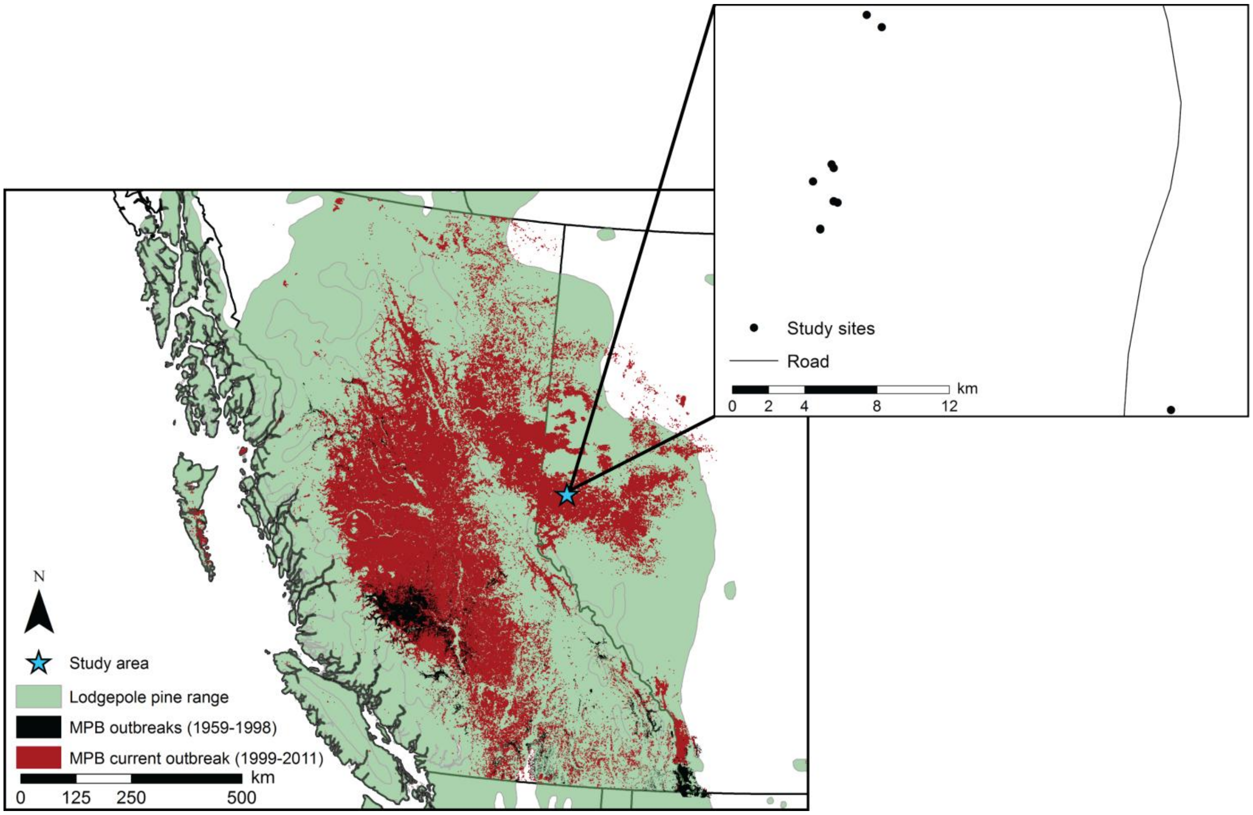


Figure 2.2. Schematic diagram of the sampling design and relative position of each sample taken for measurement in lodgepole pine (*Pinus contorta*) forests in western Alberta. Study sites are the principle experimental unit (*N*=11).

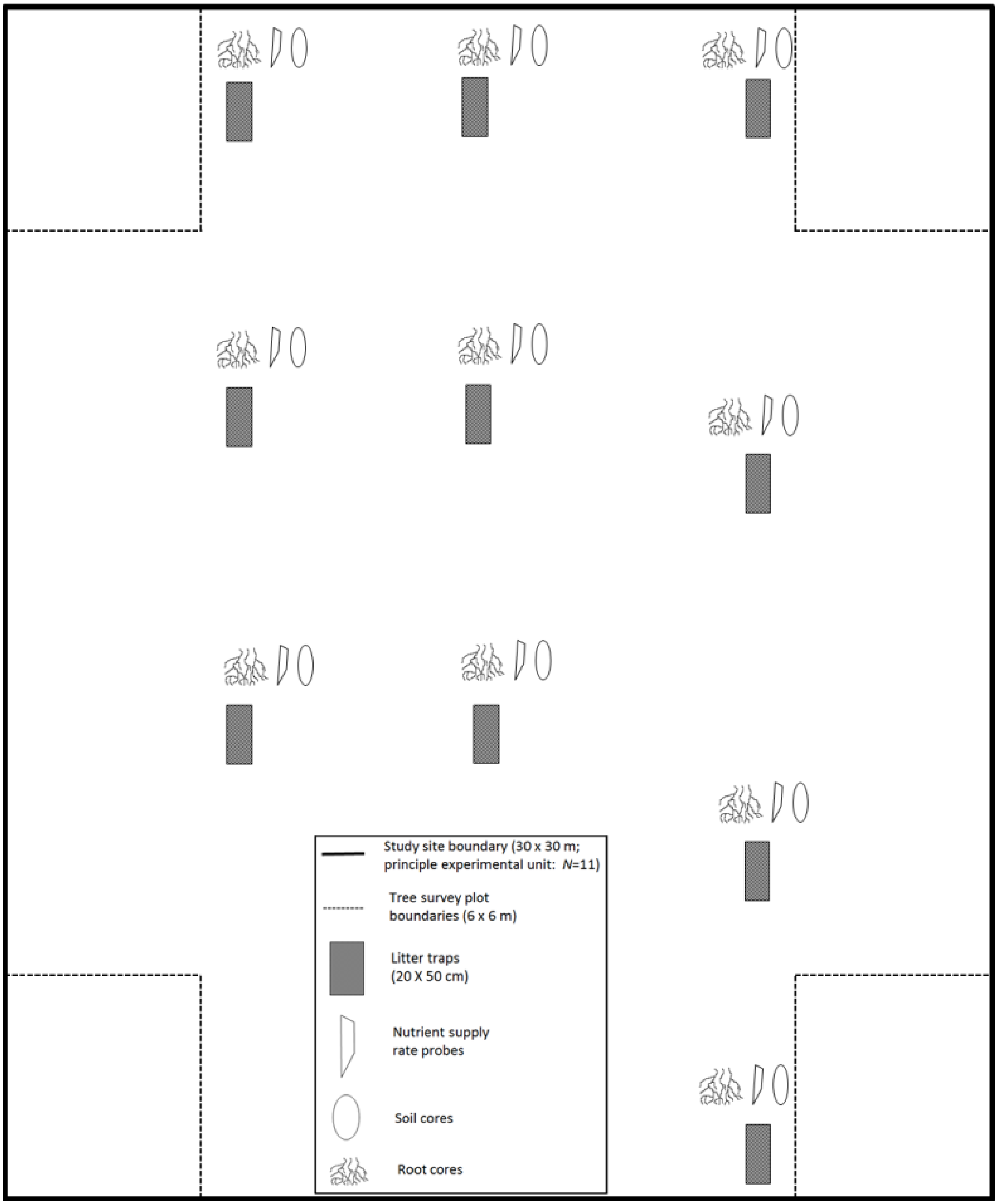


Figure 2.3. Relationship between supply rates of soil nitrate and tree mortality in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2011 (dashed line; circles) and 2012 (solid line; triangles). Points are raw data values.

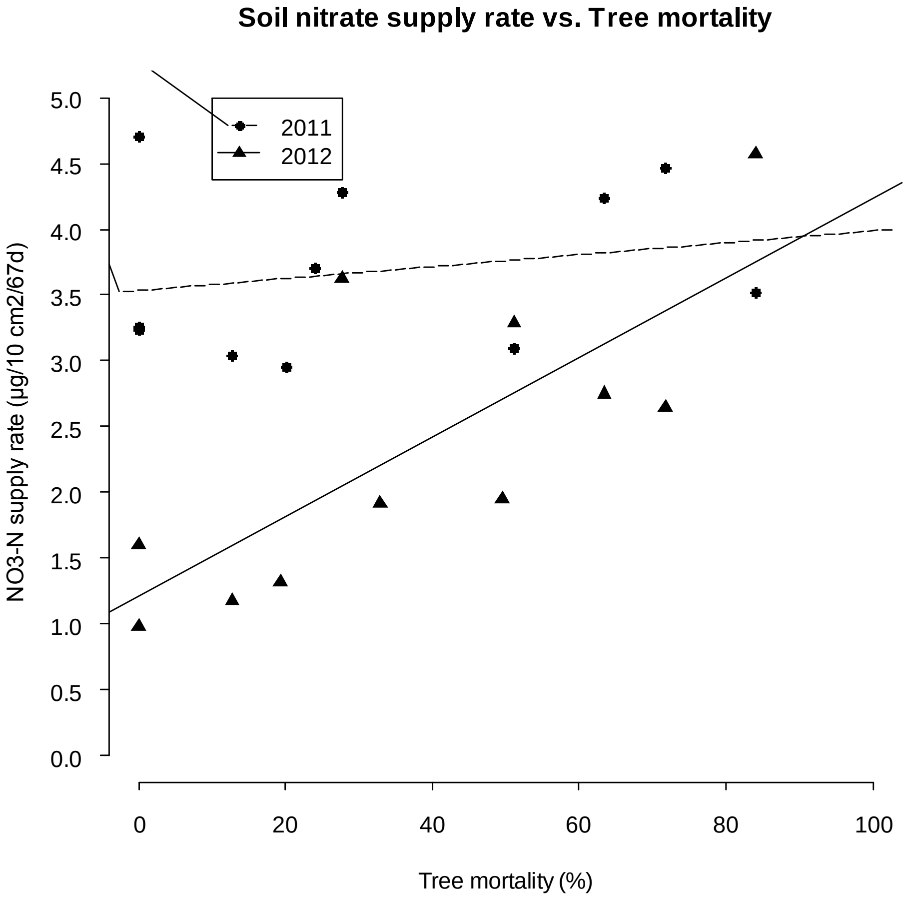


Figure 2.4. Relationship between supply rates of soil total nitrogen and tree mortality in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2011 (dashed line; circles) and 2012 (solid line; triangles) . Points are raw data values.

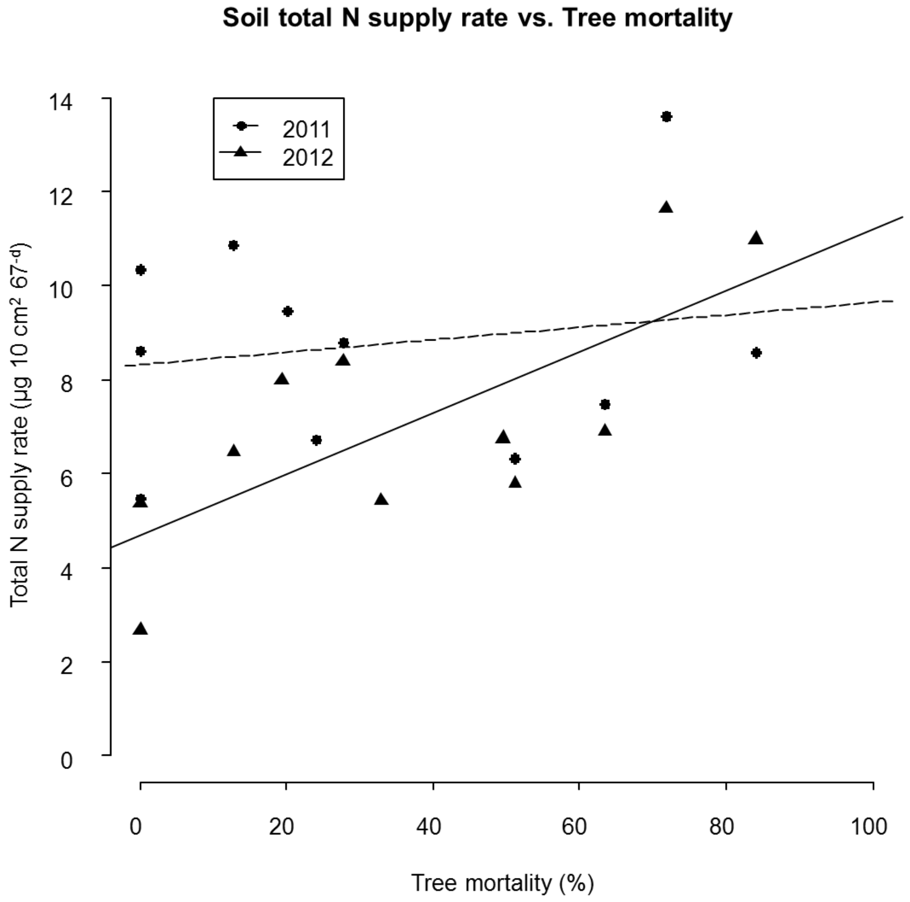


Figure 2.5. Relationship between supply rates of soil potassium and tree mortality in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2011 (dashed line; circles) and 2012 (solid line; triangles). Points are raw data values.

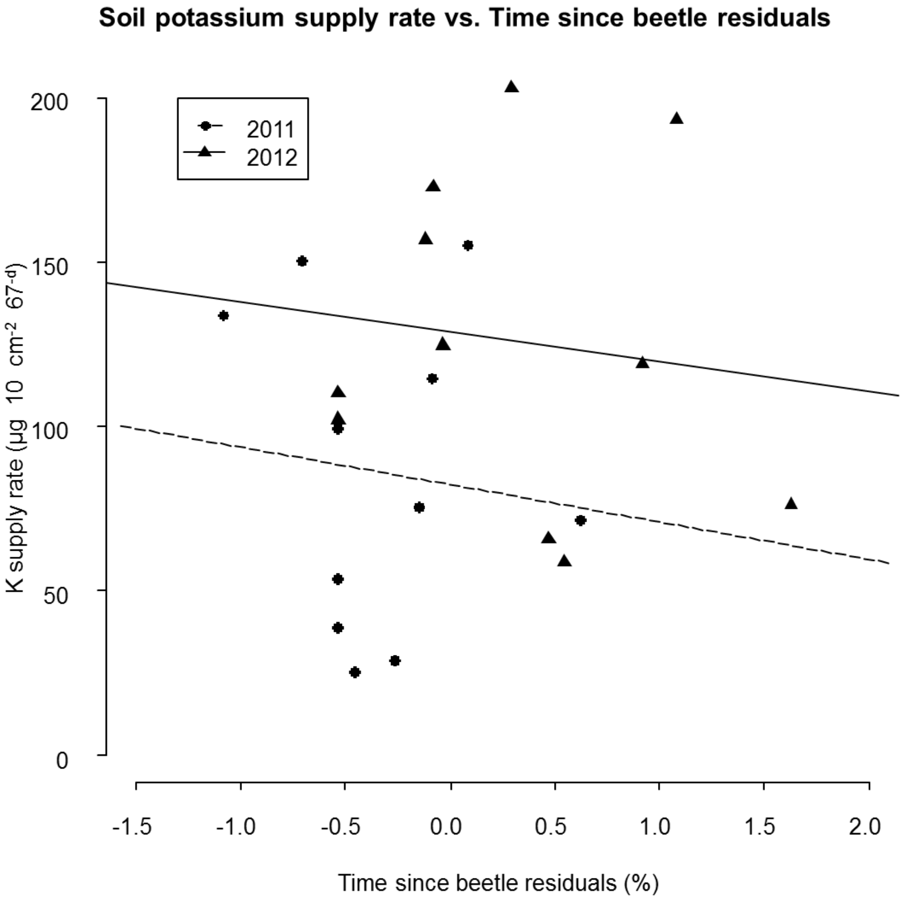


Figure 2.6. Results of a Principle Component Analysis ordination illustrating macro-scale patterns amongst, and pairwise correspondence between, supply rates of individual soil elements. Soil samples were collected in lodgepole pine (*Pinus contorta*) forests in western Alberta.

