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Ecology of understory and below-ground communities in lodgepole pine forests under changing disturbance regimes

by

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Dedication

Dedicated to my parents: my mom who has always believed in me and encouraged me to succeed at whatever I attempt in life, and to my dad who said ‘it’s really the PhD that counts’ as I was completing my MSc! I may not agree entirely, but I am here because of you ☺

Abstract

As climate changes and disturbance regimes shift, there is a need to better understand and anticipate potential impacts of both natural and anthropogenic disturbance agents on forest ecosystems. Lodgepole pine forests in western Canada are experiencing an unprecedented mountain pine beetle (MPB) outbreak, and the ecosystem-level effects of ongoing expansion of MPB into novel habitats east of the Canadian Rockies are unknown. Another way ecosystems are being disturbed is through management practices that are attempting to enhance timber production by using introduced species in plantations; lodgepole pine has been introduced around the world as an important timber species, but its invasive potential in some areas, such as Sweden, remains unclear.

To better understand the ecological impacts of disturbance in lodgepole pine ecosystems I investigated: i) fine-scale patterns in understory plant and microbial communities in mature lodgepole pine forests; ii) effects of simulated MPB attack and salvage harvest on above- and below-ground dynamics of these forests; iii) potential for pine regeneration after MPB attack in newly invaded stands; and iv) effects of the introduction of lodgepole pine to Sweden on forest floor properties and processes.

In mature undisturbed lodgepole pine forests I identified four fine-scale plant communities, primarily influenced by below-ground factors; four structural microbial communities, primarily influenced by the understory composition; and four functional microbial communities that were not strongly associated with any environmental factors measured in my study. I found short-term resistance to ecosystem change after simulated MPB attack, compared with more immediate ecosystem changes in response to salvage harvest. Regeneration of lodgepole pine seedlings appears unlikely to occur in the short term after MPB attack without active silvicultural intervention. In northern Sweden, introduced lodgepole pine had minor ecosystem-level effects compared with the native

pine; the impacts of species introductions are likely functions of both regional influences and ecological differences between the introduced and native species.

Overall, my thesis provides novel insights into the ecology of lodgepole pine forests in the face of changing disturbance regimes and forest management practices, demonstrating the important ecological roles that both above- and below-ground properties and processes play in these forested ecosystems.

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Abbreviation/Acronym	Description
CLPP	Community-level physiological profiling
dbRDA	Distance-based redundancy analysis
DWM	Downed woody material
ISA	Indicator species analysis
MPB	Mountain pine beetle
MSIR	Multiple carbon-source substrate-induced respiration
NMS	Non-metric multi-dimensional scaling
PLFA	Phospholipid fatty acid
PrCoord	Principal Coordinates Analysis
PRS	Plant Root Simulator
SIR	substrate-induced respiration

Chapter 1. Introduction to ecology of understory and below-ground communities in lodgepole pine forests under changing disturbance regimes

1.1. Introduction

Lodgepole pine (*Pinus contorta* Douglas ex Loud. var. *latifolia* Engelm.) forests are highly valued for their timber, wildlife habitat, and recreational use. Lodgepole pine has a wide climatic and geographical range in North America, from latitude 31° - 64° North, and West to East from the Pacific Ocean to the Black Hills of South Dakota (Lotan and Critchfield 1990). It is a fire-adapted, shade-intolerant species throughout much of its range, and can rapidly invade sites after disturbance, resulting in even-aged stands which may persist for up to 200 years in the absence of colonization by later-successional trees (Lotan and Critchfield 1990). Lodgepole pine stands are also susceptible to other disturbance agents, including attack by mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae). Because lodgepole pine forests often grow as monocultures, they are an ideal test system for evaluating ecosystem properties and processes at the within-stand spatial scale that are independent of differences among multiple canopy species types.

The forest canopy is an important driver of understory community composition and biodiversity, as well as below-ground processes (e.g., Légaré et al. 2002, Macdonald and Fenniak 2007), and it affects the nutrient and water availability of the stand (e.g., Prescott 2002). The forest canopy affects the availability of light, nutrients and moisture for use by the understory plant community, as well as soil pH and soil temperature (Prescott 2002, Hart and Chen 2006). The canopy also affects the understory plant community through its deposition of surficial litter, which can influence soil pH, and affects both the understory and the below-ground faunal and microbial communities (e.g.,

Hannam et al. 2006, reviewed by Barbier et al. 2008). Forest structural attributes such as basal area and stand height, independent of canopy species type, can also be associated with forest understory composition (e.g., Légaré et al. 2002). With mortality or loss of the overstory canopy as a result of disturbance, we would expect associated changes in other ecosystem properties and processes; but the direction and magnitude of these changes is unknown for many forest types, including for the lodgepole pine forests of Alberta.

The importance of understory vegetation as an integral component of the ecological functioning and biodiversity of forests has been demonstrated, although it remains an under-appreciated component of forest ecosystems (Gilliam 2007). The forest understory community of non-tree plants growing beneath the canopy contains the majority of plant biodiversity in boreal forests, and it may play an important role in determining canopy composition through its interactions with regenerating tree species (George and Bazzaz 2003). The understory plant community plays important roles in influencing both community and ecosystem properties above- and below-ground (Nilsson and Wardle 2005, Hart and Chen 2006). Understory vegetation may comprise less than 1% of the biomass of the temperate forest, yet can still comprise 90% or more of the plant species richness, and may supply up to 20% of the forest floor foliar litter, which is often of higher nutrient content than that of the tree foliar litter (Gilliam 2007). Persistent dense understories can be an important filter in determining the future successional trajectory of forests by reducing or delaying tree regeneration and growth (Lieffers et al. 1993b, Royo and Carson 2006). The forest understory also plays an important role in providing browse and habitat for a variety of other biota (e.g., Carey and Johnson 1995, Work et al. 2004). The composition and biodiversity of these ecologically important understory plant communities is driven by both above- and below-ground properties and processes, with complex interactions among these factors (e.g., Reich et al. 2012). However, there is still considerable uncertainty about which above- and below-ground factors are important

drivers of understory plant communities, their relative importance, and how these vary among ecosystem types and at different spatial scales, including at the micro-habitat scale within monoculture stands, such as lodgepole pine forests.

Below-ground forest floor (i.e. organic matter) properties and processes are also essential components of forested ecosystems that are coupled with above-ground properties and processes (Wardle et al. 2004). The forest floor is an important habitat for microbes, as well as a large portion of the arthropod biodiversity in forests (e.g., Niemelä et al. 1993). Biogeochemical cycles and the turnover of organic matter in forested ecosystems are largely influenced by the composition and activity of soil microbial communities (Zelles 1999). Tree species, stand age and soil type have all been shown to influence the soil microbial community structure and composition; in turn, influencing nutrient cycling within forested ecosystems (e.g., Bauhus et al. 1998). As well, forest management influences the soil microbial community structure (e.g., Mummey et al. 2010), and deposition of downed woody material and litter (e.g., Bigler and Veblen 2011). Thus, changes in above- ground properties, including vegetation mortality in the overstory and understory after disturbance, are likely to influence the below-ground microbial community and associated properties and processes (e.g., Xiong et al. 2011); below-ground responses to canopy mortality resulting from MPB in novel habitats remain unknown.

When species are introduced into new regions, there is great uncertainty whether the trait differences of the introduced species or regional factors, such as climate or edaphic properties, will serve as the dominant control of ecosystem properties or processes. Given its wide climatic and geographical amplitude, lodgepole pine has been introduced outside its native range as a forestry plantation species. When an exotic tree species like lodgepole pine is introduced outside its native range, it has the potential to alter the forest ecosystem in a variety of ways, compared with native species within the

same region. Lodgpole pine has been shown to be a strong invader in many parts of the world where it has been introduced, particularly in Southern Hemisphere ecosystems (Ledgard 2001, Langdon et al. 2010), where it has altered community and ecosystem-level properties (Simberloff et al. 2010). For this reason, introduction of lodgepole pine can be viewed as a disturbance in these regions. Despite its' importance as a plantation species, there is uncertainty and concern about the invasive potential of lodgepole pine in regions where it has been introduced, as it has become a weedy species in southern hemisphere regions where it has established self-perpetuating populations over large areas (Richardson 1998).

Disturbance has important ecological effects in forests, influencing the abundance, composition, and distribution of vegetation through modification of the physical environment and the spatial and temporal distribution of resources (Tilman 1982; Bazzaz 1983; White and Pickett 1985; Chaneton and Facelli 1991). Disturbance temporarily reduces live plant biomass (Reader et al. 1991), releases resources (light, space, soil moisture, nutrients) to surviving plants (Canham and Marks 1985), and permits new species to colonize the site (Grime 1973; Collins 1987). The availability of resources for use by other plant species will depend on both the quantity and size of trees affected (Franklin et al. 1987).

Lodgepole pine forests historically initiated as even-aged pioneer forests after stand-replacing wildfire. However, this historical disturbance regime is being modified by MPB (*Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae). Mountain pine beetle is considered the most destructive forest insect in western North America with a recent epidemic in British Columbia resulting in mortality of 710 million cubic meters of timber over a cumulative affected area of ~ 18.1 million hectares and the area and impacts continue to grow (Furniss and Carolin 1977, Safranyik and Carroll 2006, BC 2012). MPB kills trees by girdling them through mass attack; it has a highly

evolved mutualistic relationship with ophiostomoid "blue stain" fungi which it transports on its body; these fungi play an important role in the successful colonization of attacked trees (Kim et al. 2005). The impact of MPB as a disturbance on a forest increases with increasing numbers of MPB present within it. MPB at endemic levels targets stressed trees. At epidemic levels, MPB differs from other types of disturbance agents, such as fire or harvest, in multiple ways including that it leaves the overstory standing, and understory and soil layers intact (Burton 2008). MPB overstory tree mortality typically removes moderate levels of canopy cover and basal area over a period of a few years (Cole and Amman 1980; Romme et al. 1986, Stone and Wolfe 1996**).**

The current latitudinal and elevational range of MPB in western Canada is not restricted by the availability of suitable host trees; instead range expansion of MPB North and East has been limited by climate (Carroll et al. 2006). In 2005 MPB moved east across the Rocky Mountains into northern Alberta – British Columbia (Canada) and quickly spread through the extensive stands of lodgepole pine in Alberta. Attack in the novel host jack pine (*Pinus banksiana* Lamb.) has been confirmed (Cullingham et al. 2011), and with the range of jack pine extending across the boreal from Alberta to the East Coast, continued expansion of MPB further east and north across the boreal is possible, although this will depend on both suitable climate and host availability (Nealis and Peter 2008). The long-term implications of the expanding range of this disturbance agent are uncertain, but MPB is likely to remain in Alberta (Schneider et al. 2010).

While much research was conducted on impacts of MPB attack on vegetation following the recent epidemic in BC forests, little research on the effects of MPB on lodgepole pine forests east of the Canadian Rockies has been completed. There are significant differences in hydro-climatic regimes (e.g., longer growing season in BC) and ecosystem structure between MPB-attacked forests in BC and Alberta, which are likely to produce significantly differing effects of MPB on vegetation dynamics and below-ground

processes. It is hypothesized that the major abiotic change in the MPB-attacked forest ecosystem in the red-attack stage is stand hydrology, with an overall increase in water availability after MPB attack (Knight et al. 1991, Schnorbus 2011). This can, in turn, modify other abiotic and biotic parts of the ecosystem, including the understory vegetation and below-ground dynamics. Research that examines the potential future impact of MPB on these communities in Alberta lodgepole pine forests is needed to understand the potential impacts of a shift from stand-replacing disturbances such as fire and clear-cut harvesting, to the addition of partial canopy disturbances resulting from MPB attack.

The overarching goal of my PhD dissertation is to provide novel insights into the linkages between above- and below-ground ecosystem properties and processes in lodgepole pine ecosystems and how they respond when the forest is disturbed. As climate changes and disturbance regimes change, there is a need for our society to better comprehend and anticipate the potential impacts of disturbance agents and altered disturbance regimes on forested ecosystems. This can only occur with an increased understanding of the connections between above- and below-ground ecosystem properties and processes. I studied both natural and anthropogenic disturbance agents and their effects on both above- and below-ground ecosystem properties and processes in native lodgepole pine forests in Alberta, Canada and introduced lodgepole pine plantations in northern Sweden.

Through the following five data chapters and concluding chapter, I address the linkages among above-and below-ground properties and processes in lodgepole pine forests, the effects of natural and anthropogenic disturbance agents on the vegetation and below-ground dynamics of these forests, and the impacts of the introduction of lodgepole pine to a non-native ecosystem on forest floor ecosystem properties and processes:

Chapter 2 - Fine-scale above- and below-ground heterogeneity results in diverse understory plant communities within mature lodgepole pine forests - examined patterns in the understory plant community of lodgepole pine forests and linkages of these communities with above- and below-ground environmental variables. Specifically, in this chapter I investigated how understory plant species separated into fine-scale plant community types within a lodgepole pine forest; what species were indicators of these fine-scale plant community types, and which above- and below-ground properties and processes influenced the development of these fine-scale plant community types.

Chapter 3 - Linkages between the forest floor microbial community and resource heterogeneity within mature lodgepole pine forests – examined patterns in the below-ground structural and functional microbial communities of lodgepole pine forests and whether there were linkages of these below-ground communities with above- and below-ground environmental variables. In this chapter I investigated how the microbial structure (using phospholipid fatty acids - PLFAs) and function (using respiration of carbon substrates) separated into fine-scale community types within a lodgepole pine forest; which PLFAs and carbon substrates were potential indicators of these fine-scale microbial community types, and which above- and below-ground properties and processes were associated with these community types.

Chapter 4 - Mountain pine beetle moves east: short-term resistance of above- and below-ground properties and processes to simulated mountain pine beetle attack in a novel landscape – investigated the potential for ecosystem change in newly invaded pine forests east of the Canadian Rockies attacked by MPB. I used a before-after treatment-control experimental design to examine the short-term effects of simulated MPB attack on the downed woody material, understory plant community, and below-ground properties and processes of lodgepole pine forest in the Upper Foothills of western Alberta.

Chapter 5 - Potential for lodgepole pine regeneration after mountain pine beetle attack in newly invaded Alberta stands – examined the future successional trajectory of MPB attacked and salvage logged stands in western Alberta by quantifying the presence of seedlings in the understory that may be released after MPB attack, the availability and suitability of micro-sites for pine germination, and the potential for recruitment of new pine seedlings in these micro-sites after MPB attack and salvage logging.

Chapter 6 - Tree species versus regional controls on ecosystem properties and processes: an example using introduced Pinus contorta in Swedish boreal forests - examined the ecological effects of introduction of lodgepole pine into Sweden. I compared native Canadian lodgepole pine, introduced lodgepole pine in Sweden, and native Scots pine to evaluate whether the introduction of lodgepole pine into Sweden altered forest floor properties and processes, or whether these properties were more strongly controlled by local factors in the area where the species was planted.

In the final concluding chapter I summarize the results and present management implications and potential avenues for further research.

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Chapter 2. Fine-scale above- and below-ground heterogeneity results in diverse understory plant communities within mature lodgepole pine forests

Abstract **-** Understory plant communities play critical ecological roles in forest ecosystems. The influence of above- and below-ground properties and processes on these communities has been explored at multiple spatial scales, but their relative importance in driving understory composition at micro-habitat scales, particularly in stands with homogeneous canopy composition, is less clear. An increased understanding of patterns and drivers of fine-scale variation in understory plant communities is critical for anticipating impacts of changing disturbance regimes. We examined plant communities in three mature lodgepole pine forests in Alberta, Canada, addressing the research questions: i) do species separate into fine-scale plant community types within a forest, ii) what species are indicators of plant community types at the fine scale, and iii) which above- and below-ground properties and processes influence development of these plant communities at the fine scale? In 12 0.48-ha plots we assessed abundance of understory plants, canopy tree size and cover, and downed wood biomass. Measured below-ground variables included forest floor thickness, litter cover, pH, decomposition rate, available soil nutrients, microbial phospholipid fatty acids and multiple carbon-source substrate-induced respiration. Cluster and indicator species analysis revealed four fine-scale understory plant community types. For three of the types the most significant indicators were feather moss species, and for the fourth community, the most significant indicator was a pioneer forb species. Constrained ordination showed one above-ground variable, mean tree diameter, contributed to the separation of the plant communities, while the remaining nine significant variables were below-ground variables including pH, litter cover, available boron, and microbial properties that included respiration of malic acid, and relative abundance of five phospholipids. Surprisingly, nitrogen was not significant in our analyses. This study provided novel insights into fine-scale variation in understory plant community composition within homogeneous monoculture forests. Heterogeneity in above-and below-ground variables resulted in different fine-scale community types, with below-ground factors being the primary drivers of these. Our study illustrates the important ecological roles that below-ground properties and processes play in structuring understory plant communities; findings may be applied to understanding the impacts of partial canopy disturbances on these forests under shifting disturbance regimes.

2.1. Introduction

The importance of understory vegetation as an integral component of the ecological functioning and biodiversity of forests has been demonstrated (Gilliam 2007). The understory plant community plays important roles in influencing both community and ecosystem properties above- and below-ground (Nilsson and Wardle 2005, Hart and Chen

2006). Persistent dense understories can be an important filter in determining the future successional trajectory of forests by reducing or delaying tree regeneration and growth (Lieffers et al. 1993; Royo and Carson 2006). The forest understory can also play an important role in providing browse and habitat for a variety of other biota (e.g., Carey and Johnson 1995, Work et al. 2004). However, the understory remains an under-appreciated component of forest ecosystems (Gilliam 2007).

The composition and biodiversity of these ecologically important understory plant communities is driven by both above- and below-ground properties and processes, with complex interactions among these factors (e.g., Reich et al. 2012). For example, the forest canopy affects the availability of light, nutrients, and moisture for use by the understory plant community, as well as soil pH and soil temperature (Prescott 2002, Hart and Chen 2006). The canopy also affects the understory plant community through its deposition of surficial litter, which can influence soil pH, and affects both the understory and the below-ground faunal and microbial communities (e.g., Hannam et al. 2006, reviewed by Barbier et al. 2008). Biogeochemical cycles and the turnover of organic matter in forested ecosystems is largely influenced by the composition and activity of soil microbial communities (Zelles 1999). Tree species, stand age, and soil type have all been shown to influence the soil microbial community structure and composition, thus also influencing nutrient cycling within forested ecosystems (e.g., Bauhus et al. 1998). Studies have found that forest understory composition is strongly associated with above-ground forest structural attributes such as basal area and stand height, rather than with soil nutrients (e.g., Légaré et al. 2002). However, understory communities are not always related to forest canopy types (e.g., Carleton and Maycock 1981). Thus, there is still considerable uncertainty about which above- and below-ground factors are important drivers of understory plant communities, their relative importance, and how these vary among ecosystem types and at different spatial scales, including at the micro-habitat scale

within forest stands. It is critical to explore these factors collectively to better understand drivers of the understory plant community.