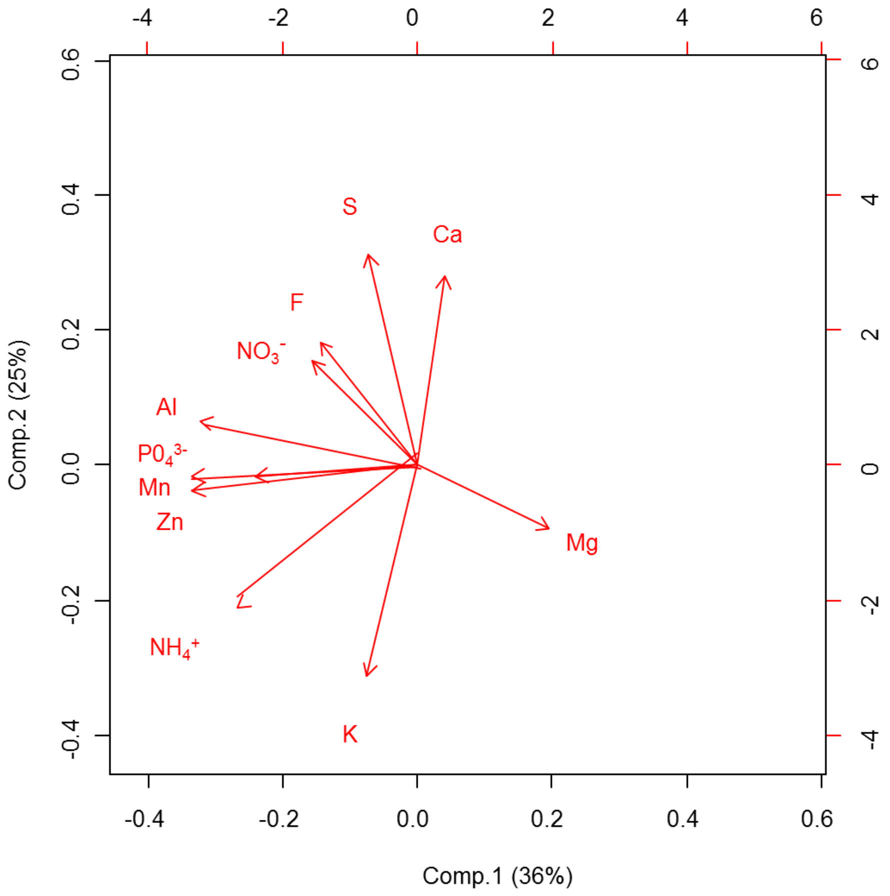


Figure 2.7. Partial a regression plot of the relationship between fine root biomass and tree mortality in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2012.

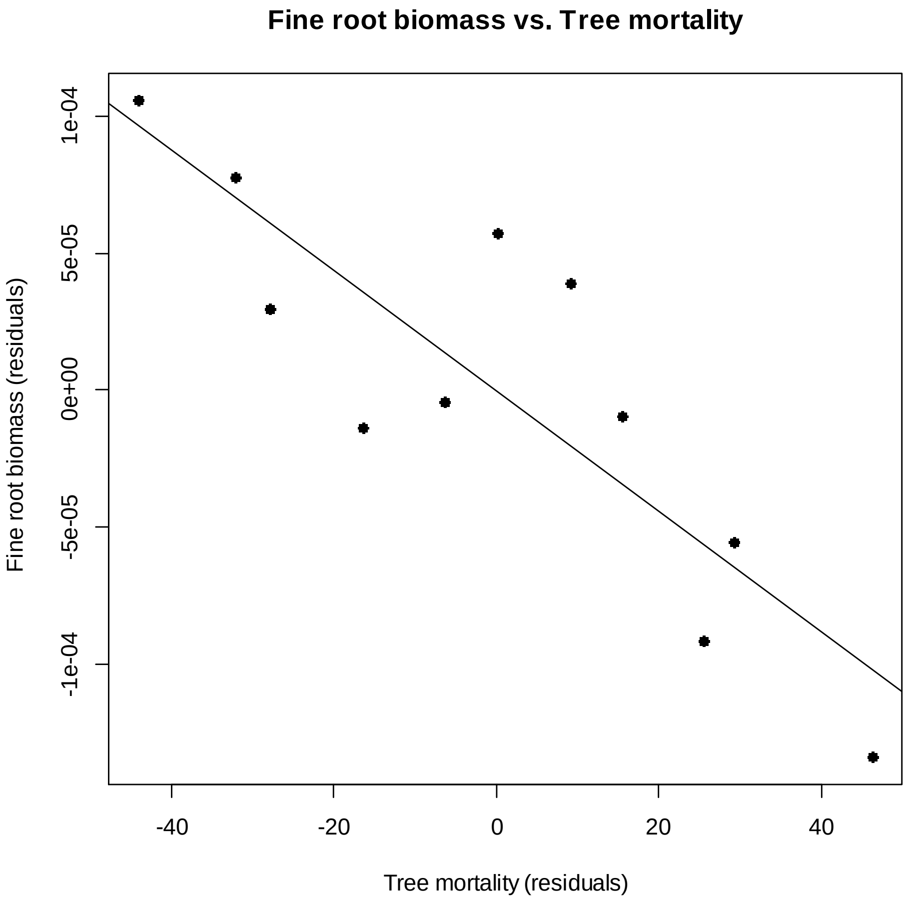


Figure 2.8. Partial a regression plot of the relationship between fine root biomass and time since beetle residuals in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2012.

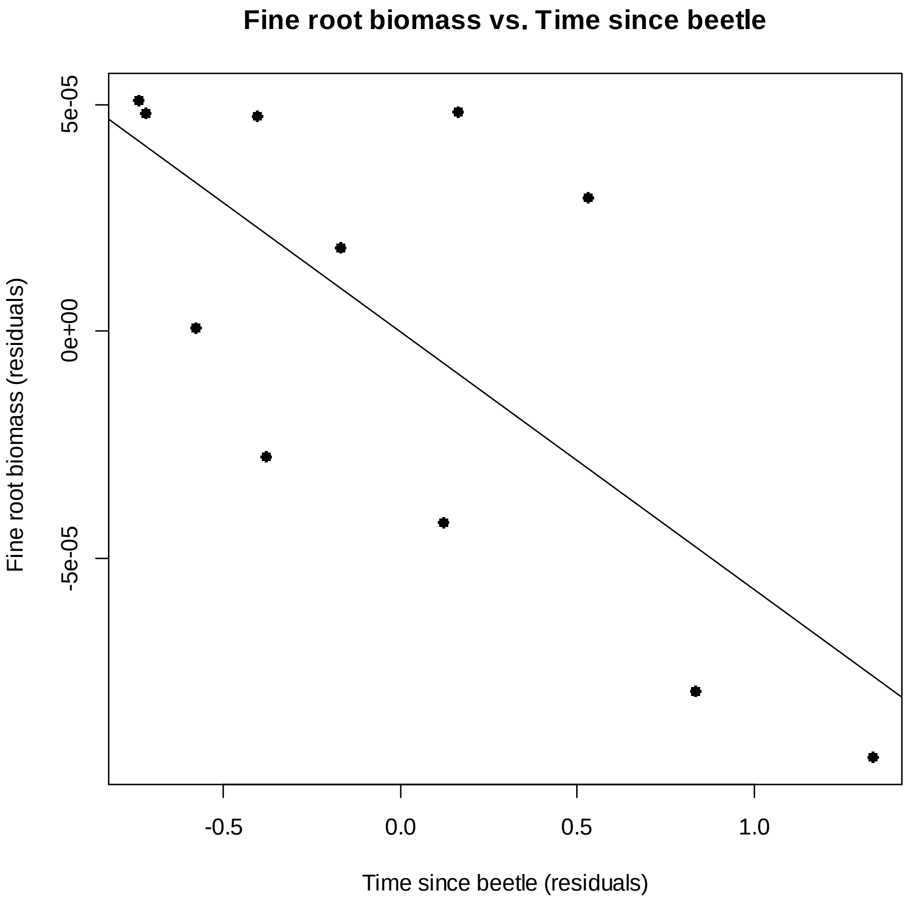


Figure 2.9. Relationship between concentrations of needle K and tree mortality during in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2011 (dashed line; circles) and 2012 (solid line; triangles). Points are raw data values.

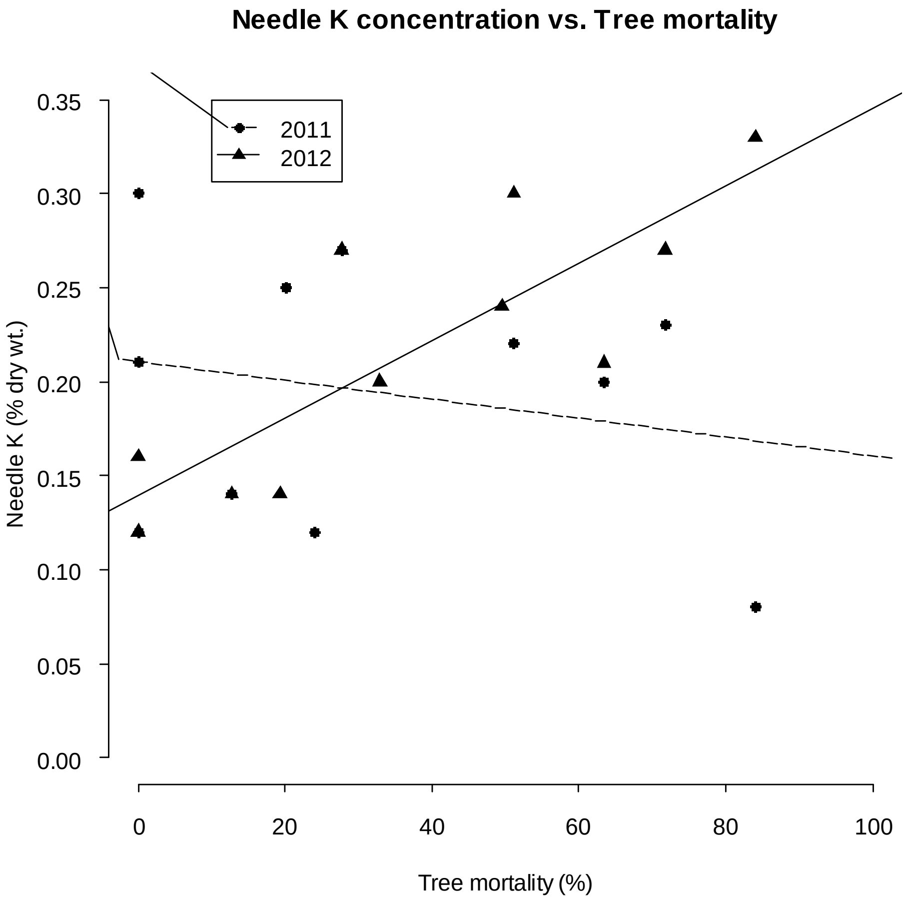


Figure 2.10. Relationship between concentrations of needle calcium and tree mortality in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2011 (dashed line; circles) and 2012 (solid line; triangles). Points are raw data values.

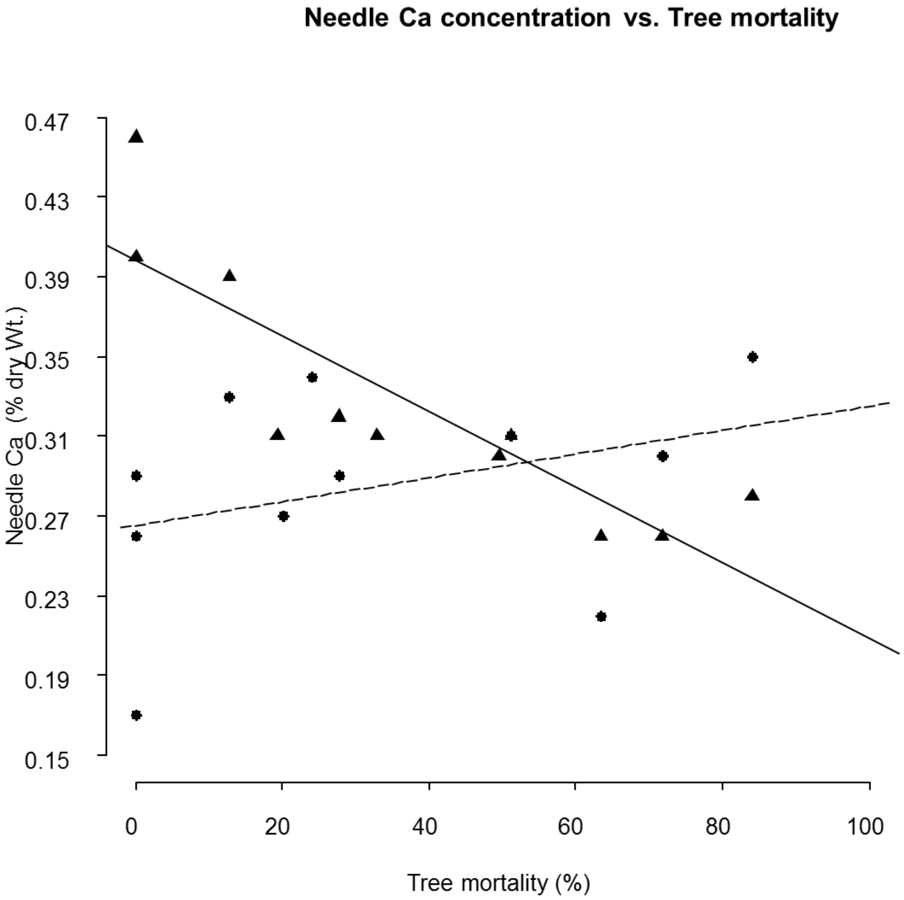


Figure 2.11. Relationship between inputs of needle-derived nitrogen and tree mortality during in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2011 (dashed line; circles) and 2012 (solid line; triangles). Points are raw data values.

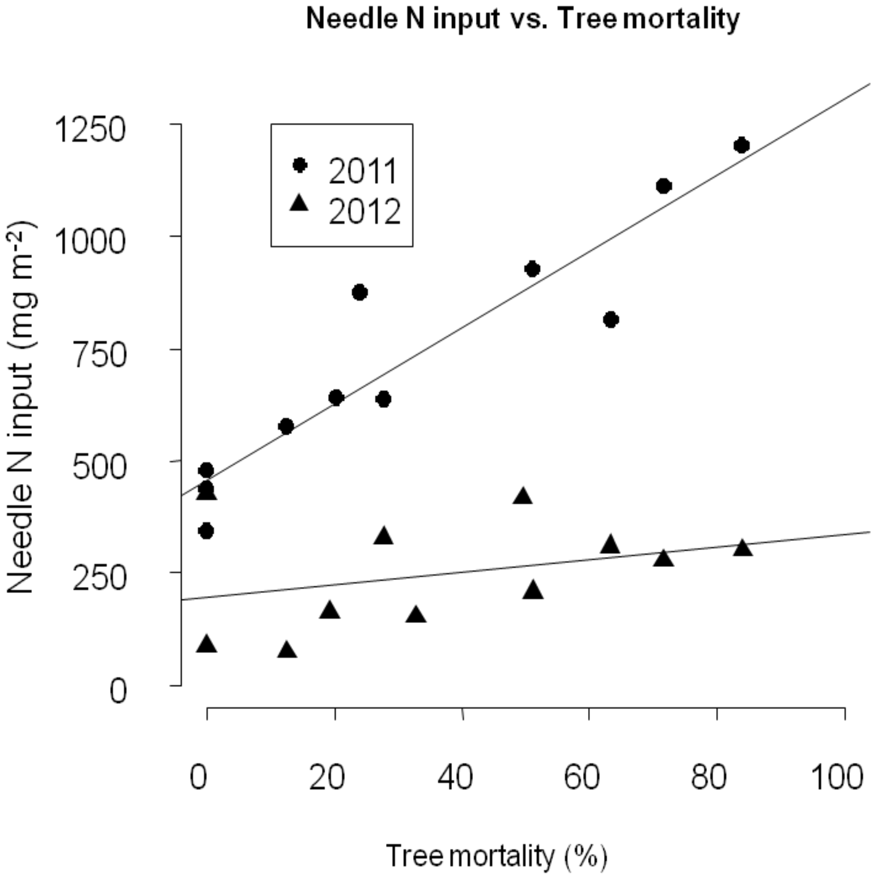


Figure 2.12. Relationship between inputs of needle-derived phosphorus and tree mortality in lodgepole pine (*Pinus contorta*) forests in western Alberta during 2011 (dashed line; circles) and 2012 (solid line; triangles). Points are raw data values.

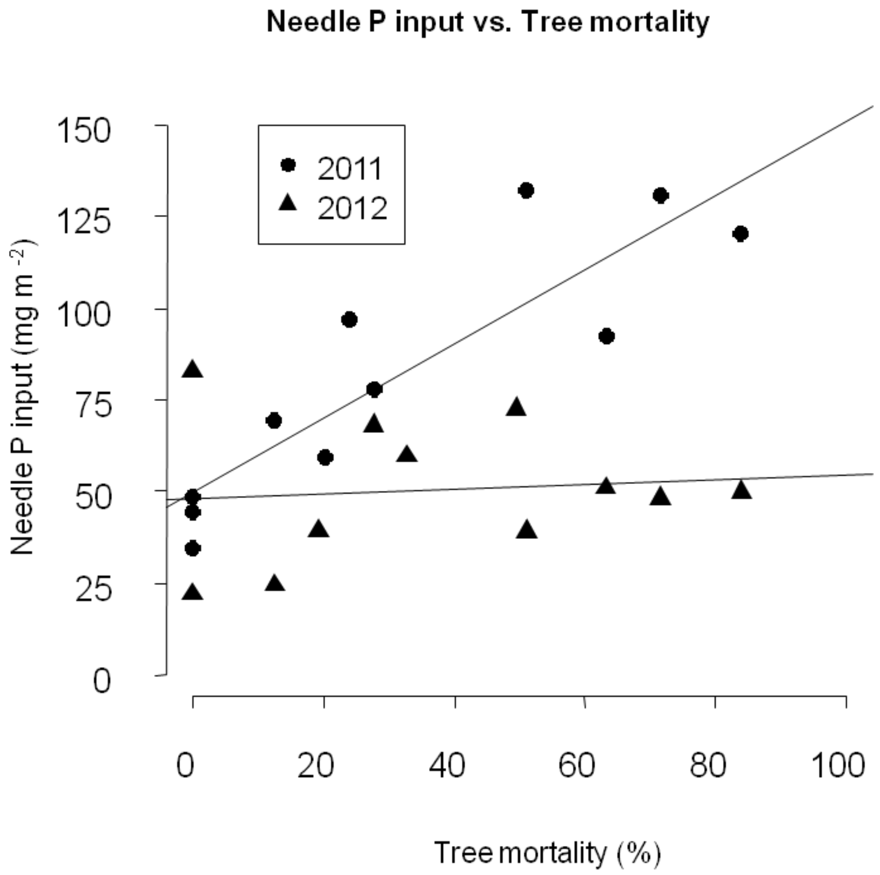
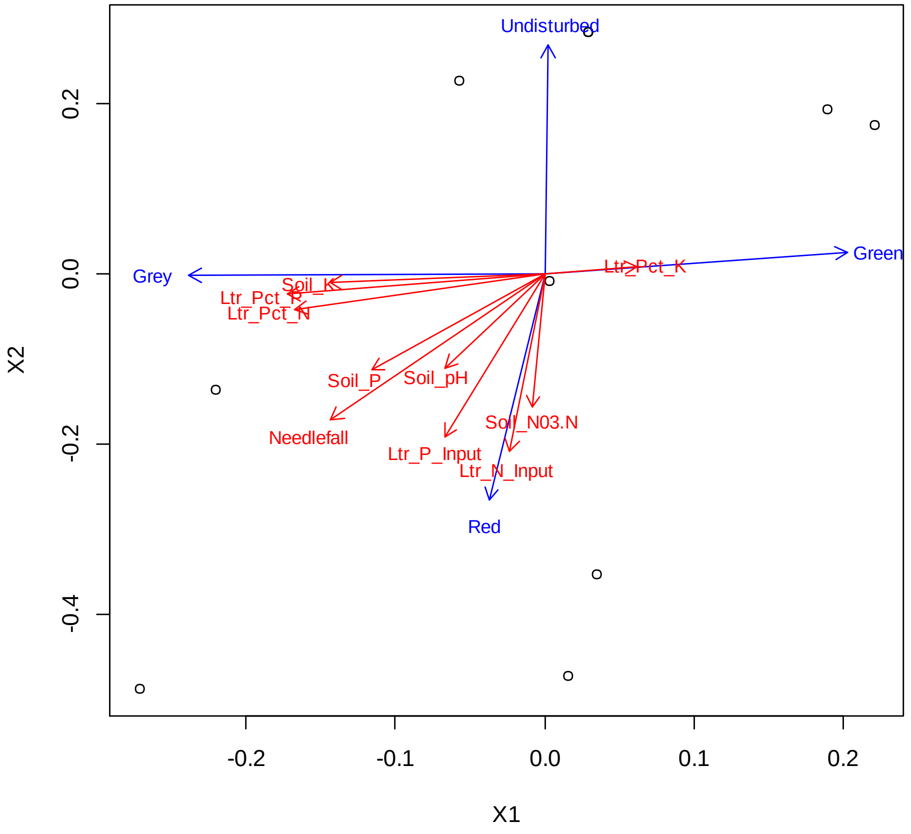


Figure 2.13 a, b. Nonmetric Multidimensional Scaling (NMDS) ordination illustrating associations between stages of MPB-induced crown decay (Undisturbed, Green-, Red-, Gray-attack) and rates for above- and belowground nutrient input and supply sampled in lodgepole pine (*Pinus contorta*) forests in western Alberta during the growing seasons of 2011 (a), and 2012 (b). Long arrows (blue) represent vectors for each of the stages of crown decay, and short arrows (red) represent each of the responses variables.



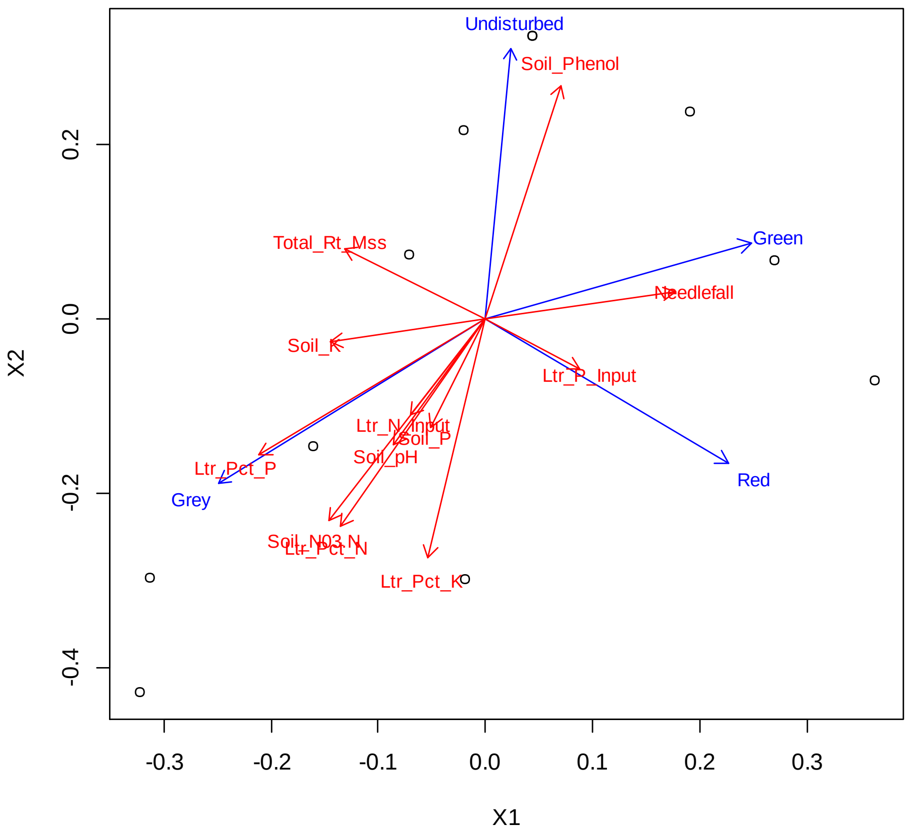

Undisturbed
Soil_Phenol
Total_Rt_Mss
Green
Needlefall
Soil_K
Ltr_P_Input
Ltr_N_Input
Soil_P
Soil_pH
Ltr_Pct_P
Grey
Red
Soil_N03_N
Ltr_Pct_N
Ltr_Pct_K
X2
X1
0.2
0.0
-0.2
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3

Table 2.1. Characteristics of forest structure and mountain pine beetle (*Dendroctonus ponderosae*) disturbance in lodgepole pine (*Pinus contorta*) forests in western Alberta by site (means). Trees were recorded as stems ≥ 12 cm diameter at breast height. Lodgepole pine (*Pinus contorta*) is coded as "PICO."

Site ID	Overstory tree age (yrs)	Pre MPB live tree basal area (m^2 ha^{-1})	Ecosite classification (Beckingham et al. 1996)	Pre MPB live tree basal area (PICO; %)	Pre MPB live tree density (trees ha^{-1})	Pre MPB live tree density (PICO; Trees ha^{-1})	MPB-Killed tree basal area 2011 (m^2 ha^{-1})	MPB-Killed tree basal area 2012 (m^2 ha^{-1})	MPB-Killed tree basal area 2013 (m^2 ha^{-1})	MPB-killed basal area 2011 (% tree basal area)	MPB-killed basal area 2012 (% tree basal area)	MPB-killed basal area 2013 (% tree basal area)	Time since beetle (Yr; TSB; 2011)
059	121	70.75	b 1.2	91.98	2154.	1737.5	0	0	0	0	0	0	0
061	120	47.41	f 1.2	92.34	903.5	834	0	9.14	13.39	0	19.29	28.24	0
KB1	123	36.4	f 1.1	97.62	625.5	486.5	0	0	29.2	0	0	80.21	0
056	121	61.67	h 1.3	94.00	2154.	1876.5	7.81	7.81	7.81	12.67	12.67	12.67	1
057	121	41.8	e 1.2	100	903.5	903.5	10.05	13.73	18.81	24.03	32.85	45.00	1
063	119	43.89	f 1.5	100	834	834	8.87	21.74	36.86	20.21	49.53	83.98	1
062A	120	44.93	e 1.1	97.88	764.5	695	12.46	12.46	12.45	27.72	27.72	27.72	2
070	123	50.55	e 3.1	60.29	625.5	417	25.83	25.83	25.83	51.09	51.09	51.09	2
062	120	54.75	f 1.4	98.37	834	764.5	34.69	34.69	34.69	63.35	63.35	63.35	2
070A	123	60.13	e 1.2	100.3	973	973	43.09	43.09	43.09	71.66	71.66	71.66	2
069	121	54.57	f 1.4	100	834	834	45.86	45.86	45.86	84.04	84.04	84.04	2

Table 2.2. Results from Pearson product-moment correlation tests to determine the significance and direction of relationships between lodgepole pine forest and soil parameters and the percentage basal area killed by the mountain pine beetle (*Dendroctonus ponderosae*).