Bartels and Chen (2010) reviewed the relative roles of resource (e.g., light, soil nutrients, and soil moisture) quantity and resource heterogeneity in structuring understory plant communities and suggested that at the stand level resource quantity may be the primary driver of species diversity, whereas resource heterogeneity may be the primary driver at broader scales; however, they did not consider the within-stand scale. The linkages with above- and below-ground ecosystem properties and processes at the within-stand level, especially for stands with a more homogenous canopy, remain poorly understood compared with these larger stand- and landscape-levels that have greater heterogeneity in canopy structure and composition. While the relative magnitude of spatial resource heterogeneity is likely to be lower at the within-stand level, compared to a larger landscape scale, we still expect fine-scale variability in nutrient availability, light transmission, and soil moisture (e.g., Frelich et al. 2003). In turn we would expect this fine-scale variability to translate to heterogeneity in understory plant community composition within a forest stand, because of species differences in response to environmental resource conditions (e.g., Rowe 1956). This partitioning of species among the heterogeneous resource conditions within a single stand may lead to finer-scale plant community types. However, studies examining patterns in understory plant community composition at the within-stand scale have been primarily at the canopy patch scale, comparing among coniferous and deciduous patch types (e.g., Chávez and Macdonald 2010), rather than looking at intra-stand variation within homogenous single canopy species forest types. We hypothesize that at the within-stand scale, the understory plant community composition of stands that lack canopy heterogeneity are likely to be more driven by below-ground factors than by above-ground factors.

An increased understanding of the drivers of understory plant community composition at the within-stand scale will provide us with much needed insights to sustainably manage our forests into the future, which is particularly relevant in the face of changing disturbance regimes. For example, lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.), which has historically experienced dramatic reorganization of the understory plant community after stand-replacing fire, is experiencing increased levels of partial- and complete- stand disturbances by mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), which kills trees without directly impacting the understory plant community in the short term after attack (Burton 2008). Lodgepole pine has a wide climatic and geographical range in North America and across a wide portion of its range grows as a monoculture forest that is highly valued for its timber, wildlife habitat, and recreational use. These forests provide a valuable study ecosystem for examining how understory plant communities might respond to on-going interactions between the attacked canopy and below-ground factors in the face of partial canopy disturbances that leave the understory undisturbed.

The overarching goal of this study was to examine the patterns of fine-scale variation in understory plant community types within a homogenous forest type and their relationships with resource and environmental heterogeneity within these forests. We addressed three key research questions regarding the relationships between understory plant community composition and overstory and below-ground properties and processes in lodgepole pine forests of Alberta: i) are there fine-scale plant community types within mature lodgepole pine forests, ii) if so, what species are indicators of these fine-scale plant communities, and iii) which above- and below-ground properties and processes influence the development of these fine-scale plant community types?

2.2. Methods

Study area

The study area was located in the Upper Foothills natural sub-region of Alberta (Natural Regions Committee 2006) in lodgepole pine forests near Robb, AB. This area is characterized by pure lodgepole pine forests with serotinous cones, along with mixed conifer stands of white spruce (*Picea glauca* (Moench) Voss) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). Stand ages in this region are generally younger than 100-120 years old reflecting the regional disturbance regime of relatively frequent stand-initiating wildfire (Beckingham et al. 1996). The climate is temperate continental with mean daily maximum air temperatures during the growing season ranging from a daily maximum of 16.2 ^{0}C in May, to 20.6 ^{o}C in August. Mean monthly precipitation during the growing season is as follows: 57.9 mm (May), 106.7 mm (June), 106.2 mm (July), and 82.2 mm (August), with a mean annual precipitation of 562.4 mm (30 year climate normal 1971-2000). The study stands were approximately 110-120 years old and were located on brunisolic gray luvisolic soils (Soil Classification Working Group 1998). The study area was classified as ecosite UF e1.1 – Pl/green alder/feather moss (Beckingham et al. 1996). The upper canopy included only lodgepole pine; there were a very few white and black (*Picea mariana* (Mill.)) spruce, trembling aspen (*Populus tremuloides* Michx.), and balsam fir (*Abies balsamea* (L.) Mill) in the lower canopy. Notably, advance regeneration was absent or present in very low numbers (i.e., < 10 seedlings or saplings – ha^{-1} – see Chapter 5). The understory was dominated by feather mosses, including *Pleurozium schreberi* (Brid.) Mitt., *Ptilium crista-castrensis* (Hedw.) De Not., and *Hylocomium splendens* (Hedw.) Schimp., and the hair cap moss *Polytrichum commune* Hedw.. Common forbs included *Cornus canadensis* L. and *Linnea borealis* L., common small shrubs included *Rosa acicularis* Lindl. and *Vaccinium myrtilloides* Michx.; *Alnus crispa*

(Aiton) Pursh was the dominant tall shrub, and the common graminoid was *Calamagrostis montanensis* (Michx) Beauv..

A total of three forest study units (i.e., blocks in a statistical sense) ranging in size from 4.8 – 8.8 ha were sampled during the growing season of 2008 (Table 2-1). The study units we selected were relatively flat topographically, were similar to one another, and covered by fairly homogenous mature lodgepole pine forest representative of the dominant forest cover type in the region. Within each study unit we established four 0.48 ha (60-m x 80-m) plots. Each plot was surrounded by a minimum of 20-m (~ one tree height) of similar composition pine forest in order to minimize edge effects. Plots were placed as close together as possible within the constraint of ensuring uniform overstory stand conditions within each plot. Within each plot we established nine systematically-located nested sample points that were used as the center-points for sampling the overstory, downed wood, understory, and below-ground (n=3 blocks * 4 plots * 9 sample points =108 sampling points). These sample points were located 20-30 m apart from one another to reduce spatial auto-correlation.

Data collection

Above-ground

The overstory plant community was sampled using 8-m fixed-radius (0.02 ha) circular subplots centered at each of the sample points. Standard forest mensuration data were collected for all trees (i.e., with dbh $\geq$ 5 cm and ht > 1.3 m) within each subplot (i.e., live/dead status, species, and dbh). These data were used to calculate basal area and stem density, separated into live/dead status.

To estimate canopy cover, hemispherical photographs were taken in the middle of the growing season (mid-July) at each of the sample points using a digital Nikon Coolpix 4500 with FC-E8 fisheye lens. Photographs were taken approximately 1.4 m above the forest floor, with the camera leveled on a tripod and the bottom of the camera

oriented towards North. We analyzed canopy photographs using SLIM (Spot Light Intercept Model v. 3.01), using batch processing to analyze photos with manual color threshold adjustments by plot to optimize differences between canopy and sky. The program calculated gap fraction, which measures the area of overhead view (in percent) which constitutes canopy gaps. We subtracted gap fraction from 100 to provide an estimate of canopy cover at each sample point.

The downed woody material (DWM) was measured at each sample point using the line intersect method (Brown 1974; Brown et al. 1982; Van Wagner 1968, 1982). Line transects ran from each sampling point out 8 m at a randomly selected angle to guard against a possible orientation bias. The diameter of each DWM piece at the point of intersection with the line transect was measured using calipers and categorized into diameter size classes as follows: 0-0.5 cm, 0.5-1.0 cm, 1-3 cm, 3-5 cm, 5-7 cm, and > 7 cm (as adopted by the Canadian Forest Service; McRae et al. 1979, Van Wagner 1982). Pieces in diameter size classes 0-0.5, 0.5-1, and 1-3 cm were counted along the first 2 m length of each transect, size classes 3-5 and 5-7 cm along the first 4 m length of each transect, and for all pieces $\geq$ 7 cm we recorded diameter, length, and decay class (i.e., 1-5, based on Table 8.1 in VRI 2007) along the full 8 m. Biomass of DWM (Mg ha^{-1}) for each of the size classes was calculated using the equation and coefficients for central Alberta foothills lodgepole pine stands (Delisle and Woodard 1988, Nalder et al. 1997). For the large pieces ($\geq$ 7 cm diameter) we also calculated the biomass of sound (i.e., decay classes 1 and 2) and rotten (i.e., decay classes 3-5) wood separately. We calculated the total biomass of DWM by summing up the biomass for all size classes. Percent cover of DWM was estimated during assessment of understory communities (see below).

We sampled the understory plant community (i.e., forest floor mosses, forest floor lichens, forbs, graminoids, and shrubs – see Appendix 2-I for detailed list) within 1-m x 1-m quadrats located at each of the sample points. Percent cover (0-100) of each

species/taxa was estimated to the nearest $1/10^{th}$ percent for species with < 1% cover and to the nearest 1% for species with > 1% cover. For species that could not be identified in the field, voucher specimens were collected for identification through comparison with University of Alberta herbarium samples. Species scientific names were confirmed using the USDA Plants database (http://plants.usda.gov/). Cover estimates were also recorded for litter, tree/snag boles, downed woody material (diameter $\geq$ 3 cm), exposed mineral soil, and rock. We calculated understory species richness and diversity (i.e., Shannon Index, Magurran 1988) per quadrat.

We measured tall shrubs and saplings (i.e., > 1.3 m ht and < 5 cm dbh, e.g., *Alnus crispa*) in 4-m radius circular subplots centered at each of the sampling points. To estimate basal area of tall shrubs and saplings within the plots, we measured the stem basal diameters for shrubs and saplings rooted within the subplot and for shrubs that weren't rooted in the subplot but had canopy overhanging the subplot and thus were expected to potentially influence the properties within the subplot.

Below-ground

The thickness of the forest floor (excluding the recent litter fall, or L layer, but including both Fibric and Humic layers – i.e., F/H, mm) was measured in each of the four corners of the nine understory vegetation quadrats within each plot.

We installed Plant Root Simulator (PRS) probe ion exchange membranes (Western Ag Innovations, Inc., Saskatoon, SK, Canada) to measure soil nutrient availability. The anion exchange PRS™-probes simultaneously adsorbed all nutrient anions, including NO_3^-, PO_4^{3-}, and SO_4^{2-}. Cation exchange PRS™-probes simultaneously adsorbed nutrient cations such as B^+, NH_4^+, K^+, Ca^{2+}, and Mg^{2+}. A chelating pre-treatment of the anion PRS™-probe also permitted the adsorption of micronutrient metals such as Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}. We installed four pairs (pair = 1 cation and 1 anion exchange membrane) of PRS probes vertically at the four corners of each of the nine understory

quadrats within each plot. The top of the ion exchange membrane was placed at the interface between the forest floor and mineral soil. Probes were installed for the duration of the growing season (mid-June to mid-September 2008). After probes were removed at the end of the growing season, they were cleaned with deionized water and shipped to Western Ag for analysis; the four probe pairs from individual quadrats were pooled prior to elution and analysis. The PRS probes were eluted with 0.5 M HCl prior to nutrient analysis. A segmented flow Autoanalyzer III (Bran and Lubbe, Inc., Buffalo, NY) was used to colorimetrically quantify NH_4^+, NO_3^-, and PO_4^{3-}, and ICP spectroscopy (PerkinElmer Optima 3000-DV, PerkinElmer Inc., Shelton, CT) was used to quantify Al^{3+}, B^+, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{2+}, K^+, Mn^{2+}, Mg^{2+}, $Pb^{2/4+}$, SO_4^{2-}, and Zn^{2+}. The PRS-probe nutrient supply rates were calculated (µg -10 cm^2 -burial $length^{-1}$). The methods used to quantify the eluate from the probes have minimum detection limits (MDL) for each element (Al=0.4, B=0.2, Ca=2, Cd=0.2, Cu=0.2, Fe=0.4, K = 4, Mn=0.2, Mg=4, NH_4^+=2, NO_3^-=2, Total N=2, P=0.2, Pb=0.2, S=2, Zn= 0.2 µg - 10cm^2 burial $length^{-1}$). The few samples for which the measured value was below the MDL were still included in analysis using the values measured for them, because censoring data below MDL can bias the dataset (Western Ag personal communication). We did not analyze several elements because their calculated nutrient supply rates were predominately below the minimum detection limits [Cd (n=62/108<MDL), Cu (n=79/108 < MDL), NO_3^- (n=58/108 < MDL), and Pb (n=35/108<MDL)].

Decomposition rate was measured at each of the sampling points within each plot. Nylon mesh bags (1.5 mm x 1.5 mm mesh size) with four 90-mm diameter Whatman cellulose filter papers were buried at the forest floor-mineral soil interface for the growing season (same time span as PRS probes). Filter papers were oven dried for 1 day (pre-burial) and 3 days (post-burial) at 70°C and weighed before and after being

buried. Decomposition rate was calculated as 100 minus the percentage of original filter paper biomass remaining at removal.

Forest floor samples were collected to measure pH, microbial multiple carbon source substrate-induced respiration (MSIR), and phospholipid fatty acid analysis (PLFA) of the below-ground microbial community, as described below. We collected forest floor samples (i.e., the entire thickness of the combined F and H layers) from each of the four corners of the nine understory quadrats per plot using aseptic techniques (sampling equipment was washed with 70% ethanol between quadrat samples) and combined them to form a single homogenous sample (~ 50 g) per quadrat. These were then divided into portions to be used for pH and MSIR, which were placed in ziplock bags, and portions for PLFA analysis, which were immediately placed in sterile Whirlpak™ bags. Samples were kept cool on ice until transferred back to the lab. Upon arrival at the lab, samples for MSIR and pH were sieved (4 mm) and kept refrigerated (4°C) in plastic bags prior to analysis. PLFA samples were stored at -86°C and then freeze-dried prior to PLFA extraction.

Forest floor pH was measured potentiometrically in a saturated paste in equilibrium with a soil suspension of a 1:4 soil:liquid mixture. We used 0.01 M $CaCl_2$ in place of water following the instructions for measuring pH of field-moist organic samples as described in Kalra and Maynard (1991).

Functional composition of microbial communities relates to their activity particularly in the carbon cycle. MicroResp™ multiple carbon source substrate-induced respiration (MSIR) offers a convenient, rapid and sensitive method for the determination of the community-level physiological profile for each forest floor sample using a 'whole soil' technique that uses the 96-well microtitre plate format that Biolog (Biolog Inc.) does (Campbell et al. 2003, Chapman et al. 2007). We prepared detection agar plates containing a gel-based bicarbonate buffer with indicator dye that responded to the pH

change within the gel resulting from carbon dioxide evolved from the soil (Cameron 2008). The plates were stored in a closed desiccation chamber in the dark when not being used for analysis.

To estimate mean forest floor moisture content within each study unit to use for MSIR analysis, we sub-sampled ~1 g of each field-moist sieved forest floor MSIR sample and then combined all 36 samples within each study unit into a single sample. Each of the three pooled study unit samples were weighed (fresh) and then dried for 48 hours at 65° C and reweighed (dry). Percent dry weight was calculated ((dry/fresh)*100), and soil moisture content was calculated as 100 – percent dry weight; this moisture content was then used for calculating substrate concentrations. Each respiration substrate was prepared as 30 mg of substrate per gram of water (Cameron 2008); a separate set of substrates was prepared for each of the three study units because of observed differences in soil moisture content among them. Fifteen substrates commonly used in carbon MSIR analysis and thought to be associated with plant root exudates (e.g., Garland and Mills 1991, Stevenson et al. 2004) were used: five amino acids (L-alanine, L-arginine, glutamine, L-lysine, γ aminobutyric acid), six carbohydrates (n-acetyl glucosamine, L-(+)-arabinose, D-(+)-galactose, glucose, mannose, trehalose), four carboxylic acids (citric acid, L-malic acid, oxalic acid, 3,4-dihydroxybenzoic acid), and water as a control to measure basal respiration. Substrates at prepared concentrations were stored at 4° C for the duration of the respiration analysis.

Field-moist sieved forest floor samples were incubated in a dark chamber at 25°C for ~24 hours prior to analysis. Forest floor samples were added to the 96-well microtiter deep well plates after 30 µl of each substrate was dispensed (three replicate substrate wells per sample, two forest floor samples per deep well plate). The deep well plate was then hermetically sealed with a gasket, face-to-face, with the detection plate, such that each well interacted with the opposite well of the detection plate. The two plates were

incubated in the dark at 25° C for six hours. The colour change in the detection plate was then read on a standard laboratory microplate reader (detection plate read before and after 6 hrs of incubation, absorbance = 570 nm) and respiration rates were calculated (μg CO_2-C g^{-1} hr^{-1}). A maximum of 16 samples could be analyzed in a day, so samples were randomly selected each day to reduce bias associated with differences in time since collection, and all analyses were completed within two weeks of when the samples were collected. One sample had five carbon substrate respiration rates below basal respiration and was excluded from analysis. Catabolic evenness was calculated using the Simpson-Yule index ($1/\sum p_i^2$, Magurran 1988), where p_i is the respiration response for an individual substrate as a proportion of total respiration rates from all substrates for a given forest floor sample (Degens et al. 2000).\

Microbial phospholipid fatty acid (PLFA) analysis produces a lipid profile of microbial communities. We transferred 0.30 g of each freeze dried forest floor sample to a muffled test tube and then analyzed each of them for PLFAs following the detailed methods described in Hannam et al. (2006). To summarize, we analyzed forest floor samples by extraction with a single-phase chloroform mixture, lipid fractionation on a solid-phase-extraction Si column and then subjected them to a mild methanolysis using a modified Bligh and Dyer extraction (Bligh and Dyer 1959, Frostegård et al. 1991, White and Ringelberg 1998). The resulting fatty acid methyl esters were then analyzed using an Agilent 6890 Series capillary gas chromatograph (GC; Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to identify individual fatty acids. Fatty acids were designated X:YωZ, where X represents the number of carbon atoms, Y represents the number of double bonds, and Z indicates the position of the first double bond from the aliphatic (ω) end of the molecule. The suffixes c and t indicate cis and trans geometric isomers. The prefixes ‘a’ and ‘i’ refer to anteiso

and iso branching and Me and OH specify methyl groups and hydroxyl groups, respectively. PLFAs that were present in 5% or less of the samples were excluded from analysis. PLFAs for 16:1ω9c and 16:1ω11c were combined and 18:2 ω6,9c and 18:0a were combined for analysis as they could not be distinguished by the GC. We excluded two samples with <85% peak matching from analysis. There were a total of 54 PLFAs included in the final analysis and these were also summed to provide a measure of total PLFA biomass (nmol g^{-1} forest floor). PLFAs that have been previously identified as associated with soil microorganisms were combined into PLFA biomarker groups for fungi, bacteria, actinomycetes, and arbuscular mycorrhizae. The fungal PLFAs 18:1ω9c, 20:1ω9c, and 18:3ω6c were used to estimate the contribution of fungi (Myers et al. 2001, Hamman et al. 2007), and 16:1ω5c was used to estimate arbuscular mycorrhizae (Frostegård and Bååth 1996, Olsson 1999). Bacterial PLFAs included 10:0 3OH, 12:0, 12:0 2OH, 12:0 3OH, 14:0, i14:0, 15:0, a15:0, i15:0, i16:0, i17:0, a17:0, 17:0, cy17:0, 18:1 ω5c, 18:1ω7c (Bååth et al. 1992, Frostegård and Bååth 1996, Olsson and Alstrom 2000, Myers et al. 2001, Hassett and Zak 2005). The ratio of fungal to bacterial PLFAs was used to estimate the relative contributions of fungi and bacteria. The 10-methyl branched fatty acids (10me16:0, 10me17:0 and 10me18:0) were used to quantify actinomycetes (Kroppenstedt 1985, Brennan 1988). Aside from the measurement of total biomass of PLFAs and the biomass for biomarker groups, all measured PLFAs were expressed on a mol% basis to standardize for differences in the total amount of forest floor PLFAs among samples.