	Model statistics		**Explanatory variables**	
Response variable	***r***	***P***	**Variable**	**d.f.**
Topography			*2011*	
Elevation (m)	-0.5117	0.1076	% Beetle Kill 2011	1,9
Elevation (m)	-0.4106	0.2097	TSB 2011	1,9
Slope (%)	0.3944	0.2299	% Beetle Kill 2011	1,9
Slope (%)	0.4759	0.139	TSB 2011	1,9
Aspect (0-360 ^{o})	0.1587	0.6411	% Beetle Kill 2011	1,9
Aspect (0-360 ^{o})	0.1667	0.6241	TSB 2011	1,9
Forest Structure				
Pre-MPB live basal area	0.2254	0.5052	% Beetle Kill 2011	1,9
Pre-MPB live basal area	0.1976	0.5603	TSB 2011	1,9
Soils texture				
% Sand	0.4197	0.1987	% Beetle Kill 2011	1,9
% Sand	0.4059	0.2155	TSB 2011	1,9
% Silt	-0.4052	0.2164	% Beetle Kill 2011	1,9
% Silt	-0.4893	0.1266	TSB 2011	1,9
% Clay	-0.1863	0.5834	% Beetle Kill 2011	1,9
% Clay	-0.0322	0.9251	TSB 2011	1,9
Topography			*2012*	
Elevation (m)	-0.3806	0.2482	% Beetle Kill 2012	1,9
Elevation (m)	-0.2923	0.3831	TSB 2012	1,9
Slope (%)	0.5372	0.0883	% Beetle Kill 2012	1,9
Slope (%)	0.5986	0.0617	TSB 2012	1,9
Aspect (0-360 ^{o})	0.0454	0.8946	% Beetle Kill 2012	1,9
Aspect (0-360 ^{o})	0.1054	0.7576	TSB 2012	1,9
Soils texture				
% Sand	0.4021	0.2203	% Beetle Kill 2012	1,9
% Sand	0.3269	0.3264	TSB 2012	1,9
% Silt	-0.4974	0.1196	% Beetle Kill 2012	1,9
% Silt	-0.4778	0.1372	TSB 2012	1,9
% Clay	-0.0127	0.9705	% Beetle Kill 2012	1,9
% Clay	0.1009	0.7677	TSB 2012	1,9
Soil surface temperature				
Average (^{o}c)	-0.064	0.85	% Total BK 2012	1,9
Maximum (^{o}c)	-0.31	0.353	% Total BK 2012	1,9
Minimum (^{o}c)	0.512	0.107	% Total BK 2012	1,9
Range (^{o}c)	-0.45	0.165	% Total BK 2012	1,9

Table 2.3. Results from linear mixed effects models used to determine the direction, effect size, precision, and significance of main effects between and interactions among mountain pine beetle disturbance (*Dendroctonus ponderosae*) (i.e., tree mortality and time since beetle) and responses in pine needle litter (a) nutrients, (b) biomass, and (c) nutrient input, and soil (a) nutrient supply rates, (b) moisture, and (c) pH sampled in lodgepole pine-dominated (*Pinus contorta*) forests in western Alberta. Fixed factors included sampling year (2011 -2012), percent tree mortality, and time since beetle residuals. Co-linearity between percent tree mortality and time since beetle necessitated the replacement in the models of that factor with its residuals that result from being regressed against percent tree mortality. Significance codes are: "." $P < 0.1$; "*" $P < 0.05$; "**" $P < 0.001$; "***" $P < 0.001$

Fixed factor	**Year (yr)**			**% Beetle Kill (%BK)**			**TSB Residuals (TSBr)**			**Yr X %BK**			**Yr X TSBr**		
Test statistic	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***
Needle litter															
Needlefall g/m^2	**-0.65 ± 25.33**	**-0.03**	**11.16***	3260 ± 1607	2.028	2.31	**-13163 ± 90948**	**-0.14**	**21.42****	**-1.621 ± 0.80**	**-2.03**	**5.76.**	6.53 ± 45.21	0.14	0.02
Nitrogen% dry wt.	**-0.44 ± 0.0993**	**-4.41**	**31.32****	**-8.34 ± 6.00**	**-1.39**	**9.89***	**472 ± 361**	**1.31**	**11.55***	0.00 ± 0.00	1.39	2.03	-0.23 ± 0.17	-1.3	0.08
Phosphorous% dry wt.	0.004 ± 0.01	0.32	0.05	**0.57 ± 0.85**	**0.67**	**7.66***	16.11 ± 43.13	0.37	1.91	-0.00 ± 0.00	-0.67	0.01	-0.01 ± 0.02	-0.37	1.44
Potassium% dry wt.	-0.03 ± 0.05	-0.61	1.13	-0.14 ± 3.15	- 0.045	1.98	**-207.57 ± 165.43**	**-1.25**	**7.29.**	**0.00 ± 0.00**	**0.046**	**10.46***	0.10 ± 0.08	1.25	0.32
Magnesium% dry wt.	**0.03 ± 0.01**	**2.30**	**48.05****	-1.16 ± 0.81	-1.44	3.2	**79.34 ± 50.36**	**1.58**	**9.52***	0.00 ± 0.00	1.44	0.16	-0.03 ± 0.02	-1.57	0.61
Calcium% dry wt.	**0.08 ± 0.04**	**1.96**	**6.97.**	1.59 ± 2.53	0.63	2.2	280 ± 157	1.79	0.088	**-0.00 ± 0.00**	**-0.63**	**0.08***	-0.13 ± 0.07	-1.78	0.97
Nitrogen input (g/m^2)	**-36.71 ± 14.99**	**-2.45**	**55.21****	**1415 ± 881**	**1.61**	**21.36****	**35860 ± 54843**	**0.65**	**36.63****	**-0.70 ± 0.44**	**-1.60**	**12.73***	-17.83 ± 27.26	-0.65	0.05
Phosphorus input (g/m^2)	**0.42 ± 2.33**	**0.18**	**12.02***	**332 ± 137**	**2.42**	**12.76***	**1220 ± 8535**	**0.14**	**6.55.**	**-0.16 ± 0.06**	**-2.42**	**10.84***	-0.60 ± 4.24	-0.14	1.15
Potassium input (g/m^2)	**1.90 ± 6.37**	**0.30**	**8.879***	958 ± 429	2.23	1.378	**-31075 ± 22371**	**-1.39**	**4.90.**	-0.47 ± 0.21	-2.23	0.685	15.44 ± 11.11	1.38	1.74
Belowground															
Soil moisture (0-20 cm)	-4.66 ± 4.08	-1.14	1.90	-135 ± 306	-0.44	3.0	8491 ± 13645	0.62	0.59	0.06 ± 0.15	0.44	0.48	-4.21 ± 6.78	-0.62	0.00
Soil pH (0-20 cm)	**-0.59 ± 0.17**	**-3.49**	**97.04*****	22.3 ± 10.3	2.16	1.18	**-811 ± 615**	**-1.32**	**45.49****	-0.01 ± 0.00	-2.16	0.22	0.40 ± 0.30	1.31	0.15
Nutrient supply: Ca	165 ± 138.1	1.19	0.45	8196 ± 10730	0.76	1.5	-591451 ± 454518	-1.30	2.84	-4.1 ± 5.3	-0.76	1.87	293 ± 225	1.30	0.47
Nutrient supply: Fe	-24.31 ± 7.22	-3.37	2.749	-1410 ± 439	-3.21	0.55	68680 ± 26235	2.62	4.13	0.70 ± 0.21	3.21	0.01	-34.13 ± 13.04	-2.61	0.23
Nutrient supply: K	**37.26 ± 15.08**	**2.47**	**58.50****	**-724 ± 1683**	**-0.43**	**41.94****	**75362 ± 46933**	**1.61**	**43.90****	0.36 ± 0.84	0.43	0.57	**-37.45 ± 23.32**	**-1.61**	**10.39***
Nutrient supply: Mg	**28.94 ± 23.52**	**1.23**	**14.33***	-1797 ± 1627	-1.10	1.48	7704 ± 81716	0.09	1.26	0.89 ± 0.81	1.10	1.79	-3.84 ± 40.62	-0.09	0.03
Nutrient supply: Mn	-47.87 ± 21.82	-2.19	0.19	-3283 ± 1533	-2.14	1.13	143016 ± 75292	1.89	0.70	1.63 ± 0.76	2.14	0.97	-71.08 ± 37.42	-1.89	0.05
Nutrient supply: NH_4-N	-2.06 ± 1.25	-1.66	1.02	-139 ± 98.29	-1.42	0.4	3137 ± 4074	0.77	0.00	0.07 ± 0.04	1.42	2.91	-1.56 ± 2.03	-0.76	0.16
Nutrient supply: NO_3-N	**-2.12 ± 0.66**	**-3.23**	**27.65****	-26.11 ± 42.32	-0.62	3.04	-2233 ± 2343	-0.95	3.36	**0.01 ± 0.02**	**0.62**	**5.78.**	1.11 ± 1.17	0.95	0.65
Nutrient supply: P	**6.84 ± 3.26**	**2.10**	**4.25.**	**376 ± 277**	**1.36**	**6.42.**	-9694 ± 10360	-0.94	0.87	-0.18 ± 0.13	-1.36	0.15	4.82 ± 5.15	0.93	1.16
Nutrient supply: Total N	**-4.39 ± 1.58**	**-2.78**	**10.57***	-171 ± 115	-1.48	0.89	1263 ± 5345	0.24	1.67	**0.08 ± 0.05**	**1.48**	**4.52.**	-0.63 ± 2.65	-0.23	0.01
Fertility PC 1	0.40 ± 0.60	0.67	2.73	**-55.02 ± 74.15**	**-0.74**	**1.06.**	2.99 ± 1884	0.00	5.95	0.02 ± 0.03	0.74	0.77	-0.00 ± 0.93	-0.00	0.56

Table 2.3. continued

Fixed factor	**%BK X TSBr**			**Yr X %BK X TSBr**		
Test statistic	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***
Needle litter						
Needlefall g/m^2	-0.65 ± 25.33	-0.03	0.03	-0.32 ± 1.17	-0.27	0.08
Nitrogen% dry wt.	-13.87 ± 9.49	-1.46	0.17	0.00 ± 0.00	1.46	2.14
Phosphorous% dry wt.	-0.00 ± 1.07	-0.00	0.70	0.00 ± 0.00	0.00	0.00
Potassium% dry wt.	6.91 ± 4.18	1.65	0.37	-0.00 ± 0.00	-1.65	2.72
Magnesium% dry wt.	-1.58 ± 1.33	-1.19	0.97	0.00 ± 0.00	1.19	1.41
Calcium% dry wt.	-5.40 ± 4.16	-1.30	1.18	0.00 ± 0.00	1.29	1.6
Nitrogen input (g/m^2)	-933.41 ± 1452.50	-0.64	0.00	0.46 ± 0.72	0.64	0.41
Phosphorus input (g/m^2)	78.91 ± 226.05	0.35	0.32	-0.03 ± 0.11	-0.34	0.12
Potassium input (g/m^2)	1256.01 ± 564.03	2.23	0.45	**-0.62 ± 0.28**	**-2.22**	**4.96.**
Belowground						
Soil moisture (0-20 cm)	-172.70 ± 322.05	-0.54	0.31	0.08 ± 0.16	0.53	0.29
Soil pH (0-20 cm)	22.40 ± 16.15	1.39	3.12	-0.01 ± 0.00	-1.38	1.92
Nutrient supply: Ca	7549.6 ± 10446.8	0.72	1.54	-3.7 ± 5.2	-0.72	0.52
Nutrient supply: Fe	**-1928.34 ± 688.10**	**-2.80**	**6.48.**	**0.96 ± 0.34**	**2.80**	**7.85***
Nutrient supply: K	246.08 ± 886.30	0.28	1.23	-0.12 ± 0.44	-0.27	0.07
Nutrient supply: Mg	-0.86 ± 2032.65	-0.00	0.53	0.00 ± 1.01	0.00	0.00
Nutrient supply: Mn	-3356.90 ± 1856.50	-1.81	1.11	1.67 ± 0.92	1.80	3.26
Nutrient supply: NH_4-N	-31.90 ± 92.47	-0.34	0.89	0.01 ± 0.04	0.34	0.11
Nutrient supply: NO_3-N	32.42 ± 60.22	0.53	0.21	-0.01 ± 0.02	-0.53	0.28
Nutrient supply: P	355.95 ± 220.79	1.61	0.08	-0.17 ± 0.11	-1.61	2.59
Nutrient supply: Total N	-7.562 ± 128.406	-0.058	0.708	0.004 ± 0.064	0.058	0.003
Fertility PC 1	17.9917 ± 34.7680	0.517	3.89	-0.0090 ± 0.017	-0.519	0.269

Table 2.4. Results from generalized linear models used to test the direction, effect size, precision, and significance of main effects between and interactions among mountain pine beetle disturbance (*Dendroctonus ponderosae*) (i.e., tree mortality and time since beetle) and responses in total mineral soils phenols, root biomass (fine, woody, and total), and soil surface temperature sampled in lodgepole pine-dominated (*Pinus contorta*) forests in western Alberta. Results are derived from data collected in 2012 only. Co-linearity between percent tree mortality (2012) and time since beetle (2012) necessitated the replacement in the models of that factor with its residuals that result from being regressed against percent tree mortality (2012). Significance codes are: "." $P < 0.1$; "*" $P < 0.05$; "**" $P < 0.001$; "***" $P < 0.001$

Fixed factor	% Beetle Kill 2012			TSB Residuals 2012			% Beetle Kill 2012 X TSB Residuals 2012			Pre MPB Live Basal Area		
Test statistic	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***	***Coef.***	***t***	***F***
Belowground												
Total soil phenols	**-4.81 ± 1.3**	**-3.71**	**15.48****	25.04 ± 74.75	0.34	1.09	-2.70 ± 2.43	-1.11	1.23	---	---	---
Fine root Mass	**-1.57e-6 ± 6.38e-7**	**-3.09**	**13.55 ***	**-1.07e-4 ± 3.62e-5**	**-2.96**	**7.55***	1.86e-6 ± 1.18e-6	1.57	2.476	**7.31e-6 ± 1.21e-6**	**6.06**	**39.57*****
Woody root mass	2.03e-6 ± 1.76e-6	1.16	0.13	-2.02e-4 ± 9.98e-5	-2.02	0.40	9.92e-6 ± 3.33e-6	2.98	3.75.	**6.31e-6 ± 3.26e-6**	**1.94**	**10.47***
Total root mass	4.64e-7 ± 1.80e-6	0.26	0.91	**-3.09e-4 ± 1.02e-4**	**-3.03**	**2.54***	**8.17e-6 ± 3.33e-6**	**2.45**	**6.01.**	**1.72e-5 ± 3.41e-6**	5.06	**29.11****
Aboveground												
Canopy openness	**0.06 ± 0.03**	**2.20**	**9.56***	**3.33 ± 1.74**	**1.92**	**8.19***	-0.02 ± 0.06	-0.38	0.14	-0.02 ± 0.06	-0.40	0.20
Soil Surface Temperature												
Average (^{o} c)	-6.49e-5 ± 5.75e-3	-0.01	0.00	-1.15e-1 ± 3.312e-1	-0.35	0.36	1.47e-4 ± 1.08e-2	0.01	0.0002	---	---	---
Maximum (^{o} c)	-0.01 ± 0.02	-0.76	0.73	0.00 ± 1.04	0.00	0.06	-0.01 ± 0.03	-0.17	0.028	---	---	---
Minimum (^{o} c)	**0.01 ± 0.00**	**2.45**	**4.60.**	-0.27 ± 0.28	-0.99	0.01	0.01 ± 0.01	1.27	1.61	---	---	---
Range (^{o} c)	-0.03 ± 0.02	-1.42	2.08	0.25 ± 1.04	0.24	0.09	-0.02 ± 0.03	-0.49	0.24	---	---	---

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CHAPTER THREE

Disrupted mutualisms limit seedling growth performance following mountain pine beetle outbreak in naïve lodgepole pine forests

3. Introduction

3.1.1 Seedling regeneration and mountain pine beetle outbreak

Currently, the conifer-dominated forests of western North America are experiencing the most extensive and severe outbreak of mountain pine beetle (*Dendroctonus ponderosae*; MPB hereafter) in recorded history (Safranyik et al. 2010; Man 2012);infestations have occurred across approximately 28 M hectares of lodgepole pine (*Pinus contorta* var. latifolia Engelm) and lodgepole x jack pine hybrid (*Pinus banksiana* Lamb.) forest (Safranyik et al. 2010; Alberta Sustainable Resource Development 2012; British Columbia Ministry of Forests, Lands and Natural Resource operations 2012).

Recovery of forests to meet minimum standards of stocking density and volume relies on regeneration of seedlings; however, recent studies have shown that regeneration and establishment of conifer seedlings across beetle-killed stands has been variable in terms of patterns of growth (Collins et al. 2011), abundance (Astrup et al. 2008; Coates et al. 2010), and composition (Caldwell et al. 2013). For example, Coates et al. (2010) reported that, across all biogeoclimatic zones surveyed in central British Columbia, 70% of stands exceeded a threshold of 1000 stems per ha, of which 74-79% were lodgepole pine. Moreover, among beetle-affected stands in Colorado, Collins et al. (2011) reported increases in annual growth rates of lodgepole pine seedlings and advance regeneration, while modelling efforts by Caldwell et al. (2013) predicted recovery of lodgepole pine-dominated stands after only 70 years. In contrast, Astrup et al. (2008) reported low abundances of lodgepole pine seedlings in beetle-impacted stands of central British Columbia, citing a low availability of seedbed substrates as a major contributing factor.

Consistent with these findings, McIntosh and Macdonald (2013) noted a low abundance of seedbed substrates and lodgepole pine recruitment in their study of field simulation of MPB disturbance in the insect's expanded range in western Alberta. This lead to the suggestion that lodgepole pine may decline in the near term without direct intervention in the beetle's newly expanded range. However, broader explanations for these patterns have drawn primarily on observational evidence to make mechanistic conclusions that are relatively simplified, or site specific (e.g., ecological releases; limitation on seedbed substrates). As a result, ecological mechanisms underlying regeneration remain poorly understood. Lacking is experimental evidence testing how biotic and abiotic conditions associated with MPB disturbance influence seedling regeneration.

Associated with MPB disturbance are rapid increases in light and needle deposition (Chp 1), with potential impacts on regeneration. Increased light intensity is known to impact regeneration for many trees, particularly shade-intolerant species such as lodgepole pine (Landhausser and Lieffers 2001). An increase in the quantity and nutrient concentration of needle biomass at the soil surface may affect seedlings by altering resource abundance and environmental conditions at the forest floor and in mineral soils. Specifically increased needle biomass may absolute amounts of decomposition and nutrient cycling (Morehouse et al. 2008; Griffin et al. 2011), reduce moisture loss through direct evaporation (Griffin et al. 2011), and moderate extremes of soil temperature (Griffin et al. 2011); positive or negative effects on the germination and survival of seedlings may be realized.

Among the various biotic interactions potentially affected by MPB infestation, ectomycorrhizal (ECM) fungal symbioses may be a critical yet overlooked factor underlying patterns of seedling establishment (Smith and Read 2008). Ectomycorrhizal fungi form physical connections with all dominant western and boreal conifers.

Ectomycorrhizal fungi generally improve overall tree health, but can be essential to the growth and establishment of seedlings (Smith and Read 2008). Severe disturbance by MPB may impact ECM fungal communities due to reductions in carbon (C), and changes to belowground physical and chemical conditions (e.g., temperature; mineral nutrient concentrations) (Bending and Read 1995; Calvo-Polanco et al. 2009. Recent work by Treu et al. (In-press) demonstrated that the diversity of ECM sporocarps declined in response to increasing severity of MPB disturbance. Limitation in C supply may have been a causal mechanism underlying this decline, as previous studies have reported a strong link between stand-levels photosynthetic rates and ECM fungal fruiting production (Hogberg et al. 2001; Kuikka et al. 2003). ECM fungal species functionally complement one another relative to extracelluar enzyme activities, as noted by Jones et al. (2010). Thus, shifts in the composition of ECM fungal communities could result in functional changes, of which the impacts on seedlings are unknown.

The impacts of MPB-driven tree mortality on interactions between seedlings and fungi –ectomycorrhizas—are poorly understood but are likely to be at least equally influential to seedling growth and establishment as is a pulse of soil resources (Marjanovic et al. 2005; Smith and Read 2008). Access for seedlings to a relatively increased abundance of belowground resources may, however, depend strongly on the strength of ECM facilitation (Plassard et al. 1994). Such ECM-mediated resource acquisition may depend on the species composition, diversity, and overall abundance of ECM fungi, as well as the specific host-fungus combinations (Marjanovic et al. 2005). These characteristics, in turn, may depend on how individual species and communities of ECM fungi—and their tree hosts—respond to a myriad of shifts in resources and environmental conditions associated with beetle disturbance.

Due to the complexity of shifting resources and conditions above- and belowground following MPB disturbance, as well as a suite of confounding environmental factors inherent to most field studies, field–based investigations have substantial limitations relative to inferring key physioecological drivers of seedling growth and establishment. Therefore, I used a greenhouse experiment to test the main effects and interactions of (1) light intensity, (2) pine needle litter presence and origin, and (3) ECM fungi colonization and origin on first-year growth performance and following-year flush potential of conifer seedlings. I selected lodgepole pine and white spruce (*Picea glauca* (Moench) Voss) because these species are dominant and widely distributed throughout the historic and expanded range of MPB. Both species play a significant ecological and economic role across the MPB-affected landscape, yet their long-term viability is now uncertain. .

3.2 Methods

3.2.1 Experimental overview

In May 2011, I accessed field sites within the lower foothills natural sub-region of northwestern Alberta (118° 59'W; 54°39'N; 1027 m) located roughly 60 km southwest of the city of Grande Prairie (Chp One, Fig. 2.1). I selected 11 lodgepole pine-dominated sites forming a gradient of MPB-induced tree mortality (0 – 84% overstory tree mortality; see Chp One, section 3.1). In both July and September 2011, I surveyed stand structure and beetle activity in each stand to calculate the density and basal area by stem type, species, and of beetle kill. Among the 11 sites, I selected two that were uninfested with MPB, and two that were severely infested (63-84% morality).

3.2.2 Soil and litter collection

Samples of litter and soils were collected from ten locations ≤ 1 m outside of the boundaries of existing research plots (See Chapter One, section 2.2) located in each of the four stands. At each location, I measured and marked a 0.5 x 0.5 m area on the forest floor within which needle litter lacking visible decay (i.e., relatively fresh litter) was collected and placed into paper bags for transport to the University of Alberta (U of A). I then removed remaining subsurface litter and the organic soil horizon to expose the soil A Horizon, and collected mineral soils to a vertical depth of 12 cm. Soil samples were kept on ice until shipment to the U of A for storage at 4° C; litter samples were also stored at the same temperature and U of A facility. Bulk litter samples were pooled by their respective disturbance class, oven-dried at 60° C for 48 h, sorted to remove fragmented pine and non-pine particles, weighed and separated into 12 g portions, and returned to cold storage at 4° C. Bulk soil samples were pooled by their respective disturbance class, sifted with a 4 mm sieve to remove non-mineral soil particles, and returned to cold storage at 4° C.

3.2.3 Greenhouse experiment

In November 2011, I initiated an experiment in a transparent glass-encased greenhouse. I filled 1 L pots with 700 ml of a sterilized 70:30 coarse sand:topsoil mixture. Mixtures were inoculated with field soil by adding and mixing 20 ml (28.6% of plot volume) of the sampled forest soil into each of the pots. Twenty surface-sterilized seeds of lodgepole pine and white spruce from a common seedlot were sown into their respective pots. Following germination, seedlings were thinned to one individual per pot and growth under factorial combinations including two additional treatments: 1) lodgepole pine litter addition (by origin), and 2) light alteration. Specifically, litter was manipulated by adding 12 g of field-collected litter from each stand type (i.e., MPB infested and uninfested), as well as a no litter control. Light was manipulated by reducing with shade cloth (65% shade) ambient light supplemented from lamps (ambient light, hereafter) for 12 hrs daily (8 AM – 8PM). Average monthly temperature ranged from 22 to 25 ^{o}C.

To test the impacts of the experimental treatments on both establishment success and flush potential, seedlings were randomly assigned for harvest and measurement at one of two stages of growth: 1) active growth, harvested in early September prior to bud set (nine and half-month old seedlings), and 2) dormancy, harvested in early November following but set (eleven and half-month old seedlings), achieved through continuous outdoor exposure beginning in early September and lasting two months. Prolonged diurnal exposure to sub-freezing temperatures provides a biological cue that elicits physiologically adaptive responses—collectively dormancy—that promote seedling survival during periods of resource scarcity and physical constraints on biochemical processes (Kozlowski and Pallardy 2002). Treatments were replicated eight times in a full factorial, completely randomized block design yielding a total of 576 pots (2 species x 3 levels of ECM inoculation x 3 levels of litter x 2 levels of light x 2 harvest levels x 8 replicates).

3.2.4 Harvest one: active growth

After 9.5 months half of the seedlings were excised at the root collar, separated between stem and foliar tissues, wrapped in aluminum foil, placed in a cooler over dry ice, and brought to storage at -40 ° C. Intact roots systems and potting media were placed individually into plastic freezer bags and brought to storage at -40 ° C. Seedlings were freeze dried for 48 h and weighed to determine biomass by tissue type.

3.2.5 Harvest two: dormancy

The remaining unharvested seedlings were moved outdoors and placed onto a fully exposed rooftop until being harvested two month later. Individuals were positions such that the relative blocking design and shade treatments were maintained outdoors. At harvest, seedlings were excised at their root collar and separated between tissues of shoot (stem and foliar) and root; roots were rinsed gently with tap water to remove soil, and both tissue types placed into labelled paper envelopes. I oven dried tissues for 1 h at 100 ° C, and then for 48 h at 70° C and weighed them by tissue type. I ground using a Wiley Mill (Thomas Scientific, Inc., Swedesboro, NJ, USA) to ≤ 400 µm particle size and delivered ground samples to the Forest Ecology Laboratory (Department of Renewable Resources, U of A) for determination of water-soluble sugar and starch concentrations. Concentrations of total non-structural carbohydrate (NSC) reserves (i.e., sugars and starches) present in whole-seedling tissues following the onset of dormancy can help predict overwintering survival (Landhausser et al. 2012) as well as the magnitude of growth likely to occur during spring flush the following year (Landhausser et al. 2012). Extracts of soluble sugars, obtained using triple extraction with hot 80% ethanol, were mixed with phenol-sulfuric acid, and measured calorimetrically with a spectrophotometer (Genesys 10S UV-Vis; Therma Scientific, Madison, WI, USA) at a 490 nm absorbance (Chow and Landhausser 2004). Starch concentrations were measured by digesting the remaining tissue with the enzymes α-amylase and amyloglucosidase, and reacting them with perioxidase-glucose and oxidase-*o*-dianisidine for colorimetric measure at 525 nm absorbance (Chow and Landhausser 2004). Soluble sugar and starch estimates are presented at the whole-plant level.

3.2.6 Statistical analysis

To test the main effects and interactions among light, litter, and soil inoculation on seedling growth and establishment, while accounting for variance linked to spatial blocking and split-plot arrangement of the light treatments, I used linear mixed effects models in R (Pinheiro and Bates 2000; R Development Core Team, 2013). Fixed factors included light, litter, and fungi, while the random factor was block, with plot nested within the block by light interaction (Zuur et al. 2009). To determine statistical differences among treatment groups, I performed post-hoc comparisons using Fischer's

Least Significant Difference Tests, and adjusted p-values using Bonferroni Correction to account for multiple inference (Dunn 1961). To meet the assumptions of my parametric tests, including normality and homoscedasticity of statistical errors, I log transformed response variable data prior to all analysis.

3.3 Results

3.3.1 Seedling biomass

Seeding total biomass for each of the species and stages of growth varied in response to the main effects of, and interactions between, soil inoculation and light manipulation (Table 3.1). Across each species and stage, seedlings of greatest mass were typically associated with ambient light and soil inoculation (Figs. 3.1, 3.2, 3.3, 3.4). However, the impact of inoculation on biomass was more pronounced for lodgepole pine than for white spruce; inoculated lodgepole pine seedlings in ambient light were consistently heavier than uninoculated control seedlings in ambient or reduced light (Figs. 3.1, 3.3). In white spruce, biomass of inoculated seedlings typically exceeded those uninoculated only in ambient light (Figs. 3.2, 3.4). In pre-dormant lodgepole pine, mass was greatest for unshaded seedlings inoculated with ECM fungi originating from undisturbed stands (Fig. 3.1). In dormant lodgepole pine, mass was lowest for shaded seedlings grown with inocula from disturbed stands, while that of shaded seedlings grown with inocula from undisturbed stands was equivalent to that of unshaded, inoculated seedlings (Fig. 3.3). In both pre-dormant and dormant white spruce, mass was greatest for unshaded, inoculated seedlings, irrespective of origin (Figs. 3.2, 3.4).

3.3.2 Non-structural carbohydrates

In lodgepole pine, concentrations of sugar, starch, and total NSCs varied in response to the main effects of, and interactions between, soil origin and light intensity (Table 3.1). Control seedlings grown in ambient light without soil inoculation were consistently lowest in sugar, starch, and total NSC reserves (Figs. 3.5, 3.6, 3.7). Concentrations of sugar in lodgepole pine were significantly reduced in the absence of soil inoculation, irrespective of light intensity, with the lowest concentrations observed in unshaded, control seedlings without soil inoculation (Fig. 3.5); this group also showed the lowest concentrations of total NSCs (Fig. 3.7). Starch concentrations for lodgepole pine were lowest for shaded, control seedlings, while all other groups were equivalent (Fig. 3.6).

Similar to lodgepole pine, concentrations of total NSCs in white spruce were influenced by interactions between light and soil inoculation (Table 3.1). In terms of main effects, concentrations of sugar were highest for seedlings grown with soil inocula originating from disturbed stands (Figs. 3.8).

3.4 Discussion

Seedling of lodgepole pine and white spruce treated to differential combinations of light, litter, and soil inoculation varied markedly in multiple parameters of growth that underlie establishment success and flush potential**.** Across all treatment factors, soil inoculation had the strongest impact on growth, with light ranking as the second most important factor. Interactions between light and soil inoculation also impacted the growth of each seedling species, with compounding effect observed in once instance. When compared to white spruce, the main effects of, and interactions between, soil inoculation and light intensity impacted a wider variety of responses for lodgepole pine. In fact, such interactions in lodgepole pine affected all the growth responses tracked in the experiment. Soil inoculation and ambient light generally increased total biomass and NSC reserve status of seedlings of lodgepole pine and, to a lesser extent, white spruce. Overall, I found some indirect evidence for potential alteration of ECMs following MPB disturbance. This was manifested in reductions to seedling total biomass and NSC reserve status in several instances. Consequently, my finding suggests that disturbance-induced changes to ECM fungal communities, in concert with altered understory light intensity and litter inputs, may potentially constrain lodgepole pine seedling establishment after MPB disturbance. Below I discuss the importance of these results on seedling establishment and growth.