We calculated the microbial metabolic quotient for each forest floor sample as the ratio of soil basal respiration (i.e. SIR with water as the substrate) to microbial biomass (i.e., the total PLFA biomass) (qCO_2 - Anderson and Domsch 1978b).

Statistical analyses

We found that 16 understory species were very uncommon/infrequent, occurring in < 5% of quadrats, compared with 24 species/taxa that occurred in 5% or more of the quadrats (see Appendix 2-I for detailed species/taxa list). The uncommon/infrequent species were excluded from analysis because their sample sizes were too small to analyze patterns in their relative abundance among the quadrats.

We used hierarchical, agglomerative clustering to analyze the 24 dominant plant species/taxa and whether they grouped into discrete plant community types. Cluster analysis is a powerful tool to interpret vegetation patterns (Urban et al. 2002), and we used a flexible beta linkage method with β= -0.25 and Sørenson's distance measure. Because we did not have *a priori* types, Indicator Species Analysis (ISA; Dufrêne and Legendre 1997) was used to prune the dendrogram of the cluster analysis by using different numbers of plant community types as cut-offs (n=1-10) and then selecting the type number cut-off with the lowest average P-value for indicator species (McCune and Grace 2002). Once the final clusters had been identified, ISA was used to identify which species were contributing to the separation of the cluster plant community types, using Monte Carlo permutations (n=5000). Cluster analysis and ISA were conducted using PC-ORD (Version 5 MjM Software Design, Gleneden Beach, OR).

We used nonmetric multidimensional scaling (NMS) unconstrained ordination to examine the multivariate patterns in the understory plant community (McCune and Grace 2002). Ordination used PC-ORD (Version 5 MjM Software Design, Gleneden Beach, OR), with Sørenson as the distance measure, and we completed 100 runs with real data and 100 Monte Carlo randomized runs, starting with a six-dimensional solution and stepping down to a one-dimensional solution. We determined the number of dimensions of our final solution by evaluating the scree plot and the reduction in stress with step-down in dimensionality of the preliminary runs (McCune and Grace 2002). Stability of

the solution (stability criterion = 0.00005) was assessed by plotting stress versus iteration. After checking the optimal number of dimensions and best solution from the preliminary runs, we ran a final NMS with the number of dimensions determined from the preliminary runs, using the starting configuration that worked best in our preliminary runs and omitting the Monte Carlo test. We then calculated the Pearson correlation coefficients and R^2 for several overstory vegetation variables; multiple factors that describe the plant community including understory cover by growth form and total understory cover combined, understory species richness and Shannon diversity; downed wood material biomass by size class; and below-ground variables [forest floor thickness, litter cover, pH, decomposition, available soil nutrients, and microbial phospholipid fatty acids (PLFAs) and multiple carbon-source substrate-induced respiration, catabolic evenness, and metabolic quotient] in the secondary environmental axis with the NMS ordination axes and overlaid variables and species, using a cut-off of $R^2>0.25$ on the ordination plots. The sample points plotted in ordination space were coded by the community types created in the cluster analysis.

Distance-based redundancy analysis (db-RDA) constrained ordination was used to evaluate the relationships between the understory species and community composition that we measured within each quadrat and the above-and below-ground variables we measured. Distance-based RDA used sample scores from a Principal Coordinates Analysis (PCoA), which extracted the principal coordinates of the distance matrix in a redundancy analysis (RDA) that then used these sample scores as the species data (Legendre and Anderson 1999). The PCoA was done using our untransformed species-cover data using PrCoord (ter Braak and Šmilauer 2009). We used the Bray-Curtis distance measure and excluded negative eigenvalues in our PCoA. Prior to db-RDA analysis, all variables in the environmental matrix were standardized to unit variance (i.e., each variable was centered and standardized by bringing their means to zero and their

variances to one – Lepš and Šmilauer 2003). Environmental variables were tested for inclusion in the db-RDA with forward step-wise selection and testing for significance by 499 Monte Carlo permutations, blocked by block (α=0.05). Once we identified the significant variables from our first db-RDA, we ran a final db-RDA including all the significant variables, and we tested the significance of the first and all canonical axes. For the db-RDA Analyses we used Canoco for Windows Version 4.56 (ter Braak and Šmilauer 2009), with scaling focused on samples, species scores not post-transformed, no transformation of species data, and centering by species. We used the original matrix of species cover by sample plots as a supplemental dataset so that species could be projected in ordination space when plotting the ordinations in CanoDraw. We only displayed species and environmental variables which had Pearson correlation coefficients >0.3 with either of the first two significant ordination axes in our ordination plots. The sample points plotted in ordination space were coded by the community types identified in the cluster analysis.

For the resource and environmental variables that were significant in the db-RDA we compared the mean values among the four plant community types identified by the cluster analysis using one-way ANOVAs (Proc Mixed, SAS Institute Inc., Version 9.2 (32-bit), Cary, NC, USA: SAS Institute Inc., 2008). We first determined whether each variable met the assumptions for analysis of variance (ANOVA) using analysis of residuals and normal probability plots, and transformed response variables when necessary to meet the assumptions. When significant differences were detected, we used post-hoc linear contrasts to compare variables among the plant community types using Bonferroni-adjusted P-values (family-wise α=0.05) (Proc Mixed, SAS v9.2).

Four of the 108 subplots were excluded from analysis because of low PLFA peak matching, low respiration rates, or based on outlier analysis of environmental variables used in the ordination, so results below are only for 104 subplots.

2.3. Results

Within these mature, even-aged lodgepole pine stands there were four distinct plant community types identified by cluster analysis of data from the 104 subplots (Fig. 2-1).

The indicator species analysis revealed multiple significant indicator species for three of the plant community types; community type 3 had a single feather moss species as an indicator (Table 2-2). For community type 1 the indicators included two feather moss species and two forb species (Table 2-2). Indicator species for understory community type 2 included a feather moss species, a hair-cap moss species, one shrub species and one grass species (Table 2-2). A club-moss, two forb, and two shrub species were significant indicators for community type 4.

The unconstrained ordination illustrated the separation among the four plant community types and which species were responsible for that separation. The NMS 3-dimensional solution (final stress = 16.8 after 26 iterations) explained 77.2% of the variation in the dataset (Fig. 2-2). The NMS ordination showed correlations (with $R^2 > 0.25$) of one forb, *Chamerion angustifolium*, and four bryophyte species, *Hylocomium splendens*, *Pleurozium schreberi*, *Ptilium crista-castrensis*, and *Polytrichum commune*, with the three ordination axes. The locations of these species on the ordination plots overlain with the plant community type subplots were consistent with our findings of four community types from the indicator species analysis. Plant community type 1 subplots were located towards the upper end of axes 1 and 3 and lower end of axis 2 and were associated with higher percent cover of *Hylocomium splendens*. Plant community type 2 subplots were located towards the upper end of all three axes, and were associated with higher cover of the mosses *P. commune* and *P. crista-castrensis*. Plant community type 3 subplots were located towards the lower end of axes 1 and 2 and towards the upper end of axis 3 and were most associated with high percent cover of the feather moss *P. schreberi*.

Plant community type 4 subplots were located across axis 1, towards the upper end of axis 2, and the lower end of axis 3, and were most associated with higher percent cover of *C. angustifolium*.

The unconstrained ordination also showed the relationships between understory plant community composition and a subset of the environmental variables. Two above-ground (live stem density, mean dbh), and seven below-ground (pH, PLFAs: a15:0, 16:1 ω 5c, cy17:0, i17:0, 18:1 ω7c,) variables showed correlations (with $R^2 > 0.25$) with one or more of the three ordination axes. Also, two factors that describe the plant community: species richness and bryophyte cover were correlated ($R^2 > 0.25$) with the ordination axes (Fig. 2-2). Based on their relative locations in the plot, plant community type 1 subplots were not highly correlated with any of the variables we measured. Plant community type 2 subplots were positively associated with four PLFAs (a15:0, 16:1ω5c, i17:0, 18:1ω7c) and density of live trees. Plant community type 3 subplots were only positively associated with bryophyte cover. Plant community type 4 subplots were positively associated with two PLFAs (cy17:0, 18:1ω7c), higher species richness, pH, and larger mean dbh of live trees. Correlations for other species and environmental variables (e.g., MSIR rates, N, forest floor thickness, decomposition rate, canopy cover) with the ordination axes were low (i.e., $R^2 < 0.25$) and thus were not considered in further detail.

The constrained ordination also illustrated separation among the four plant community types, which species contributed to that separation, and showed the environmental variables that were driving the separation of the plant community types. The first four db-RDA axes, which were all significant, accounted for 84.4% of the species-environment relations (Table 2-3). The four understory plant community types separated along the first two db-RDA axes: type 1 was loaded towards the lower end of axes 1 and 2, type 2 overlapped with the other types and was centered towards the lower two thirds of axis 1 and middle of axis 2, type 3 was loaded towards the upper end of axis

1 and distributed across axis 2, and type 4 was loaded towards the lower end of axis 1 and also distributed across the middle of axis 2 (Fig. 2-3a). The relative locations of subplots of the different plant community types was consistent with the indicator species analysis: type 1 subplots were loaded in the ordination where *Hylocomium splendens* was found (but also *Vaccinium caespitosum* and *myrtilloides*), type 2 were loaded where *Ptilium crista-castrensis* was located, type 3 loaded where *Pleurozium schreberi* was located (but also *Vaccinium vitis-idaea*), and type 4 were located where *Aralia nudicaulis, Lycopodium annotinum, Chamerion angustifolium*, and *Rubus pubescens* were found (but also *Viburnum edule*). The species' locations in ordination space (on the db-RDA bi-plot) reflect their correlation relative to the environmental gradients (Fig. 2-3b).

One overstory and nine below-ground variables were significantly correlated with the understory plant community composition along the four axes and collectively explained 24.1% of the variation in the species data (Table 2-3, Fig. 2-3b). Forest floor pH and the PLFAs 18:1ω7c, 16:1 2OH, a15:0, and 14:0 were most strongly correlated with Axis 1, mean dbh of overstory trees and available boron were the variables most highly correlated with Axis 2, litter cover and the PLFA cy17:0 were most correlated with Axis 3, and respiration of malic acid was most correlated with Axis 4 (Table 2-3).

In general, the patterns in mean values for the environmental variables among the four plant community types were consistent with the relationships found in the db-RDA ordination (Table 2-4). Community type 4 had the highest pH, which was significantly higher than for community types 2 and 3, with intermediate levels in community type 1 that were also significantly higher than for community type 3. Mean dbh of trees in plots of plant community type 4 was significantly larger than for plant community types 1 and 3, with intermediate levels for community type 2. The bacterial PLFA 18:1ω7c was significantly lower in community type 3 than the other three community types. For bacterial PLFA a15:0, community type 3 had lower levels than community type 2, with

intermediate levels in community types 1 and 4. Bacterial PLFA cy17:0 was significantly lower in community type 3 than in community type 1, without significant differences between these community types and community types 2 and 4. PLFA 16:1 2OH was significantly lower in community type 3 compared with community types 2 and 4, with intermediate levels in community type 1. There were no significant differences in the bacterial PLFA 14:0, boron, litter cover, or malic acid respiration among any of the four community types (Table 2-4).

2.4. Discussion

This study provided novel insights into within-stand patterns of variation in understory community composition in mature monoculture lodgepole pine forests in the Upper Foothills of Alberta. Differences in overstory species composition may be important in driving, or being associated with variation in understory plant community structure; this has been well documented (e.g., Crozier and Boerner 1984, Hart and Chen 2006, Chávez and Macdonald 2010). Yet, even in these monoculture lodgepole pine forests, we still identified four fine-scale plant community types. Our findings show that even with a homogeneous overstory canopy species, there are still differences in the spatial partitioning of resources (e.g., microbes, nutrients) and the environmental conditions (e.g., pH) below the canopy that create micro-habitat variation influencing the patterns of variation in understory plant communities. Our study demonstrated the existence of four plant community types at this fine scale and illustrated strong relationships among these plant community types, their associated indicator species and multiple above- and below-ground resource and environmental variables. As we hypothesized, the majority of resource and environmental variables contributing to the separation of the plant communities were below-ground, compared with only one significant above-ground variable.

For these pure lodgepole pine stands we found that the only important above-ground variable contributing to heterogeneity in understory plant community composition within the stands was tree size. This variable also differed significantly among the community types, suggesting that the size of trees is the most important overstory determinant of the plant communities, rather than other overstory variables including transmission of light to the forest floor as measured by canopy cover. Our findings are in contrast to studies in near boreal pine forests in eastern North America where fine-scale plant communities have been shown to differ along light gradients (Frelich et al. 2003). Our study stands generally had moderate cover values (see Table 2-1), and transmission of light to the forest floor was high enough to allow for presence of shade-intolerant pioneer species, such as *Chamerion angustifolium,* in addition to shade-tolerant species such as *Aralia nudicaulis*, so the lack of influence of canopy cover was unlikely to be a function of low variability of light transmission levels in our study. It is unclear why the size of trees is influencing the plant communities moreso than other overstory attributes such as canopy cover.

The important below-ground variables contributing to heterogeneity in understory plant community composition within the stands included pH, boron, litter cover, microbial respiration of malic acid, and abundance of several microbial PLFAs. Of these below-ground factors, there were significant differences in pH, and PLFAs 18:1ω7c, a15:0, cy17:0, and 16:1 2OH among some of the plant community types. Malic acid is a root-derived organic carboxylic acid that can complex with Aluminum (Hue et al. 1986). Boron is an essential higher plant micronutrient, playing an important role in the formation and structure of plant cell wall complexes (Matoh 1997); rapid inhibition of plant growth occurs in response to boron deficiency (Hu and Brown 1997). The microbial community is impacted by the soil pH, and it is generally accepted that fungi are favored over bacteria at low pH (Alexander 1977). This pattern has been shown across large pH

ranges (e.g., Högberg et al. 2003), but also with narrow ranges of soil pH (e.g., Pennanen et al. 1999) such as we observed. The different PLFAs we observed at higher pH, including the bacterial PLFAs 18:1ω7c, a15:0 and cy17:0, and the PLFA 16:1 2OH, compared with the bacterial PLFA 14:0 that was associated with lower pH, are consistent with the heterogeneity in soil pH in these stands also contributing to heterogeneity in the soil microbial community composition. This heterogeneity in pH and the microbial community composition, in turn, will influence biogeochemical cycles in these stands, including the soil concentrations of plant root exudates (including malic acid) and boron. Soil adsorbs boron at high pH levels and this has been found to reduce its availability for plant uptake (Goldberg 1997), but within the narrow acidic range of pH in this study we saw an increase in available soil boron associated with increasing pH and this was likely because these stands were very acidic and boron is still highly mobile across this range of low pH levels. Litter cover was also associated with patterns in the understory plant community. Márialigeti et al. (2009) found that litter cover played a significant role in determining bryophyte assemblages and that increased litter levels were associated with reduced bryophyte cover. However, the association of lower litter cover with a particular community type (e.g., type 3) doesn't necessarily imply inhibition by litter. This result could simply be due to reduced litter production by that community type, as compared to other types which were multi-layered understories including forbs, shrubs, and/or grasses. Overall, our findings show that the heterogeneity of below-ground environmental/resource variables contributes to the existence of fine-scale plant communities within these homogenous forests.

Notably, nitrogen availability did not influence below-ground variable. Other studies have suggested that N is associated with the structuring of plant communities (e.g., Gundale et al. 2006), including in other pine systems (e.g., Frelich et al. 2003). There was substantial variation in N levels (ranged from 0-25.6 μg 10 cm^{-2} summer burial

$^{-1}$), so the lack of relationship between N and the plant community type was not a function of a low range of variability in our study. The dominant tall shrub in our stands, *Alnus crispa*, is an important N fixer that understory plants may rely on for N, especially in N-limited systems (Rhoades et al. 2001). Given the patchy distribution of alder among the 104 subplots it was surprising that we did not find a relationship between available soil N and variation in understory plant communities in this N-limited system. However, the forest floor mosses *H. splendens* and *P. schreberi* are also N-fixers and can comprise a significant portion of the N-fixation within boreal stands (Deluca et al. 2002). Therefore, the contributions of N from these forest floor mosses may have balanced out with the N contributions of *A. crispa*. Alternatively, it could be that other nutrients, such as boron, are more important in influencing the development of these fine-scale plant communities. While Mackenzie and Quideau (2010) found linkages between nitrogen availability and vegetation and soil microbial communities in reclaimed soils in northern Alberta, their study also showed that available boron levels may be associated with seasonal changes in the soil microbial communities.

Both the direct and indirect gradient analyses supported the classification of four fine-scale understory community types in these lodgepole pine forests; several of the indicator species for the types came out as highly correlated with the three NMS ordination axes and also with the dbRDA ordination axes. However, there was also overlap among the four understory plant communities, as seen in both the NMS and dbRDA ordination plots. We expected this overlap because none of the indicator species were unique to a particular community type, rather the differences among the plant community types were largely a reflection of differences in relative abundance (cover) of species. This shows that these species have fairly broad tolerances to both the resource and environmental properties and processes within these stands. However, there were still differences in tolerance among species such that the fine-scale variation in both the

environmental and resource properties and processes in these forests resulted in changes in relative abundance that could be detected as distinct plant community types with significant indicator species. Our findings are consistent with how species tolerances to availability of resources have been shown to influence boreal understory plant community composition (Hart and Chen 2008).

Plant community type 1 was characterized by two forest floor mosses combined with two low-growing, evergreen, woody species in microsites with a higher density of smaller trees. *Hylocomium splendens* has been used as an indicator of moisture availability within forests (Caners 2010), and has been identified as a very moist forest species (Rowe 1956). *Linnea borealis* has been classified as a fire avoider, and is generally considered a forest generalist that can tolerate a wide range of light, moisture and nutrient conditions (Howard 1993). *Cornus canadensis* habitat is typically cool and moist, but like *L. borealis* it also tolerates a wide range of moisture and nutrient conditions, and because it is rarely restricted to particular moisture conditions it is not commonly identified as an indicator species (Gucker 2012). *Dicranum polysetum* requires intermediate light and moisture levels (Caners 2010). In our study *D. polysetum* percent cover was overall quite low (mean <2%) and so it appears to be a less biologically meaningful indicator compared with the other three indicator species, which were much more abundant within the first community type. Community type 1 did not have any strong relationship with the environmental/resource variables based on the overlay of variables in the NMS or dbRDA ordination plots, suggesting it is more closely associated with other factors that we did not measure in this study.