3.4.1 Effects of soil inoculations and light on seedling biomass

For trees, the period of seedling establishment, which typically occurs within the first few weeks to months after germination, is the most critical phase of survival and growth (Smith et al. 1997). The probability of mortality is high in this phase due to heightened vulnerability to disease and herbivore agents, as well as abiotic stressors, such as drought (Smith et al. 1997).

In my study, both pre-dormant and dormant lodgepole pine, total seedling biomass responded to interactions between light and soil inoculation. Specifically, growth stage at harvest mediated the light intensity under which relative benefits to biomass accumulation were conferred by ectomycorrhizas formed with fungal associates from healthy stands. In the predormancy phase, relative benefits of reference versus disturbance-associated ectomycorrhizas occurred under ambient light, whereas for dormant seedlings, relative benefits occurred in shade. It is plausible that the combined effects of reference ectomycorrhizas and ambient light contributed to relative growth rates that, although initially higher than those of disturbance-associated ectomycorrhizas, were eventually matched by dormancy onset. The relative growth advantage realized by dormant, shaded lodgepole pine grown in with disturbance-associated ecomycorrhizas is a critical finding, as it likely provides the nearest approximation in my study of reference conditions (i.e., an undisturbed forest state). In contrast, despite the relative growth benefits for unshaded seedlings forming reference ecotycorrhizas, the conditions associated therein may be rather exceptional in the field, as they emulate the intersection of rare physioecologcal factors—an absence of disturbance, yet high levels of light—across MPB-disturbed stands.

In locations where one or more unattacked trees surround pre-existing canopy gaps, stable ECM communities associated with ‘hub’ trees may be potential sources of colonization leading to infection by a diversity of ECM associates of seedlings establishing within light gaps (Beiler et al. 2010). Across both growth periods in my study, there was greater consistency in growth patterns in response to soil inoculation than to light. For example, ambient light alone failed to enhance biomass of lodgepole pine in the pre-dormant or dormant stages, whereas colonization by ECM communities from healthy stands enhanced growth for each stage. This finding suggest that even as direct and diffuse light increase during early stages of forest recovery (i.e., 3-5yr after outbreak), proximity to a limited number of dispersed live lodgepole pine trees hosting stable ECM communities may become an increasingly important legacy feature contributing to seedling establishment (Beiler et al. 2010; Swanson et al. 2011).

For white spruce, although biomass accumulation was also impacted by light and soil inoculation, the origin of ECM fungal associates that conferred a relative benefit flipped relative to lodgepole pine. For pre-dormant seedlings, any soil inoculation associated with ambient light yielded the seedlings with the highest biomass overall. However, shade-grown seedlings forming symbioses with disturbance-associated ECM fungal symbionts realized a relative benefit to biomass accumulation over control seedlings, but this benefit did not occur when seedlings received reference inocula (i.e., inocula from controlled stands). In the dormant stage, comparisons of all inoculated seedlings across light levels showed that only seedlings forming ectomycorrhizas associated with disturbance yielded biomasses that were greater than shade-grown inoculated seedlings;

this relative benefit over the shaded groups did not occur for the ambient light-grown seedlings forming reference ectomycorrhizas.

3.4.2 Effects of soil inoculations on NSCs

Carbon metabolism is an essential and prominent physiological activity in plants serving many roles (Kozlowski and Pallardy 2002). A relatively large fraction of plant C exists in the form of NSC (Kozlowski and Pallardy 2002). Accumulation and storage of NSCs is a physiologically adaptive function enabling plants to buffer temporal imbalances between C supply and demand, thus facilitating essential functions including growth, respiration, reproduction, and defense (Chapin et al. 1990; Sala et al. 2012).

My findings support the occurrence of ECM-mediated NSC reserve accumulation, as well as demonstrate differential resistance between lodgepole pine and white spruce to alterations in NSC status by MPB disturbance-associated experimental treatments. My results showed that NSC reserves of lodgepole pine resisted shifts in NSC reserves, while those of white spruce were relatively sensitive to such shifts. In contrast to NSC reserves of lodgepole pine, which though enhanced by ECM colonization where not influenced by origin, those of white spruce responded most positively to ectomycorrhizas formed with disturbance-associated ECM fungal communities. In lodgepole pine, reductions in biomass, but maintenance NSCs, in response to disturbance-associated ECM fungal infection, may be a consequence of C competition between actively regulated NSC stores and actively consuming NSC sinks (i.e., growth). Light may also be an important factor leading to shifts C allocation as well. The prioritization to reserve accumulation by lodgepole pine indicates a trade-off in C allocation between maintaining NSC reserves, potentially sufficient to buffer against environmental stochasticity versus allocation of C to growth.

3.5 Conclusion

Deep uncertainly surrounds the cumulative impact of MPB epidemics on forest ecosystem services—timber production, water quality and availability, C sequestration, and maintenance of wildlife populations. Natural recovery of MPB-killed forests will rely on ecological mechanisms influencing the growth, establishment, and survival of tree seedlings. To better understand the relative role of shifting ecological resources, conditions, and interactions on seedling establishment and growth into the following year, I performed a greenhouse experiment to emulate a subset of conditions documented in MPB-killed stands. My results showed that light availability and ECM fungal symbiosis substantially and consistently impact seedling biomass and its organ-wise allocation, and NSC reserve status. Seedlings of lodgepole pine colonized by ECM fungal associates from healthy stands tended to outperform seedlings colonized by disturbance-associated ECM fungi, in terms of accumulation of biomass, but not NSCs. In contrast, white spruce seedlings colonized by ECM fungal communities from disturbed stands tended to outperform seedlings colonized by ECM associates from healthy stands. My contrasting findings between the two trees species suggests that MPB disturbance may have triggered shifts in ECM communities, and that such shifts may limit the establishment success of lodgepole after disturbance, while simultaneously improving tree species such as white spruce. However, resistance of lodgepole to reductions in NSC concentrations indicate that this species may be more resistant than white spruce to secondary stressors during forest recovery. Thus, the relative success of each species after widespread MPB outbreak may depend on the frequency and magnitude of secondary stressors (e.g., drought; pathogens), and the species' respective adaptive strategy for C allocation, which appear, in the establishment stage, to be in opposition to one another.

Figure 3.1. Total biomass of lodgepole pine (*Pinus contorta*) harvested during active growth after grown under different combinations of light intensity ((i.e., 65% shade ("shade")), and full sun light ("sun")) and source-specific soil inoculation ((i.e., incocula from healthy ("healthy"), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) ("Red-attack")).

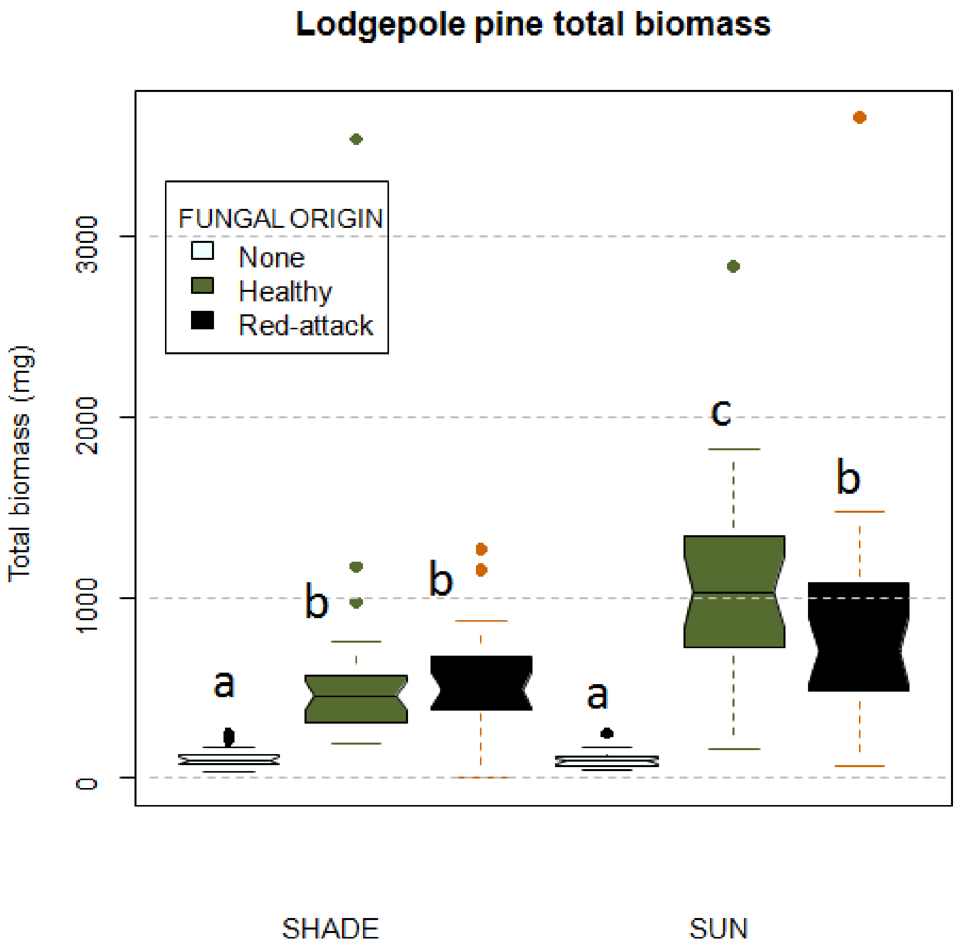

Figure 3.2. Total biomass of white spruce (*Picea glauca*) harvested during active growth after grown under different combinations of light intensity ((i.e., 65% shade ("shade")), and full sun light ("sun")) and source-specific soil inoculation ((i.e., incocula from healthy ("healthy"), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) ("Red-attack")).

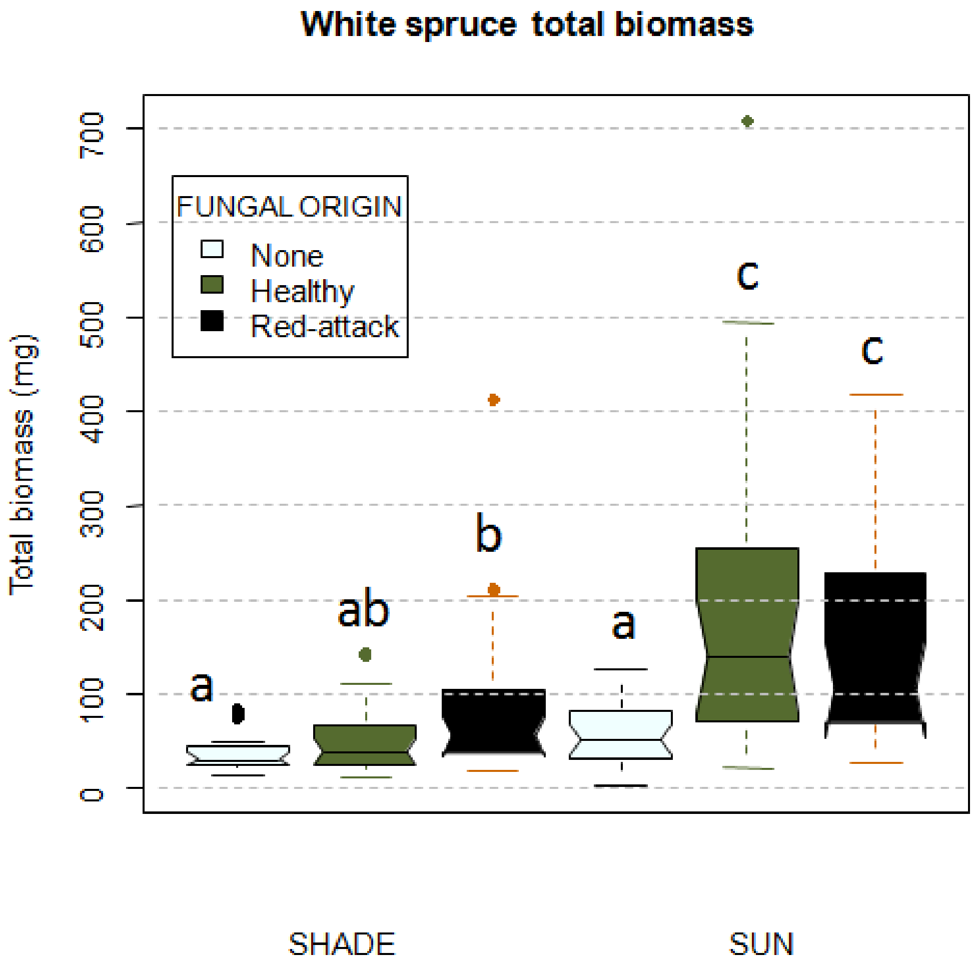

Figure 3.3. Total biomass of lodgepole pine (*Pinus contorta*) harvested during dormancy after grown under different combinations of light intensity ((i.e., 65% shade (“shade”)), and full sun light (“sun”)) and source-specific soil inoculation ((i.e., incocula from healthy (“healthy”), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) (“Red-attack”)).

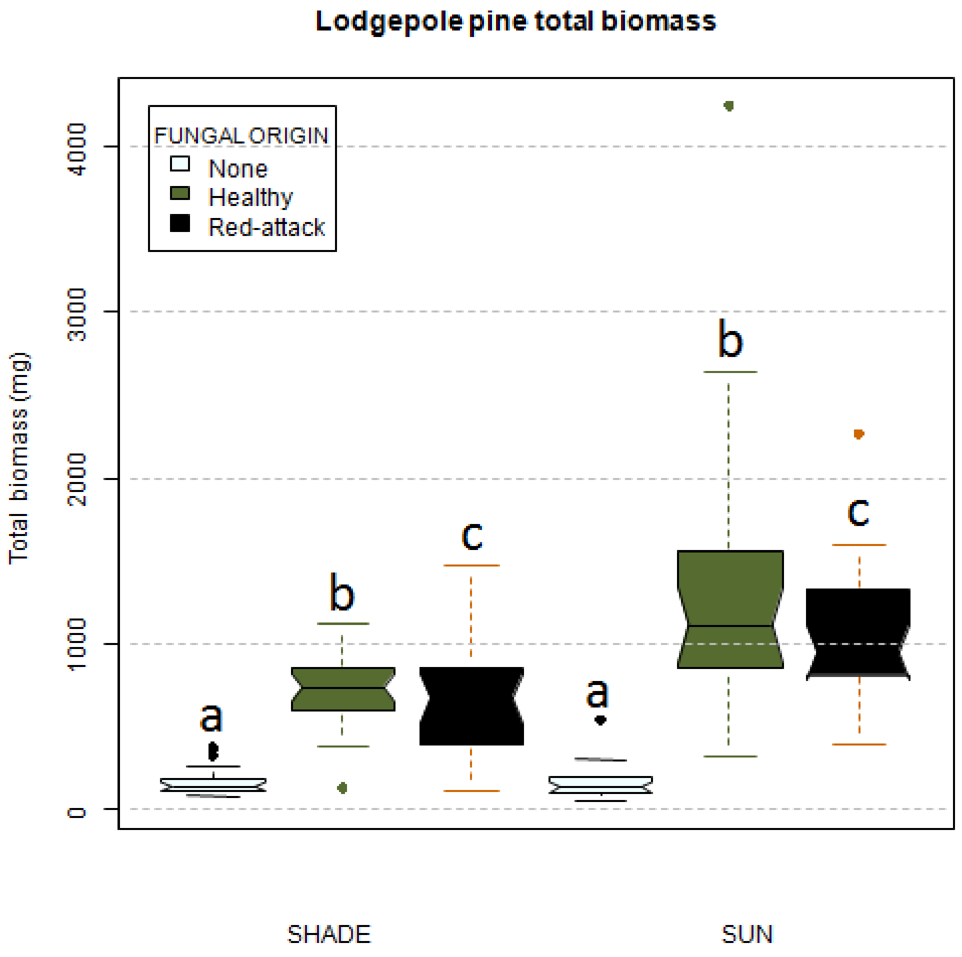

Figure 3.4. Total biomass of white spruce (*Picea glauca*) harvested during dormancy after grown under different combinations of light intensity ((i.e., 65% shade ("shade")), and full sun light ("sun")) and source-specific soil inoculation ((i.e., incocula from healthy ("healthy"), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) ("Red-attack")).

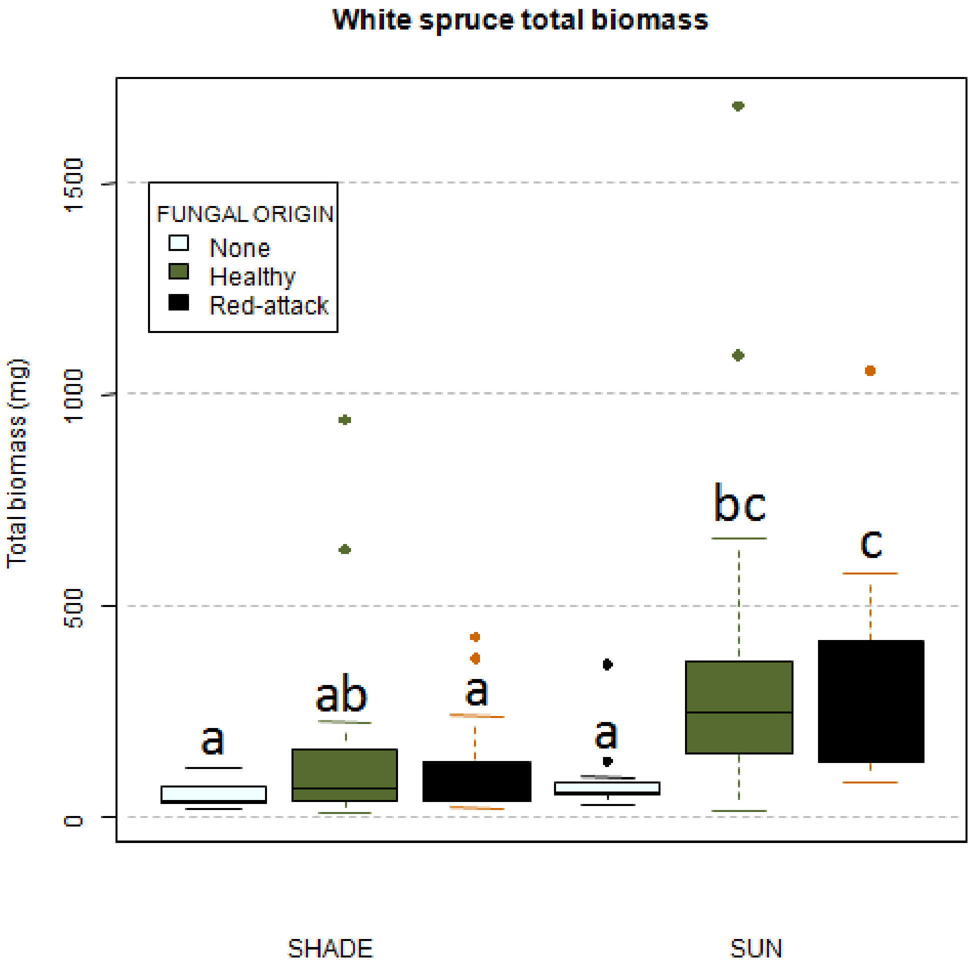

Figure 3.5. Sugar concentration of lodgepole pine (*Pinus contorta*) harvested during dormancy after grown under different combinations of light intensity ((i.e., 65% shade ("shade")), and full sun light ("sun")) and source-specific soil inoculation ((i.e., incocula from healthy ("healthy"), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) severely MPB-disturbed stands ("Red-attack")).

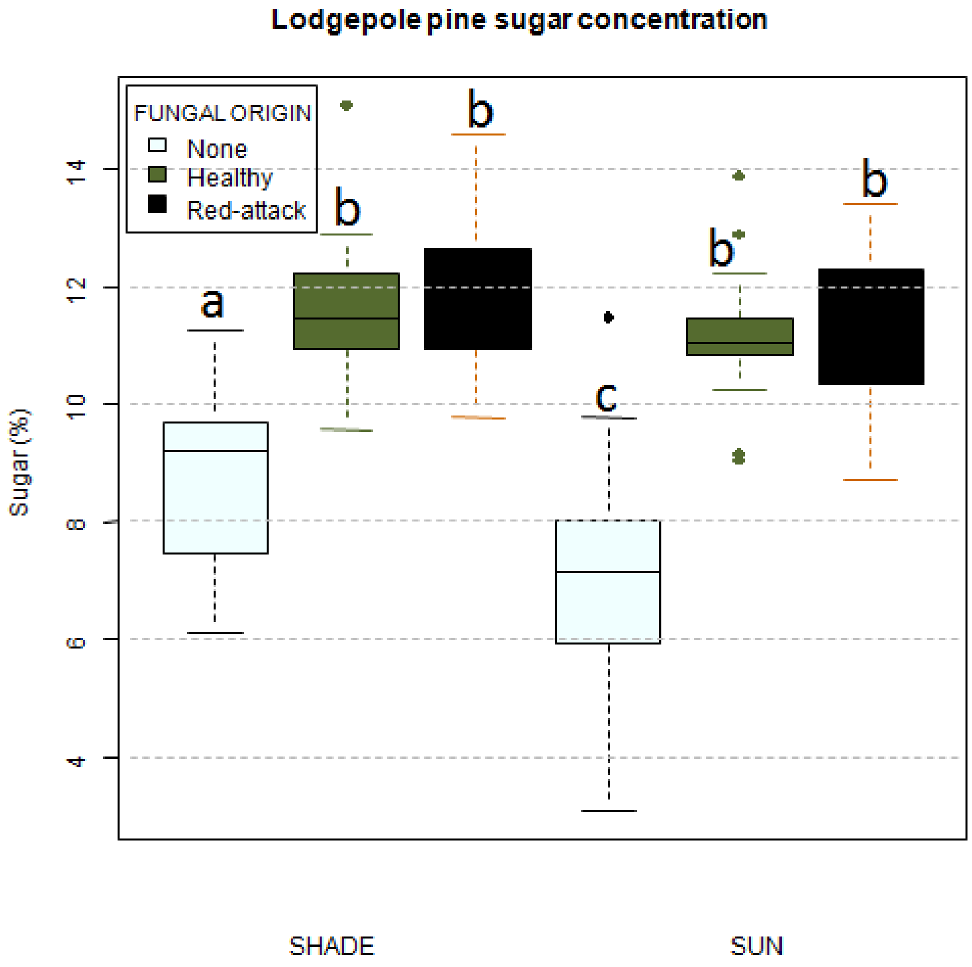

Figure 3.6. Starch concentration of lodgepole pine (*Pinus contorta*) harvested during dormancy after grown under different combinations of light intensity ((i.e., 65% shade ("shade")), and full sun light ("sun")) and source-specific soil inoculation ((i.e., incocula from healthy ("healthy"), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) ("Red-attack")).

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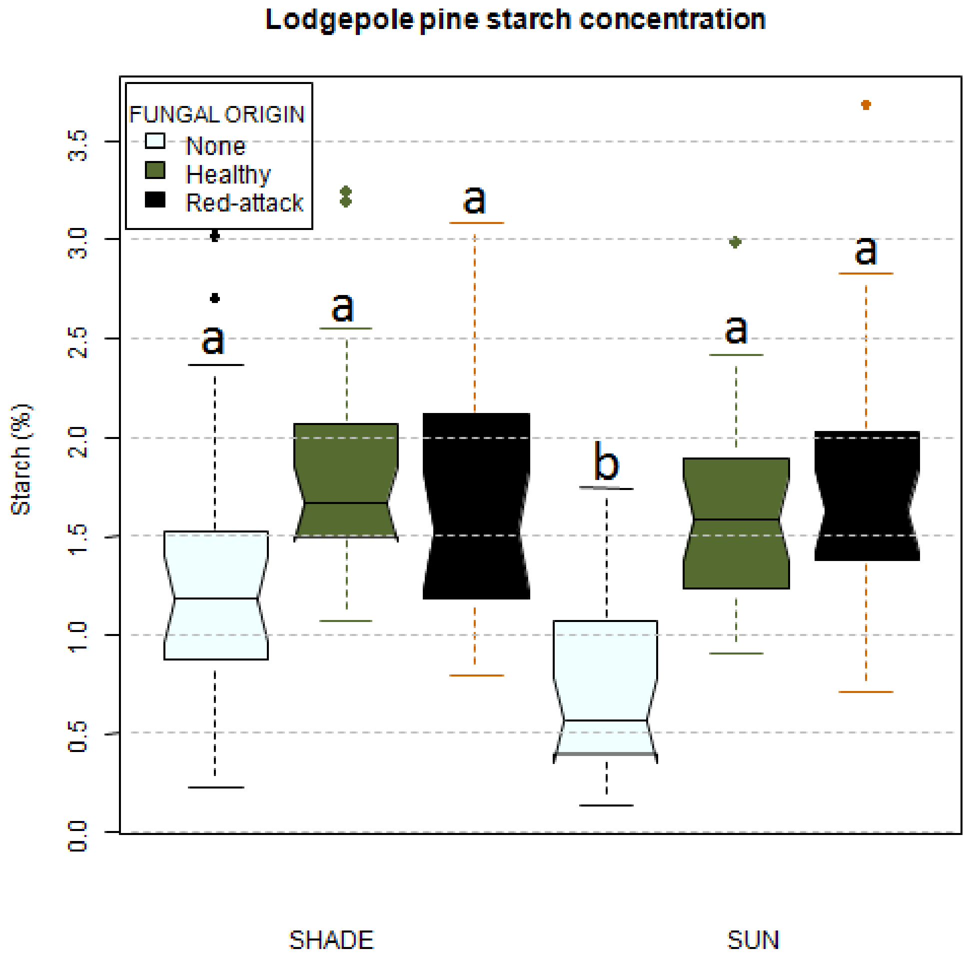

Figure 3.7. Total non-structural carbohydrate concentration of lodgepole pine (*Pinus contorta*) harvested during dormancy after grown under different combinations of light intensity ((i.e., 65% shade (“shade”)), and full sun light (“sun”)) and source-specific soil inoculation ((i.e., incocula from healthy (“healthy”), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) (“Red-attack”)).

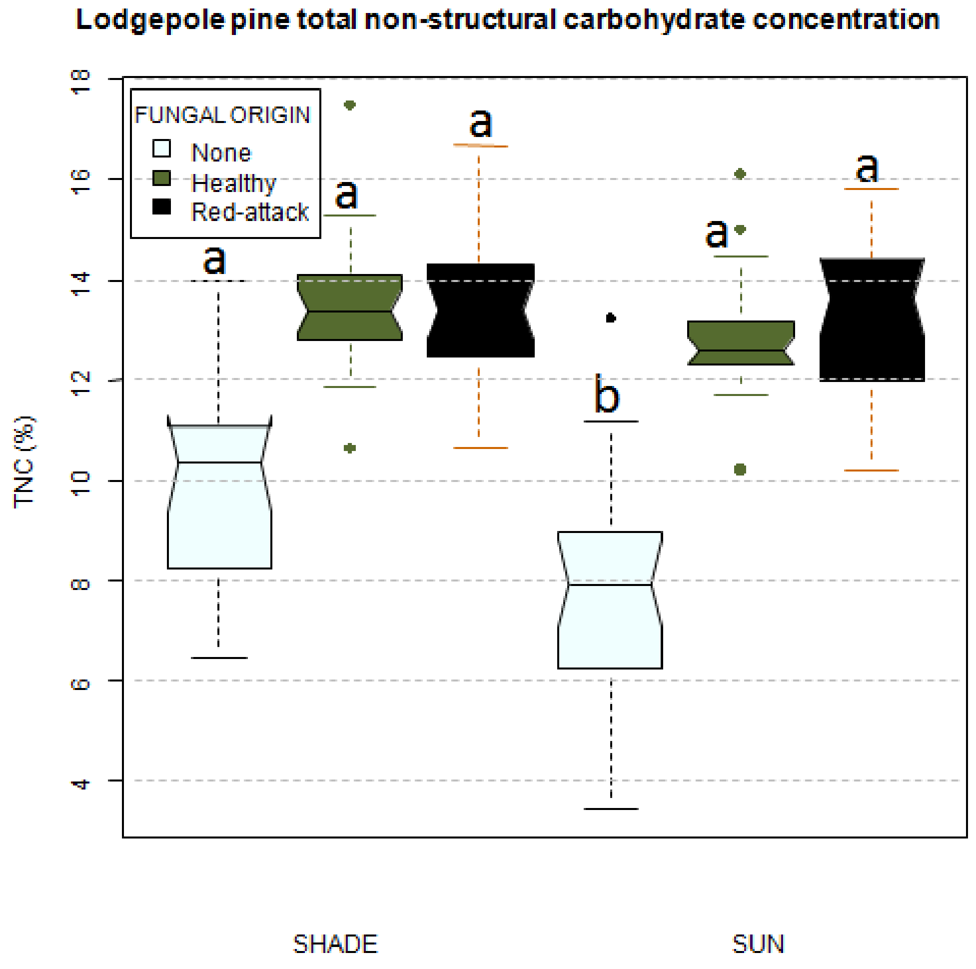

Figure 3.8. Sugar concentration of white spruce (*Picea glauca*) harvested during dormancy after grown under different combinations of source-specific soil inoculation ((i.e., incocula from healthy ("healthy"), or stands severely disturbed by mountain pine beetle (*Dendroctonus ponderosae*) ("Red-attack")).