Plant community type 2 was characterized by two forest floor mosses along with a broad-leaved evergreen shrub and a grass. *Ptilium crista-castrensis* is most frequently found in humid/mesic conifer woods in Alberta (R. Belland personal communication). Comparing its light and moisture requirements to *Pleurozium schreberi* and *Hylocomium*

splendens, Caners (2010) classified *P. crista-castrensis* as requiring slightly higher moisture and tolerating slightly lower light; this may be contributing to its separation from the other moss species. *Rhododendron groenlandicum* is a species that is associated with moist acidic soils, requiring constant moisture for germination (Karlin and Bliss 1983). We observed large pockets of *P. commune* in very wet depressions in these forests, which also corresponds to this community being associated with high soil moisture conditions. However, the grass species indicator *Calamagrostis montanensis* has been associated with relatively dry sites, which would be inconsistent with this community type being associated with moist micro-habitats, although *C. montanensis* can be found growing near *C. canadensis*, which is associated with moist micro-habitats (Marr et al. 2011). In the NMS ordination we saw a positive association between plant community type 2 and the presence of four PLFAs (a15:0, 16:1ω5c, i17:0, 18:1ω7c) and density of live trees, but in the dbRDA ordination plot we saw instead a relationship with only below-ground variables, in particular there were differences in the relative abundance of the bacterial PLFA a15:0 and PLFA 16:1 2OH that appear to be drivers of this plant community. Although we did not directly measure soil moisture, the autoecological properties of the indicator species, in particular the two moss species and *R. groenlandicum*, support this community type likely being associated with moister micro-habitats.

The third plant community type was dominated by one common feather moss, and was associated with several below-ground variables. This plant community was positively associated with the PLFA 14:0, and was negatively associated with forest floor pH, boron, and PLFAs a15:0, cy17:0, 16:1 2OH and 18:1ω7c. This community type was also associated with lower species richness compared with the other community types. Consistent with our findings, Anderson et al. (1995) examined bryophyte species along environmental gradients and found that *P. schreberi* was associated with more acidic

conditions, as compared to *H. splendens* and *P. crista-castrensis*. Reduced boron availability was associated with community type 3 (although not significantly different), thus the plant species in this community may be adapted to tolerating reduced boron levels. Our findings suggest that these more acidic soils and associated relationships of pH with the microbial community along with the availability of boron may be contributing to lower species richness in this plant community type.

The fourth understory community type was a rich plant community with a variety of herbs and short shrubs associated with larger trees and several below-ground variables. This plant community was most positively asssociated with pH and two microbial PLFAs (18:1ω7c and 16:1 2OH). *Chamerion angustifolium* was by far the highest indicator for this type; as a pioneer species that has been associated with gaps (Chávez and Macdonald 2010) this could suggest this is an earlier-successional type that may be associated with local small-scale disturbances within the pine forests where this light-demanding species can occur (Haeussler et al. 1990, Humbert et al. 2007). If these micro-habitats were more recently disturbed then this community may result from the lack of moss species having had time to colonize the area, thus accounting for the dominance by forb and shrub species, as *C. angustifolium* is considered a poor competitor that does not invade previously occupied understory cover, although it can persist across a wide range of pH, moisture, and nutrient levels (Haeussler et al. 1990). *Lycopodium annotinum* is a circumboreal species associated with coniferous forests but appears to have a wide amplitude for light, nutrients and moisture (Matthews 1993), so its importance as an indicator of this plant community type is unclear. *Aralia nudicaulis* is a shade-tolerant forb that also has wide ecological amplitude (Pavek 1993), although it has also been shown to be sensitive to disturbance and associated with primary forests (Whitney and Foster 1988). Wild roses, including *Rosa acicularis*, tolerate a wide range of soil conditions, with the exception of moist poor draining and extremely acidic soils and

while they can tolerate shade they grow better under higher light conditions (Haeussler et al. 1990). *Rubus pubescens* is present in mature forests, and widespread but rarely abundant in the understory (Whitney 1986). Overall, it appears that the indicator species for this community type, aside from *C. angustifolium* are species with wide ecological tolerances but the environmental conditions associated with this plant community type, including the least acidic soils and large diameter trees are contributing to the composition of this plant community type and its higher species richness compared with the other plant community types.

In our study we found moss species were the most significant indicators for three of the four plant community types. Our findings are in contrast to a study by Frego and Carleton (1995), who found no evidence of habitat partitioning among moss species, including three of the moss indicators in our study, *D. polysetum, P. schreberi* and *P. crista-castrensis,* across gradients of temperature, vapour pressure deficit, precipitation, litterfall, or photosynthetically-active radiation. Our results show that the above- and below-ground variables we measured in this study may better delineate these community types, compared with the variables previously measured.

Overall, this study provided new insights into the relationships of understory plant communities and micro-habitat resources and environmental conditions at the within-stand scale for a single species forest. Our findings demonstrate the importance of spatial resource/environmental heterogeneity in structuring understory plant communities at the within-stand level, even for monoculture forest types. This study illustrates the significant role that below-ground environmental and resource variables, in particular the soil microbial community, play in the structuring of plant communities at the within-stand scale for these homogeneous forests. In the face of shifting disturbance regimes, such as the shift from dramatic forest floor changes after fire to overstory mortality that leaves the understory community undisturbed in forests attacked by insects such as

mountain pine beetle, our findings have important implications for understanding the effects these partial canopy disturbance events will have in these forests. The type and intensity of disturbance and its relative impacts on the canopy, forest floor, and associated below-ground properties and processes will play an important role in determining the structure and composition of future understory plant communities of these forests.

Table 2-1. Summary of site characteristics of lodgepole pine (*Pinus contorta*) study units in Upper Foothills of Alberta. Given are the locations and mean values for each of the three study units; the minimum and maximum values across subplots within each study unit are in parentheses.

Study unit	Latitude/Longitude	Basal area (m^2 ha^{-1})	Density (trees ha^{-1})	Dbh (cm)	Canopy cover (%)
1	53.2248/116.8094	39.6 (26.7-56.2)	1420 (950-1900)	18.3 (5-34.7)	63.9 (56.2-86.9)
2	53.24129/116.8288	37.3 (21.6-55.1)	978 (550-1350)	21.5 (6.6-43.3)	59.2 (51.4-70.7)
3	53.22647/116.8212	40.3 (27.1-54.0)	1182 (450-1850)	20.1 (8.0-38.3)	62.1 (54.9-77.4)

Table 2-2. Results of indicator species analysis. Species that had an indicator value >20 and were significant at $\alpha = 0.05$ are listed in order by descending indicator value within each plant community type. N is the sample size for each community type. Mean cover values (± SE) for each of the indicator species for all four plant community types are also provided, with the cover values for the plant community a species was an indicator for highlighted in bold.

Community			Indicator		Cover (± SE)			
Type	N[i]	Species[ii]	value	P	Type 1	Type 2	Type 3	Type 4
1	18	HYSP	54.5	0.0002	**21.7 (5.1)**	4.3 (1.0)	2.7 (0.6)	6.7 (2.3)
		LIBO	40.2	0.0012	**15.9 (2.8)**	10.0 (1.9)	9.1 (1.6)	4.5 (0.9)
		COCA	34.7	0.02	**17.0 (3.7)**	13.4 (1.6)	9.6 (1.5)	9.0 (0.9)
		DIPO	26.8	0.01	**2.0 (0.7)**	0.4 (0.2)	0.8 (0.3)	0.6 (0.4)
2	25	PTCR	61.0	0.0002	3.6 (1.1)	**25.6 (4.1)**	1.0 (0.3)	8.4 (1.4)
		POCO	40.2	0.003	0.8 (0.4)	**10.4 (3.5)**	1.9 (1.0)	4.5 (1.6)
		CAMO	38.7	0.001	3.1 (1.6)	**15.8 (4.0)**	3.5 (1.4)	3.8 (1.2)
		RHGR	22.3	0.01	0.3 (0.3)	**11.7 (5.8)**	1.2 (1.0)	1.5 (1.0)
3	31	PLSC	63.8	0.0002	8.6 (2.4)	14.9 (3.4)	**54.1 (3.9)**	7.1 (2.0)
4	30	CHAN	80.2	0.0002	0.6 (0.3)	2.2 (0.8)	2.1 (0.9)	**19.7 (2.4)**
		LYAN	42.6	0.0002	0.2 (0.2)	2.7 (1.0)	0.1 (0.1)	**12.1 (3.0)**
		ARNU	39.0	0.0002	1.1 (0.9)	3.9 (1.8)	1.5 (0.8)	**10.3 (2.2)**
		ROAC	34.7	0.005	4.1 (1.3)	4.2 (1.0)	3.1 (0.8)	**6.7 (0.9)**
		RUPU	33.3	0.0008	0.5 (0.4)	0.6 (0.4)	0.3 (0.2)	**2.8 (1.0)**

[i] This is the number of subplots that were of that plant community type.

[ii] For species code descriptions see Appendix 2- I.

Table 2-3. Results of distance-based redundancy analysis. The trace value (sum of all the canonical eigenvalues) and the eigenvalues of the first four axes are presented, along with the species-environment correlations, and the cumulative percentage of the variance explained for species and species-environment. Inter-set correlations (Pearson) of significant above- and below-ground variables from the db-RDA step-wise forward selection (see Table 2-4 for description of variables) are ordered by their correlations (from high to low) with the first axis. The inter-set correlation values for the axis where the correlation was strongest are highlighted in bold.

	Axis 1	Axis 2	Axis 3	Axis 4
Trace: 0.262				
Eigenvalues[i]	0.133	0.037	0.032	0.019
Species-environment correlations	0.842	0.701	0.714	0.632
Cumulative percentage variance:				
Species data	14.5	18.6	22.1	24.1
Species-environment relation	50.9	65.2	77.3	84.4
Inter-set correlations†				
18:1ω7c (PLFA[ii])	**-0.626**	0.058	0.004	-0.146
pH	**-0.560**	-0.077	-0.263	0.0091
16:1 2OH (PLFA[ii])	**-0.447**	0.016	-0.054	-0.054
a15:0 (PLFA[ii])	**-0.376**	0.030	0.335	0.242
Dbh	-0.351	**0.550**	-0.143	-0.078
B	-0.284	**0.313**	-0.069	0.183
cy17:0 (PLFA[ii])	-0.275	-0.003	**-0.357**	0.013
14:0 (PLFA[ii])	**0.262**	0.136	-0.0004	0.061
Litter	-0.259	-0.153	**-0.438**	0.372
Malic acid	0.115	0.211	-0.164	**0.287**

[i] Axis 1 and all axes combined were significant at P=0.02

[ii]PLFA is phospholipid fatty acid

Table 2-4. The mean values (± SE) for each of the four plant community types of the above- and below-ground variables that were significant in the distance-based redundancy analysis (db-RDA) ordination (see Table 2-3). Different lower case letters (a, b, c) after mean values indicate significant differences for individual variables among plant community types.

			Plant Community Type			
Variable code	Description[i]	Units	1	2	3	4
pH	Forest floor pH	n/a	3.69 (0.04)ab	3.52 (0.04)bc	3.38 (0.03)c	3.80 (0.06)a
18:1ω7c	Phospholipid fatty acid	mol%	9.41 (0.27)a	9.37 (0.40)a	7.88 (0.27)b	10.64 (0.26)a
16:1 2OH	Phospholipid fatty acid	mol%	0.20 (0.05)ab	0.23 (0.04)a	0.10 (0.02)b	0.31 (0.03)a
a15:0	Phospholipid fatty acid	mol%	2.26 (0.08)ab	2.46 (0.07)a	2.20 (0.06)b	2.45 (0.06)ab
Dbh	Mean stem diameter	cm	18.8 (0.3)b	19.8 (0.5)ab	19.3 (0.3)b	22.1 (0.06)a
B	Plant available boron	µg -10 cm^2 –summer $burial^{-1}$	1.01 (0.11)	1.00 (0.09)	0.75 (0.07)	0.96 (0.09)
cy17:0	Phospholipid fatty acid	mol%	1.99 (0.06)a	1.86 (0.07)ab	1.81 (0.05)b	2.07 (0.05)ab
14:0	Phospholipid fatty acid	mol%	1.55 (0.10)	1.51 (0.05)	1.63 (0.05)	1.50 (0.04)
Litter	Cover of litter	%	55.6 (4.8)	50.8 (4.4)	42.3 (2.1)	53.0 (4.4)
Malic acid	Respiration rate	µg CO_2-C g^{-1} hr^{-1}	28.8 (2.1)	28.1 (1.2)	30.9 (1.2)	29.7 (1.1)

[i] Further details on the measurement of these variables are described in the methods.

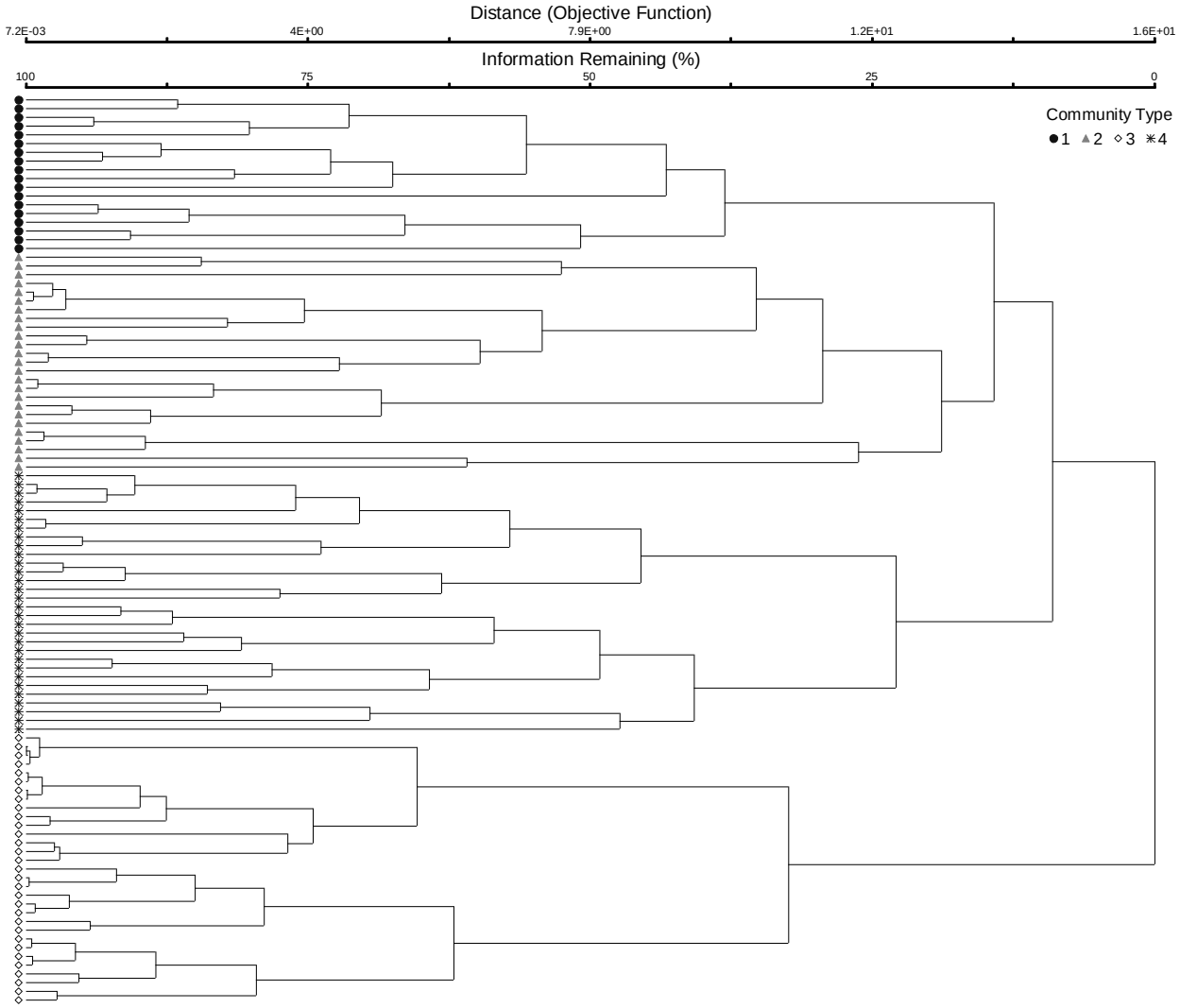

Fig. 2-1. Dendrogram of hierarchical agglomerative cluster analysis of data of species' percent covers from understory quadrats. The dendrogram was pruned to four plant community types, as shown, based on Indicator Species Analysis. Cluster analysis used a flexible beta linkage method with β= -0.25 and Sørenson's distance measure. Chaining = 1.26%.

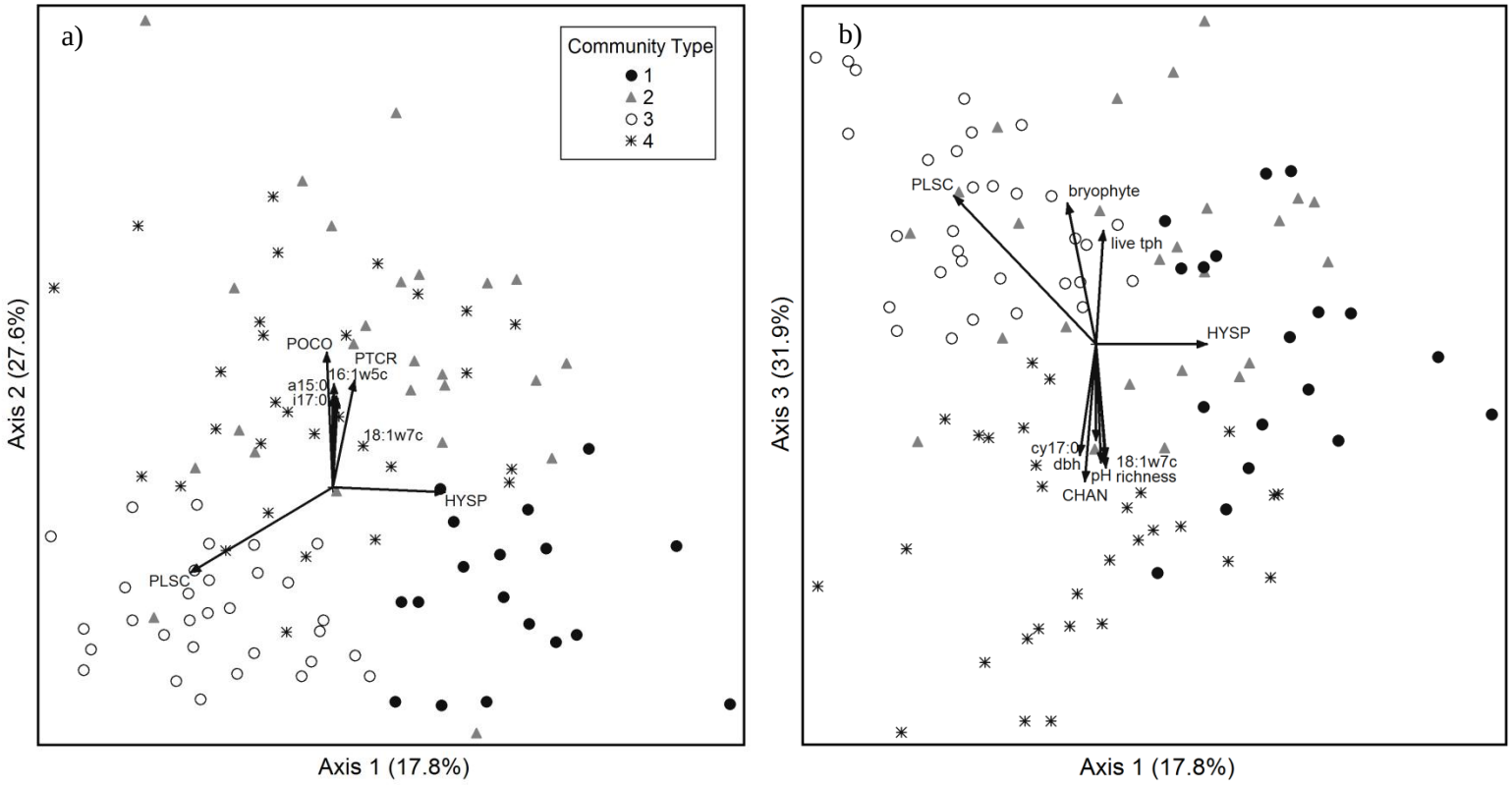

Fig. 2-2. Results of nonmetric multidimensional scaling ordination of understory plant community composition. Each symbol is a subplot, which is coded by plant community type (see Fig. 2-1). The final ordination was a 3-D solution, so two plots are presented: a) the first and second ordination axes, and b) the second and third ordination axes. The amount of variation explained by each axis is included in parentheses. The angles and lengths of the vectors for the environmental variables (description in lowercase letters, see Table 2-4 for details) overlain on the ordination vectors indicate direction and strength of associations of the variables with the ordination axes (cut-off for displayed variables was $R^2 > 0.25$). Uppercase four letter codes show the locations of species in ordination space (for species code descriptions see Appendix 2-I).

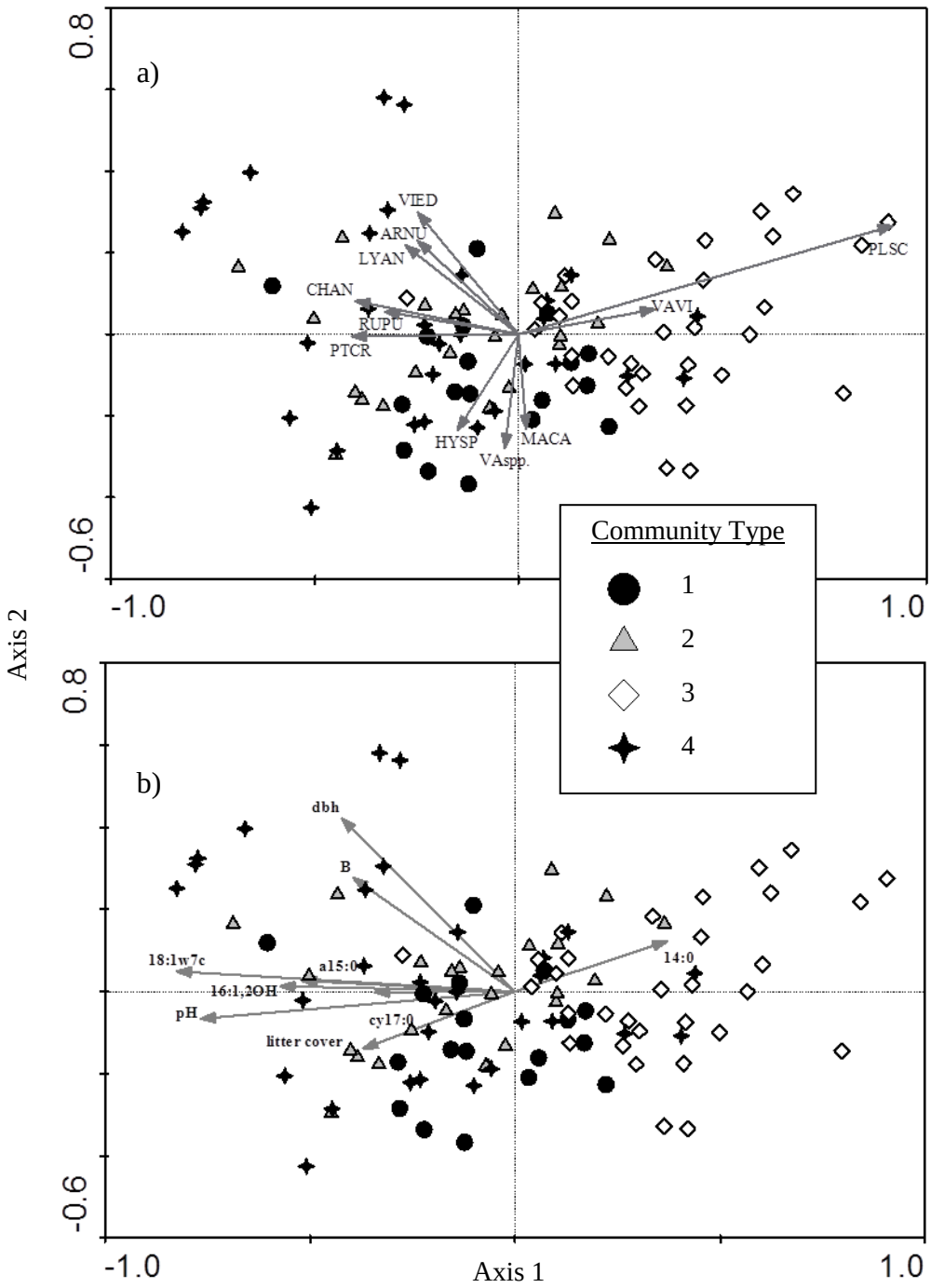

Fig. 2-3. Results of distance-based redundancy analysis of understory plant community composition delineated by the four plant community types identified by hierarchical cluster analysis : a) Uppercase four letter codes indicate the locations of plant species which had a Pearson correlation coefficient > 0.3 (see Appendix 2-I for description of species codes), and b) the direction and length of the vector for environmental variables (description in lowercase letters, see Table 2-4 for details) reflects the strength of correlation with the first two axes for variables that had a Pearson correlation coefficient > 0.3 for either of the first two axes. Each symbol is a subplot, which is coded by plant community type (see Fig. 2-1). The environmental and species scores were scaled up 3.3 and 2.5 times, respectively, to those of sample scores and some species points were moved slightly from their original location to improve readability.

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2.6. Appendix 2-I. List of species and taxa for vascular and non-vascular plants and lichens sampled in the study.

Code[i]	Genus	Species	Scientific authority	Growth form
ARCO	*Arnica*	*cordifolia*	Hook.	forb
ARNU	***Aralia***	***nudicaulis***	**L.**	**forb**
BRST	*Brachythecium*	*starkei*	(Brid.) Schimp.	bryophyte
CAAN	*Carex*	*aenea*	Fern.	graminoid
CACA	***Calamagrostis***	***canadensis***	**(Michx.) Beauv.**	**graminoid**
CAMO[ii]	***Calamagrostis***	***montanensis***	**Scribn. ex Vasey**	**graminoid**
CHAN	***Chamerion***	***angustifolium ssp angustifolium***	**(L.) Holub**	**forb**
CILA	*Cinna*	*latifolia*	(Trevir. ex Göpp.) Griseb.	graminoid
COCA	***Cornus***	***canadensis***	**L.**	**forb**
DIPO	***Dicranum***	***polysetum***	**Sw.**	**bryophyte**
DRAU	*Dryopteris*	*austriaca*	(Jacq.) Woynar ex Schinz & Thellung	fern
ELIN	*Elymus*	*innovatus*	(Beal) Pilg.	graminoid
EQSY	*Equisetum*	*sylvaticum*	L.	forb
GOOB	*Goodyera*	*oblongifolia*	Raf.	forb
HYSP	***Hylocomium***	***splendens***	**(Hedw.) Schimp.**	**bryophyte**
LIBO	***Linnaea***	***borealis***	**L.**	**forb**
LICO	*Listera*	*cordata*	(L.) R. Br.	forb
LOIN	*Lonicera*	*involucrata*	(Richards.) Banks ex Spreng.	shrub
LYAN	***Lycopodium***	***annotinum***	**L.**	**club-moss**
LYCL	*Lycopodium*	*clavatum*	L.	club-moss
LYCO	*Lycopodium*	*complanatum*	L.	club-moss
MACA	***Maianthemum***	***canadense***	**Desf.**	**forb**
MEPA	*Mertensia*	*paniculata*	(Ait.) G. Don	forb
MINU	*Mitella*	*nuda*	L.	forb
PEAP	***Peltigera***	***aphthosa***	**(L.) Willd.**	**lichen**
PEPA	***Petasites***	***palmatus***	**(Aiton) A. Gray**	**forb**
PHPR	*Phleum*	*pretense*	L.	graminoid
PLSC	***Pleurozium***	***schreberi***	**(Brid.) Mitt.**	**bryophyte**
POCO	***Polytrichum***	***commune***	**Hedw.**	**bryophyte**
POPA	*Poa*	*palustris*	L.	graminoid
PTCR	***Ptilium***	***crista-castrensis***	**(Hedw.) De Not.**	**bryophyte**
PYAS	***Pyrola***	***asarifolia***	**Michx.**	forb
PYSE	*Pyrola*	*secunda*	L.	forb
RHGR	***Rhododendron***	***groenlandicum***	**(Oeder) Kron & Judd**	**shrub**
ROAC	***Rosa***	***acicularis***	**Lindl.**	**shrub**
RUAC	*Rubus*	*aculiferus*	L.	shrub
RUPE	*Rubus*	*pedatus*	J. E. Smith	shrub

Code[i]	Genus	Species	Scientific authority	Growth form
RUPU	***Rubus***	***pubescens***	**Raf.**	**shrub**
SPBE	***Spirea***	***betulifolia***	**Pallas**	**shrub**
TRHY	*Trifolium*	*hybridum*	L.	forb
TRPR	*Trifolium*	*pretense*	L.	forb
VAspp.[iii]‡	***Vaccinium***	***caespitosum and myrtilloides***	**Michx.**	**shrub**
VAVI	***Vaccinium***	***vitis-idaea***	**L.**	**shrub**
VIED	***Viburnum***	***edule***	**(Michx.) Raf.**	**shrub**
VIRE	***Viola***	***renifolia***	**A. Gray**	forb

[i] The 24 species/taxa that were used in the community analyses are highlighted in bold.

[ii] *Calamagrostis montanensis* did not flower in our sites, so a small number of individuals may have been misidentified as *C. montanensis* that were in fact other graminoid species.

[iii] *Vaccinium caespitosum* and *Vaccinium myrtilloides* were combined for analysis because they were only identified to genus in the field.

Chapter 3. Linkages between the forest floor microbial community and resource heterogeneity within mature lodgepole pine forests

Abstract **-** Below-ground microbial communities play integral roles in the functioning of forested ecosystems. These communities are influenced by a wide variety of above and below-ground abiotic and biotic factors. Thus, variation in above- ground properties, such as vegetation composition and cover and litter cover, and below-ground properties such as soil pH and nutrient availability are likely to influence the structure and composition of the below-ground microbial community. These effects can vary with spatial scale, from the microsite to within-stand 'fine scale' to the landscape scale. By examining microbial community variation within forests dominated by just a single tree species, we can gain insights into the potential factors, other than dominant tree species, that may influence the structure and function of below-ground microbial communities at the within-stand scale. In this study we examined fine scale (within-stand) patterns in microbial communities within a single species forest type, mature lodgepole pine, and their relationships with abiotic and biotic factors within these forests. Specifically, we examined how the microbial structure (using phospholipid fatty acids - PLFAs) and function (using respiration of multiple carbon substrates - MSIR) separated into fine-scale community types; which PLFAs and carbon substrates were indicators of these fine-scale microbial community types, and which above- and below-ground properties and processes were related to these community types. At 108 sampling points in 12 0.48-ha plots we assessed abundance of understory plants, canopy tree size and cover, and downed wood biomass. We measured below-ground variables including forest floor thickness, litter cover, pH, decomposition rate, available soil nutrients, microbial PLFAs and MSIR. Cluster and indicator species analysis revealed four fine-scale structural (PLFA) and functional (MSIR) community types. The biotic and abiotic variables we measured had low explanatory power for describing the MSIR communities. The majority of factors contributing to the separation of the below-ground structural (PLFA) microbial community types were understory plant species. Our findings suggest that the spatial partitioning of understory plant species and their rhizosphere resources (e.g., root exudates, nutrients) creates heterogeneity that influences the patterns of variation in below-ground microbial communities, in particular the microbial structure. This study provided novel insights into the ecology of above- and below-ground interactions in these forests, which can be applied to predicting the consequences of ecosystem disturbances on the structure and functioning of the below-ground microbial community.

3.1. Introduction

Below-ground microbial communities play critical roles in the functioning of forested ecosystems. They actively contribute to ecosystem functions that include decomposition

(Wardle et al. 2004), soil respiration (Hanson et al. 2000), plant productivity (Van Der Heijden et al. 2008), plant nutrition (Marschner and Dell 1994), and soil fertility (Yao et al. 2000). Biogeochemical cycles and the turnover of organic matter in forested ecosystems are largely influenced by the composition and activity of soil microbial communities (Zelles 1999).

Recent technological advances have allowed us to make significant progress towards better understanding the structure and function of soil microorganisms using culture-independent methods (Leckie 2005). To characterize the structural composition of microbial communities, phospholipid fatty acid (PLFA) profiles can be used (e.g., Myers et al. 2001). These PLFA profiles are based on microorganisms having differences in their cell membrane phospholipid fatty acid composition, which can be used as indicators of the diversity of structural microbial composition (Zelles 1999). While PLFA profiles can provide information on the structural composition of the microbial community, they do not provide information on the functional composition of the microbial community. To complement PLFA profiles, community-level physiological profiles can be used to characterize the function of microbial communities; one such method is multiple carbon-source substrate-induced respiration (MSIR) using a 'whole soil' technique that avoids the bias of only capturing respiration of culturable microorganisms (Campbell et al. 2003, Chapman et al. 2007). MSIR quantifies respiration rates for a variety of carbon substrate sources that are thought to be associated with plant root exudates (e.g., Garland and Mills 1991).

The below-ground microbial community can be influenced by a wide variety of biotic and abiotic factors. Vegetation composition influences litter inputs (i.e., quality and quantity of litter), as well as plant root exudate composition, and thus can influence below-ground biota and processes regulated by these (e.g., Zak et al. 2003, Wardle et al. 2004). Increasing diversity in plant species may result in increased diversity of litter

quality and quantity and root exudates, thus increasing resource hetereogeneity for soil microbial communities; this, in turn, may result in greater diversity in these communities (Hooper et al. 2000).

Both the canopy trees and the understory vegetation must be considered when examining the influence of vegetation on soil microbial communities in forests. Effects of variation in tree species on structural and functional microbial community composition, at both the within-stand (Saetre and Bååth 2000) and among-stand scales, have been shown (e.g., Grayston and Prescott 2005); these effects are likely to be associated with differences in litter quantity and quality and root exudates among tree species. Alternatively, while vegetation may influence the microbial community, it may not influence both the structure and function; for example, Priha et al. (2001) observed differences in the structure of the microbial composition under differing tree species but no functional differences.

Abiotic environmental resources/factors including microclimate, soil pH, texture, nutrient status (e.g., C:N ratio), and moisture can also influence the structural and functional composition of microbial communities (e.g., Gilliam et al. 2011; Birkhofer et al. 2012). Indeed, some studies have suggested that factors such as seasonal patterns in moisture may be more important drivers of soil microbial community structure and function than is vegetation (e.g., Swallow et al. 2009, Liu et al. 2012). These abiotic environmental factors can be divided into two groups describing the directness of their effects on the structure and function of microbial communities: i) proximal factors that have direct effects; e.g., pH, soil moisture, and ii) site factors that have indirect effects; e.g., regional climate, parent material, time since disturbance (Brockett et al. 2012). Thus, it appears that interactions among vegetation, proximal, and site factors are likely influencing soil microbial communities (Leckie 2005), and it is of interest to separate out

the relative influences of these on the structure and function of soil microbial communities.

The below-ground microbial community is also expected to differ across a gradient of spatial scales (Ettema and Wardle 2002). At the micro-scale of mm to cm, changes in roots, organic particles, soil structure, etc. may influence the microbial community, although little research has been done at this scale (Leckie 2005). At a within-stand scale varying across meters (hereafter 'fine scale'), changes in factors such as plant community composition may affect the soil microbial community. For example, Pennanen et al. (1999) identified structural microbial communities that existed at scales of < 3- 4 meters within boreal coniferous forests and these were correlated with proximity to tree and understory plants. At a larger landscape scale, differences in forest type and topography are likely to influence the structure and function of the forest floor microbial community (e.g., Brockett et al. 2012). Thus, the spatial scale at which microbial communities are studied will be important in understanding the factors that influence microbial communities (at that scale), as well as the linkages among soil and vegetation communities (Ettema and Wardle 2002).

Understory plant communities have been identified at the fine-scale within monoculture forests, and these plant communities were related to below-ground factors including several below-ground microbial PLFAs (see Chapter 2). However, we do not know if forest floor microbial communities also vary at the fine scale in monoculture forests, nor which abiotic and biotic factors may contribute to such patterns. This is likely to depend on how overstory species composition is related to heterogeneity in other above- and below-ground ecosystem properties or processes at a fine scale. If below-ground communities are primarily driven by overstory species composition, then in monoculture forests they would not show a pattern of fine-scale microbial communities. However, if below-ground microbial communities are related to understory communities,

which do vary at this scale, or alternatively to abiotic factors that may also vary at this scale, then they will show fine scale patterning in their composition. Thus, by examining microbial community variation within forests dominated by a single tree species, we can gain insights into the biotic and abiotic factors, other than tree species, that may influence the structure and function of below-ground microbial communities at the fine scale.

An increased understanding of the ecosystem factors influencing the below-ground microbial community structural and functional composition at a fine scale will help us sustainably manage our forests into the future. This is particularly relevant in the face of shifting disturbance regimes and forest management practices. For example, lodgepole pine (*Pinus contorta* Douglas ex Loud. var. *latifolia* Engelm.) forests have historically experienced stand-replacing fire as their dominant disturbance regime. However, these forests are now experiencing increased levels of partial-canopy disturbances by mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), which kills trees without direct impacts to the forest floor (Burton 2008). Salvage harvesting, which is a common forest management response to MPB attack, could also alter the structure and composition of forest microbial communities (Chatterjee et al. 2008).

Lodgepole pine grows as a monoculture forest across a wide portion of its range, and with its' wide climatic and geographical range in North America it is highly valued for timber, wildlife habitat, and recreational use. Thus, these forests provide a valuable study ecosystem for examining how below-ground microbial communities might respond to on-going interactions between the disturbed canopy, the understory plant community, and other below-ground factors in the face of partial canopy disturbances that leave the forest floor intact. The overarching goal of this study was to examine fine scale patterns in below-ground microbial communities within a single species forest type and their relationships with resource heterogeneity within these forests. We addressed three key

research questions regarding the relationships between forest floor microbial community structural (as measured by PLFA) and functional (as measured by MSIR) composition and overstory, understory, and below-ground attributes in lodgepole pine forests of Alberta: i) Are there fine-scale structural or functional microbial communities within mature lodgepole pine forests? ii) If so, what structural and functional variables are indicators of these microbial communities? and iii) Which above-and below- ground abiotic and biotic factors contribute to the separation of these microbial communities? We hypothesized that fine-scale microbial communities would occur, but that with just one dominant overstory species, separation of the microbial communities would be primarily influenced by the above- and below-ground variation in abiotic and biotic factors, in particular those associated with fine scale heterogeneity in the understory plant communities.