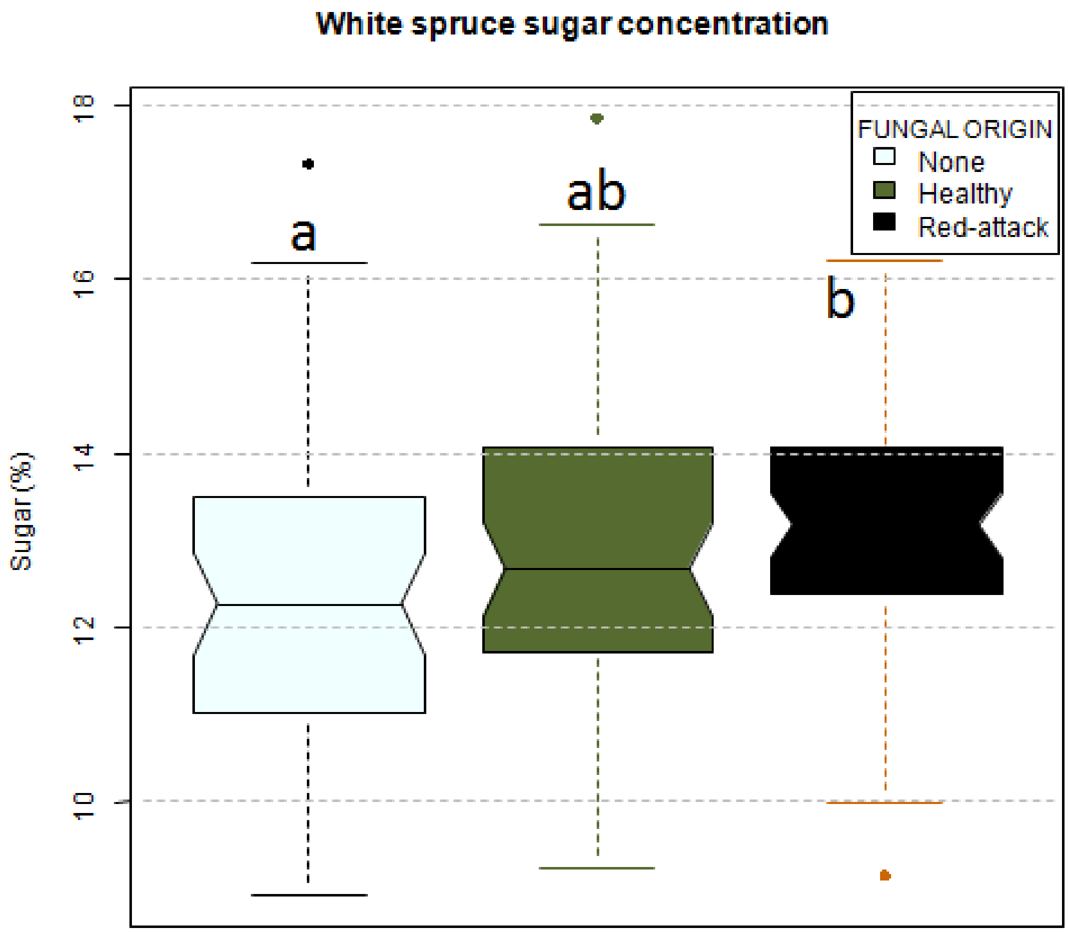

Table 3.1. Main effects and interactions of experimental combinations of mountain pine beetle (*Dendroctonus ponderosae*)-associated light, litter, and soil inoculation on biomass, root:shoot ratios, and concentrations of sugar, starch, and total non-structural carbohydrates of white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta*) seedlings harvested either during active growth or dormancy. Significance codes are: "." $P < 0.1$; "*" $P < 0.05$; "**" $P < 0.001$; "***" $P < 0.001$

Fixed factor	Light (Lg)	Litter (Lt)	Fungi (Fg)	Lg * Lt	Lg * Fg	Lt * Fg	Lg * Lt * Fg
Test statistic	*F*	*F*	*F*	*F*	*F*	*F*	*F*
<u>Response variable</u>	<u>Lodgepole pine, Harvest 1 – Pre-dormancy</u>						
Total biomass	7.63*	NS	118.74***	NS	4.41*	NS	NS
Root:shoot ratio	NS	NS	4.28*	NS	4.68*	NS	3.43*
	<u>Lodgepole pine, Harvest 2 – Dormancy</u>						
Total biomass	16.77**	NS	191.18***	NS	5.78**	NS	NS
Root:shoot ratio	NS	NS	4.16*	NS	16.56***	NS	NS
Sugar concentration	19.25**	5.36**	109.16***	NS	6.73**	NS	NS
Starch concentration	NS	NS	37.93***	NS	5.49**	NS	NS
TNC	20.60**	5.62**	105.47***	NS	7.82**	NS	NS
<u>Response variable</u>	<u>White spruce, Harvest 1 – Pre-dormancy</u>						
Total biomass	35.82**	NS	15.08***	NS	4.45*	NS	NS
Root:shoot ratio	NS	NS	NS	NS	NS	NS	NS
	<u>White spruce, Harvest 2 – Dormancy</u>						
Total biomass	20.52**	NS	20.65***	NS	3.32*	NS	NS
Root:shoot ratio	NS	3.69*	NS	NS	NS	NS	NS
Sugar concentration	NS	3.79*	3.92*	NS	NS	NS	NS
Starch concentration	NS	5.87**	16.88***	NS	NS	NS	NS
TNC	NS	5.45**	8.99***	NS	3.54*	NS	NS

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CHAPTER FOUR

Thesis discussion

4. Introduction

In chapter two, we saw that severity of, and time since, MPB disturbance were positively associated with litter-derived nutrient inputs. This, possibly in conjunction with reductions in nutrient uptake, resulted in increasing levels of soil nitrate, and overall soil fertility. These findings suggested that an additional investigation was needed to test these and other MPB disturbance-associated shifts on the potential responses of tree seedlings. In chapter three, findings from a greenhouse experiment used for this investigation were described. The findings from the greenhouse experiment elucidated the relative importance and interdependence amongst essential physioecological factors of conifer regeneration in the context of MPB outbreak. These factors include the availability of understory light, inputs of litter to the soil surface, and the strength and diversity of mutualisms with ECM fungi (Fig. 4.1). Below I discuss some of the possible causes and consequences of these results.

4.1.1 Tree mortality alters energy transfer and nutrient cycling

Mountain pine beetle epidemics result in substantial mortality of canopy trees, altering the availability of resources for auto- and heterotrophic organisms (Martin et al 2006; Treu et al. In-press). Canopy trees form prominent physical and energetic linkages between the above- and belowground environment (Hari et al. 2013). Such linkages mediate the availability of limiting resources for biota dwelling above -and belowground via energy flow and nutrient cycling between plant tissues (e.g., shoot vs. roots) and extrastructural storage pools (e.g., canopies vs. soils) (Saetre 1999; Hogberg et al. 2001). The rates of energy flow and nutrient cycling between tissues and pools, and hence the availability of resources at biotic sinks, are strongly influenced by the health, density, biomass, and composition of vegetation throughout all strata, particularly the forest canopy (Smith et al. 1997). Attack and subsequent mortality of canopy trees by MPB does, however, change the functional capacity (Hubbard et al. 2013) and structural presence (Mitchell and Preisler 1998) of those trees within the forest ecosystem. Such changes alter the availability of limiting resources as well as the strength of symbioses (e.g., ectomycorrhizas) required by vegetative strata that are crucial to long-term outcomes of ecosystem recovery, particularly tree seedlings (Jones et al. 2003).

Substantial mortality of canopy trees reduces both hydraulic conductance and gross primary productivity (GPP) (Brown et al. 2012) at the stand level; in turn, reductions in stand-level GPP are likely to reduce the overall volume of carbon that is fixed and translocated between plant tissues and re-distributed from tree host to fungal symbionts (Bidartondo et al. 2001; Heinenmeyer et al. 2006). Similarly, reductions in stand-level hydraulic conductance may lower the rates of uptake of nutrients and water from soils, thereby enhancing the supply of mineral nutrients and moisture in soils, as shown demonstrated in chapter two. In addition, mortality of canopy trees may alter the distribution patterns and abundance of physical structures that indirectly mediate absorption and re-radiation of solar energy. For example, variability in the spatiotemporal patterns of tree attack and mortality during MPB outbreaks may increase the spatial heterogeneity of the canopy structure as biomass from needles and branches fall to the soil surface (Chp 2; Simard et al. 2012). The resultant formation of spatially dispersed structural legacies such as canopy gaps and enlarged crown interstices will be associated with reductions in leaf area index and levels of light interception (Pugh and Small 2012); this effect may enhance the amount of direct and diffuse solar radiation reaching tree seedlings in the understory. Deep strata of horizontally arranged needle litter and existing mosses are a physical barrier to germination, as well as a threat to survival beyond the succulent phase of seedling development (Astrup et al. 2008; McIntosh and Macdonald 2013). Such layers may inhibit the penetration of seeds into moisture-rich substrata, despite persistent seed rain from decaying tree crowns (Teste et al. 2012). They may also increase the likelihood of heat injury on succulent stems of new germinants by impeding the dissipation of warm air via convective currents within several centimetres of the litter surface (Smith et al.1997). The abundance and diversity of ECM fungi may be affected by the above resource shifts. In fact, my experimental results suggest that such effects have occurred, representing an additional and perhaps most important mechanism by which MPB disturbance may impact seedling regeneration (Fig 4.1).

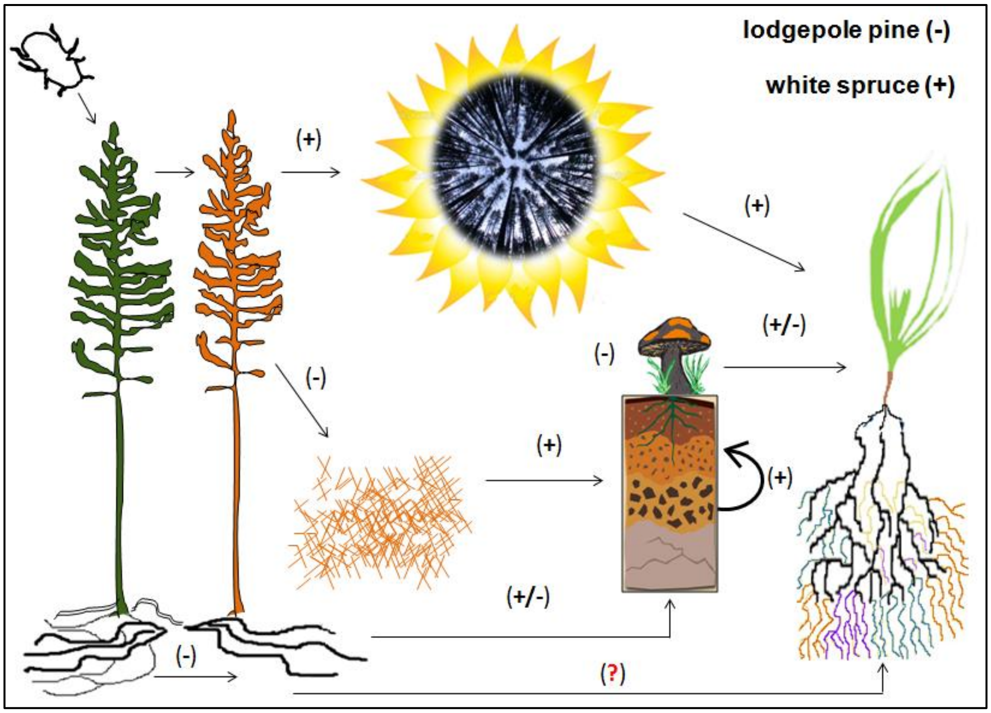
4.1.2 ECM fungal symbiosis and seedling regeneration

A wealth of evidence demonstrates that ECM symbioses benefit seedlings (Smith and Read 2008). However, to my knowledge, no prior studies evaluated the impacts of MPB disturbance on the strength of such symbioses. I found treatment-induced biomass alteration in both lodgepole pine and white spruce. This finding not only stresses the importance of ECM-mediated biomass accumulation for establishing seedlings, but also provides evidence for potential disruption to these mutualisms following MPB disturbance. My findings showed that such disruptions manifest in antagonistic effects on the growth of lodgepole pine seedlings, but contrasting positive effects on the growth of white spruce. These effects may have been linked to one or more of the following proximate causes: 1) reductions in the biomass of vegetative mycelia of ECM specialists of lodgpole pine, with a corresponding competitive advantage realized by possible specialists of white spruce; 2) compositional shifts toward species with greater relative proficiency for degrading and utilizing molecular constituents of complex organic compounds (e.g., cellulose of confer needles) (Hibbett et al. 2000; Cullings et al. 2003), but with relatively reduced proficiency for bi-directional, mutualistic exchanges.

To elaborate on the first point, differential effects may be linked to shifts in ECM fungal communities driven by reductions in lodgepole pine-adapted species (*Sensu*: Jones et al. 2010). In western Canada, MPB typically infests stands in which mature lodgepole pine has the highest relative density (Safranyik and Carroll 2007). Such stands are typically other tree-species poor (but see: Coates et al. 2010), and this attribute may be reflected in the composition of soil microbial communities with which individual tree species are commonly associated (Jones et al. 2010). From early to late seral stages of forest succession, tree-mediated changes in the physical (e.g., light), chemical (e.g., soil nutrient), and herbivore regimes may actively (e.g., ECM chemosphere development) or passively structure ECM fungal communities (Nara 2006; Twieg et al. 2009; Rasmann et al. 2010; Jones et al. 2010). As a result, seres in the succession of ECM communities may consist of species either most tolerant of, or conversely, most adapted to, features of a given monotypic host environment (Smith and Read 2008). Due to the selective mortality of the dominant tree host by MPB attack, disproportionate reductions in the abundance or function of individual ECM fungal species or guilds, displaying high host specificity toward lodgepole pine, (i.e., ECM specialists) may have occurred. Associated declines in ECM species or functional diversity may hence pose limitations to the recruitment of lodgepole pine seedlings. Moreover, reductions in competition between possible ECM specialists of lodgepole pine versus white spruce for root substrate and mineral nutrients may have improved the relative status of ECM specialists of white spruce, thereby conferring a relative growth benefit to that species, as our results suggest.

While it remains unclear how intact ECM communities as a whole will respond to severe MPB disturbance, recent evidence demonstrates a decline in their fruiting production (Treu et al. In-press). This effect is a likely indicator of reduced stand-levels photosynthetic rates (Hogberg et al. 2001; Kuikka et al. 2003) and hence carbon host-carbon supply. An emerging priority based on the findings of this thesis should be quantification of ECM community shifts on seedling regeneration in the field.

Figure 4.1. Conceptual diagram of known and inferred impacts of mountain pine beetle (*Dendroctonus ponderosae*) disturbance on canopy openness, nutrient inputs from pine needles, fine root biomass, soil chemistry, ectomycorrhizal mutualisms, and seedling growth of white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta*). Arrows indicate pathways of inferred cause and effect, "+" and "**-**" symbols represent the direction of a given linear relationship between components, and "?" indicates an unknown relationship.


lodgepole pine (-)
white spruce (+)
(+)
(+)
(+/-)
(-)
(-)
(+)
(+)
(+/-)
(-)
(?)

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