3.2. Methods

Study area

The study area was located in the Upper Foothills natural sub-region of Alberta (Natural Regions Committee 2006) in lodgepole pine forests near Robb, AB. This region is characterized by pure lodgepole pine forests with serotinous cones, along with mixed conifer stands of white spruce (*Picea glauca* (Moench) Voss) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). Stand ages are generally younger than 100-120 years old reflecting the regional disturbance regime of relatively frequent stand-initiating wildfire (Beckingham et al. 1996). The climate is temperate continental with mean daily maximum air temperatures during the growing season ranging from a daily maximum of 16.2 ^{0}C in May, to 20.6 ^{o}C in August. Mean monthly precipitation during the growing season is as follows: 57.9 mm (May), 106.7 mm (June), 106.2 mm (July), and 82.2 mm (August), with a mean annual precipitation of 562.4 mm (30 year climate normal 1971-

2000). The study stands were approximately 110-120 years old and were located on brunisolic gray luvisolic soils (Soil Classification Working Group 1998). The study area was classified as ecosite UF e1.1 – Pl/green alder/feather moss (Beckingham et al. 1996). The overstory included only lodgepole pine; there were a very few white and black (*Picea mariana* (Mill.)) spruce, trembling aspen (*Populus tremuloides* Michx.) and balsam fir (*Abies balsamea* (L.) Mill) in the lower canopy. Notably, advance regeneration was absent or present in very low numbers (i.e., < 10 seedlings or saplings – ha^{-1} see Chapter 5). The understory was dominated by feather mosses, including *Pleurozium schreberi* (Brid.) Mitt., *Ptilium crista-castrensis* (Hedw.) De Not. and *Hylocomium splendens* (Hedw.) Schimp. and the hair cap moss *Polytrichum commune* Hedw.. Common forbs included *Cornus canadensis* L. and *Linnea borealis* L., common small shrubs included *Rosa acicularis* Lindl. and *Vaccinium myrtilloides* Michx.; *Alnus crispa* (Aiton) Pursh was the dominant tall shrub and the common graminoid was *Calamagrostis montanensis* (Michx) Beauv..

Three forest study units (i.e., blocks in a statistical sense) ranging in size from 4.8 – 8.8 ha were sampled during the growing season of 2008 (Table 3-1 – see Chapter 4 for description of how site- characteristic variables (basal area, density, mean dbh, and canopy cover) were calculated). The study units we selected were relatively flat topographically, were similar to one another and covered by fairly homogenous mature lodgepole pine forest representative of the dominant forest cover type in the region. Within each study unit we established four 60-m x 80-m (0.48 ha) plots. Each plot was surrounded by a minimum of 20 m (~ one tree height) of similar composition pine forest in order to minimize edge effects. Plots were placed as close together as possible within the constraint of ensuring uniform overstory stand conditions within each plot. Within each plot we established nine systematically-located nested sample points that were used as the center-points for sampling the overstory, downed wood, understory and below-

ground (n=3 blocks * 4 plots * 9 sample points =108 sampling points). These sample points were located 20-30 m apart from one another to reduce spatial auto-correlation.

Data collection

The overstory plant community was sampled in 8-m fixed-radius (0.02 ha) circular subplots. Standard forest mensuration data were collected for all trees (i.e., with dbh $\geq$ 5 cm and ht > 1.3 m) within each subplot (i.e., live/dead status, species and dbh). These data were used to calculate basal area and stem density, separated by live/dead status.

To estimate canopy cover, hemispherical photographs were taken in the middle of the growing season (mid-July) at each of the sample points using a digital Nikon Coolpix 4500 with FC-E8 fisheye lens. Hemispherical photographs were taken approximately 1.4 m above the forest floor, with the camera leveled on a tripod and the bottom of the camera oriented towards North. We analyzed canopy photographs using SLIM (Spot Light Intercept Model v. 3.01), using batch processing to analyze photos with manual color threshold adjustments by plot to optimize differences between canopy and sky. The program calculated gap fraction, which measures the area of overhead view (in percent) which constitutes canopy gaps and we subtracted gap fraction from 100 to estimate canopy cover.

The downed woody material (DWM) was measured at each sample point using the line intersect method (Brown 1974; Brown et al. 1982; Van Wagner 1968, 1982). Line transects ran from each sampling point out 8 m at a randomly selected angle to guard against a possible orientation bias. The diameter of each DWM piece at the point of intersection with the line transect was measured using calipers and categorized into diameter size classes as follows: 0-0.5 cm, 0.5-1.0 cm, 1-3 cm, 3-5 cm, 5-7 cm and > 7 cm (as adopted by the Canadian Forest Service; McRae et al. 1979, Van Wagner 1982). Pieces in diameter size classes 0-0.5, 0.5-1 and 1-3 cm were counted along the first 2 m

length of each transect, size classes 3-5 and 5-7 cm along the first 4 m length of each transect and for all pieces ≥ 7 cm we recorded diameter, length and decay class (i.e., 1-5, based on Table 8.1 in VRI 2007) along the full 8 m. Biomass of DWM (Mg ha^{-1}) for each of the size classes was calculated using the equation and coefficients for Central Alberta foothills lodgepole pine stands (Delisle and Woodard 1988, Nalder et al. 1997). For the large pieces (≥ 7 cm diameter) we also calculated the biomass of sound (i.e., decay classes 1 and 2) and rotten (i.e., decay classes 3-5) wood separately. We calculated the total biomass of DWM by summing up the biomass for all size classes. Percent cover of DWM was estimated during assessment of understory communities (see below).

We sampled the understory plant community (i.e., forest floor mosses, forest floor lichens, forbs, graminoids and shrubs – see Appendix 2-I for detailed list) within 1-m x 1-m quadrats located at each of the sample points. Percent cover (0-100) of each species/taxa was estimated to the nearest $1/10^{th}$ percent for species with < 1% cover and to the nearest 1% for species with > 1% cover. For species that could not be identified in the field, voucher specimens were collected for identification through comparison with University of Alberta herbarium samples. Species scientific names were confirmed using the USDA Plants database (http://plants.usda.gov/). Cover estimates were also recorded for litter, tree/snag boles, downed woody material (diameter ≥ 3 cm), exposed mineral soil and rock. We calculated understory species richness and diversity (i.e., Shannon Index, Magurran 1988) per quadrat. We measured tall shrubs and saplings (i.e., > 1.3 m ht and < 5 cm dbh, e.g., *Alnus crispa*) in 4-m radius circular subplots centered at each of the sampling points. To estimate basal area of tall shrubs and saplings within the plots, we measured the stem basal diameters for shrubs and saplings rooted within the subplot and for shrubs that weren't rooted in the subplot but had canopy overhanging the subplot. The thickness of the forest floor (excluding the recent litter fall, or L layer, but including

both Fibric and Humic layers – i.e., F/H, mm) was measured in each of the four corners of the nine understory vegetation quadrats within each plot.

We installed Plant Root Simulator (PRS) probe ion exchange membranes (Western Ag Innovations, Inc., Saskatoon, SK, Canada) to measure soil nutrient availability. The anion exchange PRS™-probes simultaneously adsorbed all nutrient anions, including NO_3^-, PO_4^{3-} and SO_4^{2-}. Cation exchange PRS™-probes simultaneously adsorbed nutrient cations such as B^+, $NH_4^{+,}$ K^+, Ca^{2+} and Mg^{2+}. A chelating pre-treatment of the anion PRS™-probe also permitted the adsorption of micronutrient metals such as Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}. We installed four pairs (pair = 1 cation and 1 anion exchange membrane) of PRS probes vertically at the four corners of each of the nine understory quadrats within each plot. The top of the ion exchange membrane was placed at the interface between the forest floor and mineral soil. Probes were installed for the duration of the growing season (mid-June to mid-September 2008). After probes were removed at the end of the growing season, they were cleaned with deionized water and shipped to Western Ag for analysis; the four probe pairs from individual quadrats were pooled prior to elution and analysis. The PRS probes were eluted with 0.5 M HCl prior to nutrient analysis. A segmented flow Autoanalyzer III (Bran and Lubbe, Inc., Buffalo, NY) was used to colorimetrically quantify NH_4^+, NO_3^- and PO_4^{3-} and ICP spectroscopy (PerkinElmer Optima 3000-DV, PerkinElmer Inc., Shelton, CT) was used to quantify Al^{3+}, B^+, Ca^{2+}, Cd^{2+}, Cu^{2+}, Fe^{2+}, K^+, Mn^{2+}, Mg^{2+}, $Pb^{2/4+}$, SO_4^{2-} and Zn^{2+}. The PRS-probe nutrient supply rates were calculated (µg -10 cm^2 -burial $length^{-1}$). The methods used to quantify the eluate from the probes have minimum detection limits (MDL) for each element (Al=0.4, B=0.2, Ca=2, Cd=0.2, Cu=0.2, Fe=0.4, K=4, Mn=0.2, Mg=4, NH_4^+=2, NO_3^-= 2, Total N=2, P=0.2, Pb=0.2, S=2, Zn=0.2 µg - 10cm^2 burial $length^{-1}$). The few samples for which the measured value was below the MDL were still included in analysis using the values measured for them, because censoring data below MDL can bias your

dataset (Western Ag, personal communication). We did not analyze several elements because their calculated nutrient supply rates were predominately below the minimum detection limits [Cd (n=62/108<MDL), Cu (n=79/108 < MDL), NO_3^- (n=58/108 < MDL) and Pb (n=35/108<MDL)].

Decomposition rate was measured at each sampling point. Nylon mesh bags (1.5 mm x 1.5 mm mesh size) with four 90-mm diameter Whatman cellulose filter papers were buried at the forest floor-mineral soil interface for the growing season (same time span as PRS probes). Filter papers were oven dried for 1 day (pre-burial) and 3 days (post-burial) at 70°C and weighed before and after being buried. Decomposition rate was calculated as 100 minus the percentage of original filter paper biomass remaining at removal.

Forest floor samples were collected to measure pH, microbial multiple carbon source substrate-induced respiration (MSIR) and phospholipid fatty acid analysis (PLFA) of the below-ground microbial community, as described below. We collected forest floor samples (i.e., the entire thickness of the combined F and H layers) from each of the four corners of the nine understory quadrats per plot using aseptic techniques (sampling equipment was washed with 70% ethanol between quadrat samples) and combined them to form a single homogenous sample (~ 50 g) per quadrat. These were then divided into portions to be used for pH and MSIR, which were placed in ziplock bags and portions for PLFA analysis, which were immediately placed in sterile Whirlpak™ bags. Samples were kept cool on ice until transferred back to the lab. Upon arrival at the lab, samples for MSIR and pH were sieved (4 mm) and kept refrigerated (4°C) in plastic bags prior to analysis. PLFA samples were stored at -86°C and then freeze-dried prior to PLFA extraction.

Forest floor pH was measured potentiometrically in a saturated paste in equilibrium with a soil suspension of a 1:4 soil:liquid mixture. We used 0.01 M $CaCl_2$ in place of water following the instructions for measuring pH of field-moist organic samples as described in Kalra and Maynard (1991).

Functional composition of microbial communities relates to their activity particularly in the carbon cycle. MicroResp™ offers a convenient, rapid and sensitive method for the determination of the community-level physiological profile for each forest floor sample using a 'whole soil' technique that uses the 96-well microtitre plate format that Biolog (Biolog Inc.) does (Campbell et al. 2003, Chapman et al. 2007). We prepared detection agar plates containing a gel-based bicarbonate buffer with indicator dye that responded to the pH change within the gel resulting from carbon dioxide evolved from the soil (Cameron 2008). The plates were stored in a closed desiccation chamber in the dark when not being used for analysis.

To estimate mean forest floor moisture content within each study unit to use for MSIR analysis, we sub-sampled ~1 g of each field-moist sieved forest floor MSIR sample and then combined all 36 samples within each study unit into a single sample. Each of the three pooled study unit samples were weighed (fresh) and then dried for 48 hours at 65° C and reweighed (dry). Percent dry weight was calculated ((dry/fresh)*100) and soil moisture content was calculated as 100 – percent dry weight; this moisture content was then used for calculating substrate concentrations. Each respiration substrate was prepared as 30 mg of substrate per gram of water (Cameron 2008); a separate set of substrates was prepared for each of the three study units because of observed differences in soil moisture content among them. Fifteen substrates commonly used in carbon SIR analysis and thought to be associated with plant root exudates (e.g., Garland and Mills 1991, Stevenson et al. 2004) were used: five amino acids (L-alanine, L-arginine, glutamine, L-lysine, γ aminobutyric acid), six carbohydrates (n-acetyl glucosamine, L-

(+)-arabinose, D-(+)-galactose, glucose, mannose, trehalose), four carboxylic acids (citric acid, L-malic acid, oxalic acid, 3,4-dihydroxybenzoic acid) and water as a control to measure basal respiration. Substrates at prepared concentrations were stored at 4° C for the duration of the respiration analysis.

Field-moist sieved forest floor samples were incubated in a dark chamber at 25°C for ~24 hours prior to analysis. Forest floor samples were added to the 96-well microtiter deep well plates after 30 µl of each substrate was dispensed (three replicate substrate wells per sample, two forest floor samples per deep well plate). The deep well plate was then hermetically sealed with a gasket, face-to-face, with the detection plate, such that each well interacted with the opposite well of the detection plate. The two plates were incubated in the dark at 25° C for six hours. The colour change in the detection plate was then read on a standard laboratory microplate reader (detection plate read before and after 6 hrs of incubation, absorbance = 570 nm) and respiration rates were calculated (µg CO_2-C g^{-1} hr^{-1}). A maximum of 16 samples could be analyzed in a day, so samples were randomly selected each day to reduce bias associated with differences in time since collection and all analyses were completed within two weeks of when the samples were collected. One sample had five carbon substrate respiration rates below basal respiration and was excluded from analysis. To standardize for differences in the total forest floor respiration among samples, respiration was expressed as the respiration response for an individual substrate (p_i) as a proportion of total respiration rates from all 15 substrates for a given forest floor sample (Degens et al. 2000). Catabolic evenness was calculated using the Simpson-Yule index ($1/\sum p_i^2$, Magurran 1988).

Microbial phospholipid fatty acid (PLFA) analysis produces a lipid profile of microbial communities. We transferred 0.30 g of each freeze dried forest floor sample to a muffled test tube and then analyzed each of them for PLFAs following the detailed methods described in Hannam et al. (2006). To summarize, we analyzed forest floor

samples by extraction with a single-phase chloroform mixture, lipid fractionation on a solid-phase-extraction Si column and then subjected them to a mild methanolysis using a modified Bligh and Dyer extraction (Bligh and Dyer 1959, Frostegård et al. 1991, White and Ringelberg 1998). The resulting fatty acid methyl esters were then analyzed using an Agilent 6890 Series capillary gas chromatograph (GC; Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to identify individual fatty acids. Fatty acids were designated X:YωZ, where X represents the number of carbon atoms, Y represents the number of double bonds and Z indicates the position of the first double bond from the aliphatic (ω) end of the molecule. The suffixes c and t indicate cis and trans geometric isomers. The prefixes 'a' and 'i' refer to anteiso and iso branching and Me and OH specify methyl groups and hydroxyl groups, respectively. PLFAs that were present in 5% or less of the samples were excluded from analysis. PLFAs for 16:1ω9c and 16:1ω11c were combined and 18:2 ω6,9c and a18:0 were combined for analysis as they could not be distinguished by the GC. We excluded two samples with <85% peak matching from analysis. There were a total of 53 PLFAs included in the final analysis and these were also summed to provide a measure of total PLFA biomass (nmol g^{-1} forest floor)(see Appendix 3-I for a detailed list of PLFAs). PLFAs that have been previously identified as associated with soil microorganisms were combined into PLFA biomarker groups for fungi, bacteria, actinomycetes and arbuscular mycorrhizae. The fungal PLFAs 18:1ω9c, 20:1ω9c and 18:3ω6c were used to estimate the contribution of fungi (Myers et al. 2001, Hamman et al. 2007) and 16:1ω5c was used to estimate arbuscular mycorrhizae (Frostegård and Bååth 1996, Olsson 1999). Bacterial PLFAs included 10:0 3OH, 12:0, 12:0 2OH, 12:0 3OH, 14:0, i14:0, 15:0, a15:0, i15:0, i16:0, i17:0, a17:0, 17:0, 17:0cyclo, 18:1 ω5c, 18:1ω7c (Bååth et al. 1992, Frostegård and Bååth 1996, Olsson and Alstrom 2000, Myers et al. 2001, Hassett and Zak 2005). The

ratio of fungal to bacterial PLFAs was used to estimate the relative contributions of fungi and bacteria. The 10-methyl branched fatty acids (10me16:0, 10me17:0 and 10me18:0) were used to quantify actinomycetes (Kroppenstedt 1985, Brennan 1988). Aside from the measurement of total biomass of PLFAs and the biomass for biomarker groups, all measured PLFAs were expressed on a mol% basis to standardize for differences in the total amount of forest floor PLFAs among samples.

We calculated the microbial metabolic quotient for each forest floor sample as the soil basal respiration (i.e. SIR with water as the substrate) divided by the microbial biomass (i.e., the total PLFA biomass) (qCO_2 - Anderson and Domsch 1978).

Statistical analyses

Multivariate outlier analysis of the PLFA and MSIR datasets were conducted in PC-ORD and subplots with > 2.5 standard deviations mean distance were excluded from all statistical analyses; results below are for 100 PLFA subplots and 99 MSIR subplots.

We used hierarchical, agglomerative clustering to analyze the i) 53 PLFAs and ii) 15 MSIR carbon substrates and whether they grouped into discrete structural (PLFA) and functional (MSIR) microbial community types. Cluster analysis is a powerful tool to interpret vegetation patterns (Urban et al. 2002) and we used a flexible beta linkage method with β= -0.25 and Sørenson's distance measure. Because we did not have *a priori* types, Indicator Species Analysis (ISA; Dufrêne and Legendre 1997) was used to prune the dendrogram of the cluster analysis, with individual PLFAs and carbon substrates treated as the 'species' in ISA. We used different numbers of microbial community types as cut-offs (n=1-10) and then selected the community type number cut-off with the lowest average P-value of all PLFAs (structural microbial community) or carbon substrates (functional microbial community) (McCune and Grace 2002), that also had a minimum mean indicator value of 25 among samples. Once the final clusters had been identified,

ISA was used to identify which PLFAs or carbon substrates were contributing to the separation of the microbial community types, using Monte Carlo permutations (n=5000). Cluster analysis and ISA were conducted using PC-ORD (Version 5 MjM Software Design, Gleneden Beach, OR).

We used nonmetric multidimensional scaling (NMS) unconstrained ordination to examine the multivariate patterns in the i) structural (PLFA – mol%) and ii) functional (MSIR – proportional respiration) below-ground microbial communities (McCune and Grace 2002). For this we used PC-ORD (Version 5 MjM Software Design, Gleneden Beach, OR), with Sørenson as the distance measure and completed 100 runs with real data and 100 Monte Carlo randomized runs, starting with a five-dimensional solution and stepping down to a one-dimensional solution. We determined the number of dimensions of our final solutions by evaluating the scree plot and the reduction in stress with step-down in dimensionality of the preliminary runs (McCune and Grace 2002). Stability of the solution (stability criterion = 0.00005) was assessed by plotting stress versus iteration. After checking the optimal number of dimensions and best solution from the preliminary runs, we ran a final NMS with the number of dimensions determined from the preliminary runs, using the starting configuration that worked best in our preliminary runs and omitting the Monte Carlo test. We then calculated the Pearson correlation coefficients of the PLFAs and the MSIRs with the axes of their respective ordinations. We also calculated the Pearson correlation coefficients for variables in our secondary ('environmental') matrix with the ordination axes including: overstory vegetation variables [e.g., canopy cover, density, basal area, mean dbh]; factors that describe the plant community [understory cover by species, by growth form (i.e., graminoid, forb, shrub), total understory cover, understory species richness, Shannon diversity]; downed woody material biomass by size class; and below-ground variables [forest floor thickness, litter cover, pH, decomposition, available soil nutrients, biomass of biomarker groups of

PLFAs, fungi:bacteria PLFA ratio, catabolic evenness, metabolic quotient]. We also calculated correlations for proportional respiration of carbon substrates (with axes of the PLFA ordination only), and mol percent for each PLFA (with axes of the MSIR ordination only) to evaluate potential associations between the structure and function of the microbial community along with the other environmental factors that we measured.

Distance-based redundancy analysis (db-RDA) constrained ordination was used to evaluate the relationships between the below-ground i) structural and ii) functional microbial community compositions that we measured within each quadrat and the above- and below-ground variables we measured. Distance-based RDA used sample scores from a Principal Coordinates Analysis (PCoA), which extracted the principal coordinates of the distance matrix in a redundancy analysis (RDA) that then used these sample scores as the 'species' data (Legendre and Anderson 1999). The PCoA was done using our PLFA (mol percent) and MSIR (proportional respiration) data as 'species' data using PrCoord (ter Braak and Šmilauer 2009). We used the Bray-Curtis distance measure and excluded negative eigenvalues in our PCoA. Prior to db-RDA analysis, all variables in the environmental matrix were standardized to unit variance (i.e., each variable was centered and standardized by bringing their means to zero and their variances to one – Lepš and Šmilauer 2003). Environmental variables (excluding the microbial variables, but otherwise including the same variables as listed above for the NMS ordinations) were tested for inclusion in the db-RDA with forward step-wise selection and testing for significance by 499 Monte Carlo permutations, blocked by block (α=0.05). Once we identified the significant variables from our first db-RDA, we ran a final db-RDA including only the significant variables and we tested the significance of the first and all canonical axes. For the db-RDA Analyses we used Canoco for Windows Version 4.56 (ter Braak and Šmilauer 2009), with scaling focused on samples, species scores not post-transformed, no transformation of species data and centering by species. We used the

original matrix of PLFA or MSIR data by sample plots as supplemental datasets so that PLFAs or MSIRs could be projected in ordination space when plotting the ordinations in CanoDraw. We only displayed PLFAs or MSIRs and environmental variables which had Pearson correlation coefficients >0.4 with either of the first two significant ordination axes in our ordination plots. The sample points plotted in ordination space were coded by the community types identified in the cluster analyses.

For the resource and environmental variables that were significant in the db-RDA we compared the mean values among the i) structural and ii) functional microbial community types (identified by the cluster analyses) using ANOVAs with 'community type' as a fixed effect; 'study unit' and 'plot within study unit' as random effects (Proc Mixed, SAS Institute Inc., Version 9.2 (32-bit), Cary, NC, USA: SAS Institute Inc., 2008). We first determined whether each variable met the assumptions for analysis of variance (ANOVA) using analysis of residuals and normal probability plots and transformed response variables when necessary to meet the assumptions. When significant differences were detected, we used post-hoc linear contrasts to compare among the microbial community types using Bonferroni-adjusted P-values (family-wise α=0.05) (Proc Mixed, SAS v9.2).

3.3. Results

Within the lodgepole pine stands there were four PLFA microbial community types identified by cluster analysis of data from the 100 subplots (Fig. 3-1a). The indicator species analysis revealed multiple significant PLFA indicators for each of the PLFA community types (Table 3-2a). For community type 1 there were six PLFA indicators, which included a bacterial and a fungal PLFA; the other indicators were not associated with any particular biomarker group (see Appendix 3-I for list of PLFAs and biomarker groups). Community type 2 had four PLFA indicators, including one associated with

bacteria. In community type 3 there were 20 indicators, including a non-mycorrhizal and a mycorrhizal fungal PLFA, six bacterial associated PLFAs, and two actinomycete PLFAs. A bacterial PLFA and 3 other PLFAs were indicators for community type 4.

The unconstrained ordination illustrated the separation among the four PLFA community types; the NMS 3-dimensional solution (final stress = 11.7 after 62 iterations) explained 90.6% of the variation in the dataset (Fig. 3-2a & b). There were high correlations ($R^2 > 0.5$) for multiple PLFAs, but the only strong correlations (with $R^2 > 0.25$) of the environmental variables with the NMS ordination axes were for PLFA biomarker groups: the non-mycorrhizal fungi, mycorrhizal fungi, and bacteria functional groups, and the ratio of fungi to bacteria (Fig. 3-2a & b). PLFA community type 1 loaded towards the upper portions of all three axes and was positively associated with the mol% of 18:2ω6,9c/a18:0, fungi:bacteria ratio and the biomass of non-mycorrhizal fungi (Fig. 3-2a & b). The second community type loaded towards the lower ends of axes 1 and 2 and middle of axis 3, was positively associated with PLFAs 17:0, 18:0, 20:2ω6,9c, and 20:4ω6,9,12,15c, and was negatively associated with non-mycorrhizal fungi biomass (Fig. 3-2a & b). Community type 3 loaded towards the lower ends of axes 1 and 3 and the upper end of axis 2 and was positively associated with PLFAs 16:1ω11c, 16:1 ω 5c, i17:0, and 18:1w7c, and with mycorrhizal fungi biomass (Fig. 3-2a & b). The fourth community type loaded towards the lower end of axis 1, upper end of axis 2, and middle of axis 3 without strong associations with individual PLFAs, but negative associations with total bacteria and non-mycorrhizal fungi biomass (Fig. 3-2a & b).

The constrained ordination also illustrated separation among the four PLFA community types and showed which environmental variables were associated with these (Fig. 3-3). The first four db-RDA axes, which were all significant, accounted for 88.3% of the PLFA-environment relationship (Table 3-3a). The four PLFA community types separated along the first two db-RDA axes: type 1 was loaded towards the lower end of

axis 1, type 2 was loaded in the middle of axis 1, type 3 was loaded towards the upper end of axis 1, and type 4 was distributed towards the upper end of axis 2 (Fig. 3-3). The relative locations of subplots of the different PLFA community types were consistent with the indicator species PLFA analysis, although not all indicator PLFAs were highly correlated ($r>0.4$) with the ordination axes (Fig. 3-3a). The PLFA locations in ordination space (on the db-RDA bi-plot) also reflect their correlation with the environmental gradients (Fig. 3-3). One overstory variable (canopy cover), seven understory species, and one below-ground variable (available Al) were significantly correlated with the PLFA community composition along the four axes and collectively explained 22.2% of the variation in the PLFA data (Table 3-3a, Fig. 3-3b). Most strongly correlated with Axis 1 were the understory cover of *Calamagrostis montanensis* (negative), *Chamerion angustifolium* (positive), *Lycopodium annotinum* (positive), *Pleurozium schreberi* (negative), and *Polytrichum commune* (positive) and availability of Al (negative), understory cover of *Rosa acicularis* was most highly correlated with Axis 2 (negative), canopy cover (positive) and understory cover of *Cornus canadensis* (negative) were most correlated with Axis 3 (Table 3-3a).

In general, the patterns in mean values for the environmental variables among the four PLFA community types were consistent with the relationships found in the db-RDA ordination (Table 3-4a). Community type 1 had the highest canopy cover, which was significantly higher than type 2 and it also had significantly lower cover of *L. annotinum* compared with types 2 and 3. Understory cover of *C. canadensis* was significantly higher in type 2 than in types 1 and 4. For community type 3, understory cover of *Chamerion angustifolium* was significantly higher than for type 1, cover of *P. schreberi* was significantly lower compared with types 1 and 4, and cover of *P. commune* was significantly higher than for types 1 and 2. There were no significant differences in

available soil Al, and cover of *C. montanensis* or *R. acicularis* among any of the four PLFA community types (Table 3-4a).

There were also four MSIR microbial community types identified by cluster analysis of data from 99 subplots (Fig. 3-1b). The carbon substrate indicators included a carboxylic acid and amino acid for community type 1, an amino acid for community type 2, a carboxylic acid for community type 3, and a carboxylic acid and carbohydrate indicators for community type 4 (Table 3-2b). The unconstrained ordination illustrated the separation among the four MSIR community types; the NMS 3-dimensional solution (final stress = 18.1 after 29 iterations) explained 77.9% of the variation in the dataset (Fig. 3-2c & d). However, the only variables with strong correlations (with $R^2 > 0.25$) with the NMS ordination axes were several of the MSIR carbon substrates that comprised the primary ordination matrix (Fig. 3-2c & d). MSIR community type 1 loaded towards the middle of axis 2 and upper end of axis 3 and was positively associated with oxalic acid (Fig. 3-2c & d). The second community type loaded towards the lower ends of axes 2 and 3 and was positively associated with n-acetyl glucosamine, citric acid, and L-alanine (Fig. 3-2c & d). Community type 3 loaded towards the lower ends of axis 1 and 2 and upper end of axis 2 and was negatively associated with oxalic acid (Fig. 3-2c & d). The fourth community type loaded towards the very lower end of axis 1, lower end of axis 2, and middle of axis 3 and was positively associated with malic acid respiration (Fig. 3-2c & d).

The constrained ordination also illustrated some separation among the four MSIR community types, although the separation was much less distinct compared with the NMS ordination plots (Fig. 3-4). The first three db-RDA axes, which were all significant, accounted for 100% of the MSIR-environment relations (Table 3-3b). The four MSIR community types only partially separated along the first two db-RDA axes: community type 1 was centered in the middle of axis 1 and towards the middle and lower half of axis

2, community type 2 was spread across axes 1 and 2 and overlapped with the other types, types 3 and 4 were loaded towards the upper end of axis 1 with some overlap with types 1 and 2 (Fig. 3-4). The MSIR locations in ordination space (on the db-RDA bi-plot) also reflect their correlation with the community types (Fig. 3-4). The relative locations of subplots of the different MSIR community types were consistent with the indicator species MSIR analysis, although glutamic acid and glucose were not highly correlated ($r>0.4$) with the ordination axes, and the carbohydrate trehalose, which was not a significant indicator, was (Fig. 3-4a). The significant environmental variables included: understory cover of *Vaccinium vitis-idaea*, litter cover, and biomass of DWM size class 4, which were significantly correlated with the three axes but collectively explained only 6.7% of the variation in the MSIR data (Table 3-3b, Fig. 3-4b). The understory cover of *V. vitis-idaea* (positive) and litter cover (negative) were most strongly correlated with Axis 1, whereas DWM biomass of Class 4 was most strongly correlated (positive) with axis 3; none of the variables were most correlated with Axis 2 (Table 3-3b).

The only differences in mean values for the significant environmental variables from the dbRDA among the four MSIR community types were for cover of litter and *V. vitis-idaea* (Table 3-4b). Community type 2 had significantly higher litter cover compared with type 3, with intermediate levels in types 1 and 4. The cover of *V. vitis-idaea* was significantly higher in MSIR community type 4 compared with types 1 and 2, with intermediate levels in type 3.

3.4. Discussion

This study increased our understanding of fine scale patterns of variation in below-ground microbial community composition and function in mature lodgepole pine forests and their linkages with other forest ecosystem variables. Previous studies had shown evidence that overstory species composition (Grayston and Prescott 2005), understory plant diversity

(Hooper et al. 2000), and/or abiotic factors (Birkhofer et al. 2012) influenced below-ground microbial communities. Our results show that, even in stands with a single-species homogeneous overstory, soil microbial communities are still organized into distinct, fine-scale community types. We identified four fine-scale structural (PLFA) and four functional (MSIR) microbial community types. As we hypothesized, the majority of the environmental factors associated with these below-ground microbial communities were understory plant species. These findings suggest two potential alternative linkages between the microbial community and the understory plant community: i) this could be a result of both communities responding to some shared environmental factor, or ii) that spatial partitioning of plant species and their associated litter and rhizosphere resources (e.g., root exudates, nutrients) may create heterogeneity, which in turn influences the patterns of variation in below-ground microbial communities. We consider these further below.

In these monoculture pine forests, the only important overstory variable associated with below-ground community composition was percent canopy cover. Although overall our sample points showed only a moderate range of variation in cover values, two of the plant community types showed significant differences in canopy cover. These findings suggest that the cover of trees is an important regulator of the structure of the microbial below-ground community. Thus, even in a monoculture forest, variation in overstory properties can still influence microbial communities, perhaps through the changes in canopy cover contributing to variation in the forest floor light environment and microclimatic conditions such as soil moisture and soil temperature, that in turn may influence the structure and function of the microbial community. Interestingly, canopy cover was not a significant indicator of the understory plant community types identified in Chapter 2 (although dbh was). Thus, any influences of the overstory cover on soil microbial communities are not a result of indirect influences on understory plant

community composition, and instead appear more likely to be related to the influence of canopy cover on variation in microclimatic conditions, albeit for microclimatic factors that we did not measure in this study.

Seven understory plant species were associated with structural microbial community composition within the stands (*Calamagrostis montanensis, Chamerion angustifoium, Cornus canadensis, Lycopodium annotinum, Pleurozium schreberi, Polytrichum commune,* and *Rosa acicularis*). Interestingly, all five of these understory species were indicators of understory plant community types within these forests too (Chapter 2). There were significant differences in cover of all of these species (except *C. montanensis* and *R. acicularis*) among structural microbial community types, suggesting that these species are important in the development of these distinct microbial community types. However, it is unclear whether this linkage is associated with both the plant and microbial communities responding to some shared environmental factor(s), or that it results from variation in the litter and rhizosphere resources (e.g., root exudates, nutrients) among plant community types. The auto-ecological properties of these understory species may provide insights into whether they are indicators of particular underlying environmental conditions within the stand. *Chamerion angustifoium* is a shade-intolerant pioneer forb that is considered a poor competitor that does not invade previously occupied areas in the understory, although it can persist across a wide range of pH, moisture and nutrient levels (Haeussler et al. 1990). The forb *C. canadensis,* because it is rarely restricted to particular moisture conditions, is not commonly identified as an indicator species for understory plant communities (Gucker 2012). *Lycopodium annotinum* is a circumboreal club-moss species associated with coniferous forests but appears to have a wide amplitude for light, nutrients and moisture (Matthews 1993). Anderson et al. (1995) examined bryophyte species along environmental gradients and found that *P. schreberi* was associated with more acidic conditions, as compared to other

boreal feather mosses (which were present in our study sites, but not significantly associated with differences in microbial community types). Therefore, *P. schreberi* may be associated with structural microbial community types that persist under more acidic compared with the other microbial community types. We did not measure soil moisture associated with each forest floor sample, but we observed large pockets of *P. commune* in very wet depressions in these forests, suggesting it may also be associated with a structural microbial community type found under higher soil moisture conditions than the other microbial community types. The auto-ecological properties of *P. schreberi* and *P. commune* suggest they may be associated with pH and soil moisture respectively, whereas the other species appear to be habitat generalists with respect to environmental factors. As described above, all five of these understory species, including the three generalists, were indicators of understory plant community types within these forests. Thus differences in below-ground microbial communities may be largely a reflection of differences in the composition of the litter and/or root exudates of these understory plant species. Overall, our findings suggest that the heterogeneity of below-ground structural microbial communities is in large part driven by the existence of fine-scale variability in the species composition of the understory plant communities within these monoculture forests. Thus, the understory and the below-ground microbial community types appear to function with very close linkages to one another at the fine scale within these forests.

Interestingly, we did not observe pH as an important (strongly correlated) environmental factor in our dbRDA or NMS ordinations. The microbial community is impacted by the soil pH, and it is generally accepted that fungi are favored over bacteria at low pH (Alexander 1977). This pattern has been shown across large pH ranges (e.g., Högberg et al. 2003), but also across soil pH ranges (e.g., Pennanen et al. 1999). However, within the narrow range of pH in our study we did not detect a relationship between pH and the structural or functional microbial communities. Thus, it appears that

the understory community species composition is more important than pH in influencing the structure and function of below-ground microbial communities at the fine scale within these forests. For example, *P. schreberi*, which is associated with acidic soil conditions and was negatively correlated with pH in this study, appears to be a better indicator of microbial community type than was forest floor pH. This finding supports the important direct linkages between the understory plant and below-ground microbial community types.

While we identified four fine-scale structural microbial community types in these lodgepole pine forests, there was also some overlap among these, as seen in both the NMS and dbRDA ordination plots. We expected this overlap because the differences among the community types were largely a reflection of differences in relative abundance (mol%) of PLFAs, rather than any individual PLFAs being unique to a particular community. This suggests that microbes, as indicated by their PLFA profile, have fairly broad tolerance ranges for the properties and processes measured within these stands. However, there were still differences in their tolerance such that the fine-scale variation in these properties and processes in these forests resulted in changes in their relative abundance that could be detected as distinct structural microbial community types with significant PLFA indicators.

The four functional (based on MSIR) microbial communities we identified showed rather weak separation from one another. This may be associated with the power of MSIR to distinguish patterns at the scale of our study. An explanation for the poor separation of the microbial community types is that the functional separation of microbial communities is occurring at a scale that we did not measure in this study. We pooled forest floor from 4 samples that were within 1.4 -m of each other. If micro-scale differences in microbial communities occur at scales of mm -cm, then these would have been missed based on the spatial resolution in this study (Kirk et al. 2004). Both diurnal

and seasonal variability in temperature and moisture conditions are likely to influence the functioning of the below-ground microbial community. However, our MSIR samples represented only a single moisture and temperature combination (those during the incubation). MSIR is dependent on microbes being active under the conditions in which they are measured and it is unclear how representative the growing conditions in the lab setting were compared with those experienced in the natural forest setting (Preston-Mafham et al. 2002). Thus, the lack of strong separation among the functional microbial community types may also be a function of our MSIR results being dominated by a subset of dominant microbes that grew well in the lab conditions, rather than the lack of *in situ* patterns in functional microbial communities at the fine scale. Therefore, it may be more appropriate to evaluate MSIR patterns under the conditions that are more representative of *in situ* conditions rather than those established in Microresp MSIR protocols (e.g., incubate at lower temperatures). Finally, there is a greater likelihood of redundancy in the functional composition of microbes compared with the structural composition, and thus only large difference in functional composition (perhaps greater than occurring within mature monoculture pine stands) may be detected using this approach (Kirk et al. 2004).

The environmental variables we measured explained very little of the variation (<7% of the variability in the dataset) in the functional microbial community types; only cover of litter and *Vaccinium vitis-idaea* were significantly different among community types. Litter plays an important role in biogeochemical cycling within forests, as microbial community function depends on both the quantity and quality of litter. Higher cover of litter will provide more available substrate for decomposition processes to occur; the rate of decomposition of this litter will, in turn, be influenced by its quality and the structure and function of the microbial community that decomposes it. While *V. vitis-idaea* has been shown to be an indicator species for conifer patches in boreal mixedwood

forests (Chávez and Macdonald 2010), it is a habitat generalist that is not an indicator for particular environmental factors. Thus its influence on the microbial function is likely to be a function of its litter and root exudates rather than an association with particular environmental conditions.

The low explanatory power of the measured abiotic and biotic factors suggests that factors that we did not measure have more influence on the functional microbial communities. One such potential variable is soil moisture, which has been shown to be linked to the functioning of microbial communities (Swallow et al. 2009, Brockett et al. 2012). We expect that forest floor moisture will vary both spatially and temporally across the growing season as a function of the relative influences of snowmelt, precipitation, antecedent soil moisture conditions, and evaporative demand (Schume et al. 2003), thus influencing the microbial community both spatially and temporally. However, budgetary constraints limited our ability to quantify soil moisture at this fine scale for the duration of the growing season, and it was therefore beyond the scope of this study. A companion study conducted adjacent to our research sites that evaluated soil moisture at multiple depths within stands and across different levels of disturbance demonstrated the greatest variation in soil moisture conditions in the middle of the growing season when evaporative demand plays the critical role in regulating soil moisture (Piña 2012). Therefore, to test the influence of forest floor moisture on the functioning of fine scale microbial communities, an alternative option to expensive continuous sampling throughout the growing season could be to sample the microbial community in the middle of the growing season to optimize for potential fine scale variation in forest floor moisture, rather than at the end of the growing season as was done in this study where forest floor moisture is likely to be less variable at the fine scale.

Overall, this study provided new insights into the relationships of below-ground structural and functional microbial communities and micro-habitat resource partitioning

at the within-stand fine scale for a single overstory species forest. Our findings demonstrate the importance of spatial heterogeneity in the understory plant community in structuring below-ground microbial PLFA communities at the fine scale for monoculture forest types. However, spatial heterogeneity of above and below-ground factors in structuring functional microbial communities at the within-stand level appears to be less important, at least for the variables and spatial scale that we measured in this study. While we were unable to explicitly test the mechanisms that may be driving the relationships between the above and below-ground communities, it is evident that understory and below-ground communities operate with very close linkages to one another.

Both natural and anthropogenic disturbance agents can have substantial impacts on the structure (and composition) of the forest microbial community both through direct effects of disturbance on the forest floor (e.g., fire – Certini 2005, timber harvest - Chatterjee et al. 2008), and potentially indirect effects through disturbance to the overstory and understory composition (e.g., MPB). This study provides novel insights into the important linkages between above- and below-ground communities; knowledge of these linkages can be used to evaluate the relative influences of disturbance-induced changes on within stand factors (e.g., dead organic matter, understory plant cover, pH, soil moisture, soil nutrient supply rates) on the structure (and function) of the microbial community.

Table 3-1. Summary of site characteristics of lodgepole pine (*Pinus contorta*) study units in Upper Foothills of Alberta. Given are the locations and mean values for each of the three study units; the minimum and maximum values across subplots within each study unit are in parentheses.

Study unit	Latitude/Longitude	Basal area (m^2 ha^{-1})	Density (trees ha^{-1})	Dbh (cm)	Canopy cover (%)
1	53.2248/116.8094	39.6 (26.7-56.2)	1420 (950-1900)	18.3 (5-34.7)	63.9 (56.2-86.9)
2	53.24129/116.8288	37.3 (21.6-55.1)	978 (550-1350)	21.5 (6.6-43.3)	59.2 (51.4-70.7)
3	53.22647/116.8212	40.3 (27.1-54.0)	1182 (450-1850)	20.1 (8.0-38.3)	62.1 (54.9-77.4)

Table 3-2. Results of Indicator Species Analysis examining a) PLFA and b) MSIR microbial communities for the 3 lodgepole pine study units. Given are the a) PLFAs and b) MSIRs that had an indicator value >25 and that were significant at $\alpha = 0.05$ (Dufrêne and Legendre 1997), listed in order by descending indicator value within each microbial community type. N is the sample size for each community type. Mean a) PLFA mol % or b) MSIR proportional respiration (± SE) for each of the indicators for the microbial community types are also provided. The mean a) PLFA or b) carbon substrate values for the microbial community they were an indicator for are highlighted in bold.

Community Type	N	Variable	Indicator value	P	Mean (± S.E.) for Community Type			
					Type 1	Type 2	Type 3	Type 4
a) PLFA Community Type								
1	46	17:1ω8c	36.3	0.001	**1.08 (0.08)**	0.74 (0.17)	0.60 (0.15)	0.03 (0.03)
		18:2ω6,9c/a18:0	33	0.0002	**13.93 (0.34)**	10.12 (0.44)	8.42 (0.46)	9.75 (0.35)
		20:1ω9c	32.9	0.007	**0.83 (0.05)**	0 (-)	0.62 (0.10)	0.86 (0.08)
		15:0	27.7	0.0002	**1.03**	0.90 (0.02)	0.82 (0.02)	0.97 (0.02)
		16:0	27.2	0.0002	**15.56 (0.17)**	13.30 (0.22)	13.81 (0.25)	14.63 (0.20)
		18:1ω9c	26.6	0.0008	**13.68 (0.16)**	12.85 (0.31)	11.88 (0.31)	12.98 (0.14)
2	16	20:2ω6,9c	94.2	0.0002	0.06 (0.03)	**3.06 (0.33)**	0.13 (0.10)	0 (-)
		20:4ω6,9,12,15c	41.8	0.0002	1.57 (0.07)	**3.19 (0.33)**	1.52 (0.08)	1.36 (0.07)
		17:0	39.3	0.0002	0.74 (0.02)	**1.31 (0.11)**	0.63 (0.03)	0.65 (0.04)
		18:0	31.9	0.0002	2.71 (0.04)	**3.77 (0.18)**	2.62 (0.06)	2.73 (0.09)
3	15	20:1ω7c	52.1	0.0002	0.02 (0.01)	0 (-)	**0.15 (0.02)**	0.06 (0.02)
		16:1 2OH	37.6	0.001	0.16 (0.02)	0.17 (0.05)	**0.39 (0.05)**	0.25 (0.03)
		a17:1ω9c	37.4	0.0006	0.12 (0.03)	0.07 (0.05)	**0.50 (0.11)**	0.11 (0.04)

Community Type	N	Variable	Indicator value	P	Mean (± S.E.) for Community Type Type 1	Type 2	Type 3	Type 4
		i15:1	35.5	0.0002	0.38 (0.02)	0.39 (0.03)	**0.73 (0.08)**	0.42 (0.04)
		i17:0	31.7	0.0002	0.63 (0.01)	0.69 (0.03)	**0.94 (0.02)**	0.69 (0.01)
		18:1ω5c	30.8	0.001	0.89 (0.06)	1.33 (0.06)	**1.55 (0.06)**	1.26 (0.08)
		16:1ω11c	30.7	0.0002	0.78 (0.03)	0.92 (0.04)	**1.15 (0.04)**	0.90 (0.05)
		i14:0	30.5	0.0004	0.31 (0.01)	0.33 (0.01)	**0.42 (0.03)**	0.32 (0.02)
		16:1ω5c	30.5	0.0002	0.89 (0.06)	1.33 (0.12)	**1.55 (0.08)**	1.26 (0.09)
		10me18:0	30.2	0.01	0.61 (0.03)	0.55 (0.05)	**0.79 (0.08)**	0.66 (0.05)
		11me18:1ω7c	29.8	0.0002	0.70 (0.02)	0.76 (0.04)	**0.94 (0.04)**	0.75 (0.03)
		18:1ω7c	29.6	0.0002	8.18 (0.21)	10.71 (0.24)	**11.74 (0.19)**	8.97 (0.23)
		a15:0	28.5	0.0002	2.25 (0.05)	2.23 (0.07)	**2.74 (0.06)**	2.37 (0.07)
		i16:1	28.3	0.003	0.74 (0.02)	0.70 (0.03)	**0.88 (0.03)**	0.78 (0.04)
		10me16:0	28.2	0.002	3.51 (0.09)	3.16 (0.13)	**4.18 (0.15)**	3.97 (0.14)
		17:0c	28.1	0.0002	1.89 (0.04)	1.83 (0.07)	**2.21 (0.07)**	1.92 (0.05)
		19:0c	27.5	0.02	6.01 (0.17)	5.67 (0.21)	**6.78 (0.45)**	6.24 (0.19)
		i15:0	27.1	0.003	5.35 (0.12)	4.95 (0.16)	**5.75 (0.18)**	5.12 (0.10)
		a17:0	27	0.003	1.00 (0.02)	0.95 (0.01)	**1.10 (0.04)**	1.02 (0.02)
		16:0,2OH	25.9	0.001	0.01 (0.01)	0 (-)	**0.05 (0.02)**	0.01 (0.01)
4	23	17:1ω7c	49.6	0.0002	0.44 (0.11)	0.71 (0.21)	0.82 (0.22)	**1.93 (0.11)**
		15:1ω8c	38.6	0.0002	1.41 (0.09)	1.34 (0.11)	1.33 (0.17)	**2.56 (0.25)**
		20:0	33.2	0.0002	2.52 (0.08)	2.45 (0.16)	2.44 (0.11)	**3.69 (0.18)**
		14:0	27.7	0.01	1.51 (0.05)	1.47 (0.05)	1.51 (0.05)	**1.72 (0.05)**
b) MSIR Community Type								
1	36	Oxalic acid	27.8	0.01	**0.086 (0.002)**	0.074 (0.001)	0.083 (0.002)	0.067 (0.003)
		Glutamic acid	26.1	0.04	**0.076 (0.001)**	0.072 (0.001)	0.071 (0.002)	0.071 (0.001)
2	40	L-alanine	27.0	0.02	0.056 (0.011)	**0.066 (0.001)**	0.062 (0.001)	0.059 (0.004)
3	17	Citric acid	28.6	0.001	0.065 (0.002)	0.067 (0.001)	**0.078 (0.002)**	0.062 (0.003)
4	6	Malic acid	30.2	0.0002	0.088 (0.002)	0.088 (0.002)	0.099 (0.002)	**0.119 (0.003)**
		Glucose	26.8	0.008	0.078 (0.001)	0.076 (0.001)	0.074 (0.001)	**0.084 (0.003)**

Table 3-3. Results of distance-based redundancy analyses (dbRDAs) for the a) PLFA and b) MSIR microbial communities. The trace value (sum of all the canonical eigenvalues) and the eigenvalues of the first four axes are presented, along with the species-environment correlations, and the cumulative percentage of the variance explained for PLFAs/MSIRs and PLFA/MSIR-environment. Inter-set correlations (Pearson) of significant above- and below-ground variables from the db-RDA step-wise forward selections are listed (see Table 3-4 for description of variables), ordered by their correlations (from high to low) with the first axis. The inter-set correlation values for the axis where the correlation was strongest are highlighted in bold.

	Axis 1	Axis 2	Axis 3	Axis 4
a) PLFA Community				
Trace: 0.234				
Eigenvalues[i]	0.125	0.042	0.028	0.011
Species-environment correlations	0.730	0.569	0.542	0.544
Cumulative percentage variance				
PLFA variance explained (%)	13.4	18.0	21.0	22.2
PLFA-environment correlation variance (%)	53.4	71.6	83.6	88.3
Inter-set correlations				
PLSC	**-0.42**	0.37	0.02	0.21
Al	**-0.37**	-0.08	0.08	0.29
CHAN	**0.37**	-0.19	0.15	0.22
POCO	**0.36**	0.04	0.23	0.03
LYAN	**0.28**	0.19	-0.09	-0.11
CAMO	**0.21**	0.10	0.00	0.14
COCA	0.13	-0.17	**-0.30**	0.18
Canopy cover	-0.07	-0.07	**0.37**	-0.10
ROAC	-0.07	**-0.16**	0.11	-0.04
b) MSIR Community				
Trace: 0.065				n/a
Eigenvalues[i]	0.042	0.015	0.008	n/a
Species-environment correlations	0.602	0.464	0.398	n/a
Cumulative percentage variance				
MSIR variance explained (%)	4.3	5.9	6.7	n/a
MSIR-environment correlation variance (%)	64.6	87.9	100.0	n/a
Inter-set correlations				
Litter cover	**-0.39**	0.35	0.05	n/a
VAVI	**0.39**	0.16	0.27	
DWM - Class 4	-0.26	-0.24	**0.30**	

[i] Axis 1 and all combined axes were significant at P=0.02.

Table 3-4. The mean values (± SE) for each of the four a) PLFA and b) MSIR community types of the above- and below-ground variables that were significant in the distance-based redundancy analyses (db-RDAs) described in Table 3-3. Different lower case letters (a, b) after mean values indicate significant differences for individual variables among a) PLFA or b) MSIR community types.

			Mean (± S.E.)			
Variable	Code	Units	Type 1	Type 2	Type 3	Type 4
a) PLFA Community						
Pleurozium schreberi[ii]	PLSC	%	29.4 (4.0)b	14.1 (4.4)ab	1.9 (0.6)a	30.8 (5.4)b
Aluminum	Al	µg -10 cm^2 – burial $length^{-1}$	74.1 (2.3)	64.8 (3.8)	64.0 (2.9)	65.4 (3.3)
Chamerion angustifolium [i]	CHAN	%	2.5 (0.8)a	8.3 (2.7)b	14.9 (3.6)b	6.5 (1.5)ab
Polytrichum commune[i]	POCO	%	2.0 (0.9)a	3.0 (1.4)a	13.1 (5.3)b	5.5 (2.1)ab
Lycopodium annotinum[i]	LYAN	%	0.3 (0.1)a	7.5 (3.2)b	9.3 (3.1)b	6.7 (3.2)ab
Calamagrostis montanensis	CAMO	%	5.2 (1.4)	6.9 (4.7)	13.0 (4.1)	5.4 (2.4)
Cornus canadensis[i]	COCA	%	10.9 (1.2)a	19.1 (4.0)b	9.8 (1.8)ab	9.3 (1.3)a
Canopy cover	canopy	%	63.9 (0.8)b	58.5 (1.2)a	62.5 (2.3)ab	59.8 (0.6)ab
Rosa acicularis	ROAC	%	4.9 (0.8)	2.9 (0.9)	6.3 (1.1)	4.3 (1.1)
b) MSIR Community						
Litter cover[ii]	litter	%	49 (3)ab	57 (3)b	41 (5)a	34 (4)ab
Vaccinium vitis-idaea	VAVI	%	2.5 (0.7)a	3.1 (1.1)a	5.2 (2.3)ab	14 (7.1)b
Downed woody material – size class 4	DWM - Class 4	Mg ha^{-1}	1.9 (0.4)	1.7 (0.4)	2.2 (0.7)	2.0 (0.7)

[i] Square root-transformed for analysis

[ii] Log-transformed for analysis.

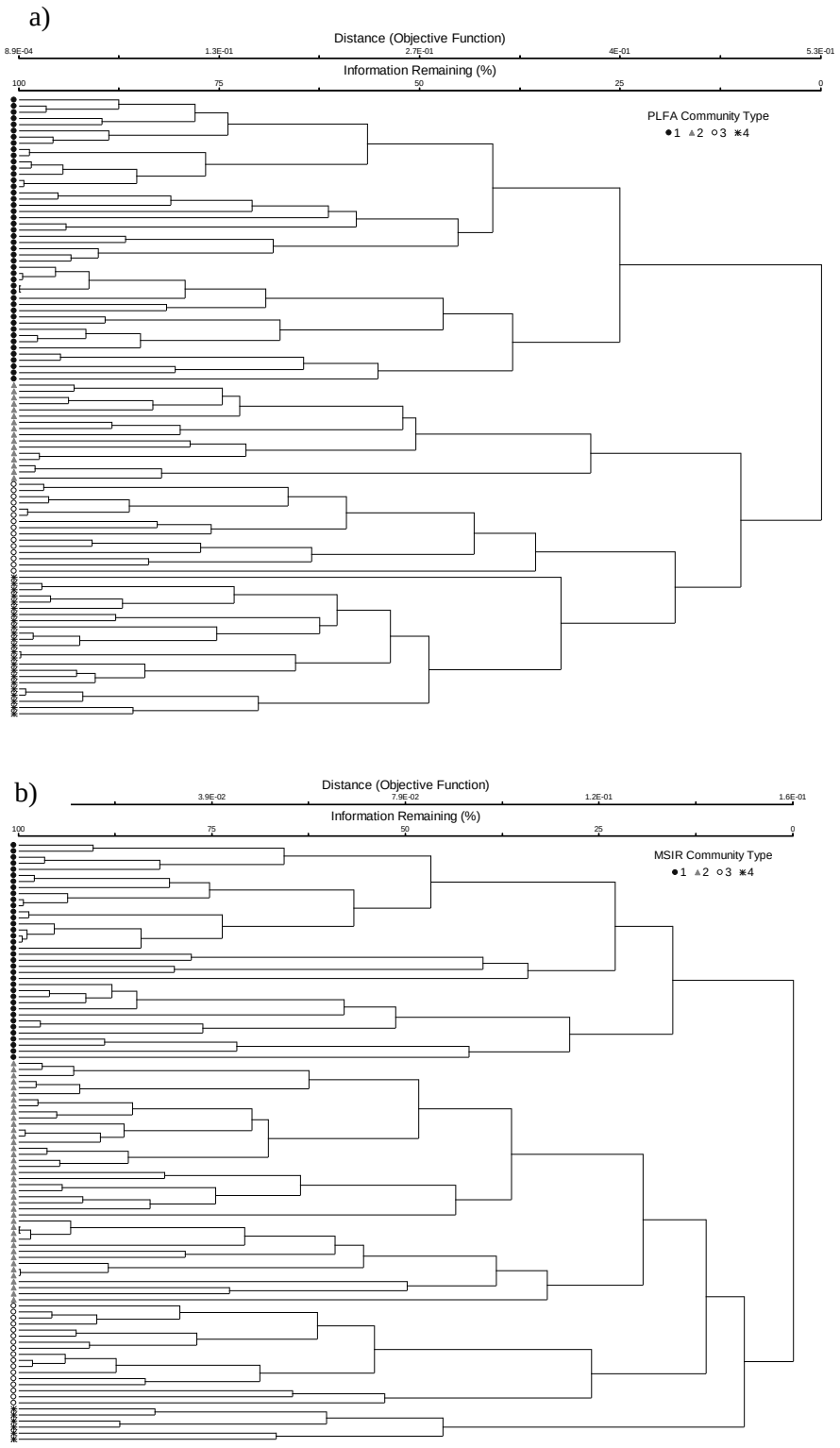

Fig. 3-1. Dendrogram of hierarchical agglomerative cluster analysis of the microbial a) PLFAs and b) MSIRs showing the four community types for each. Cluster analysis used a flexible beta linkage method with β= -0.25 and Sørenson's distance measure. Chaining =1.6% for PLFA and 2.4% for MSIR microbial communities.

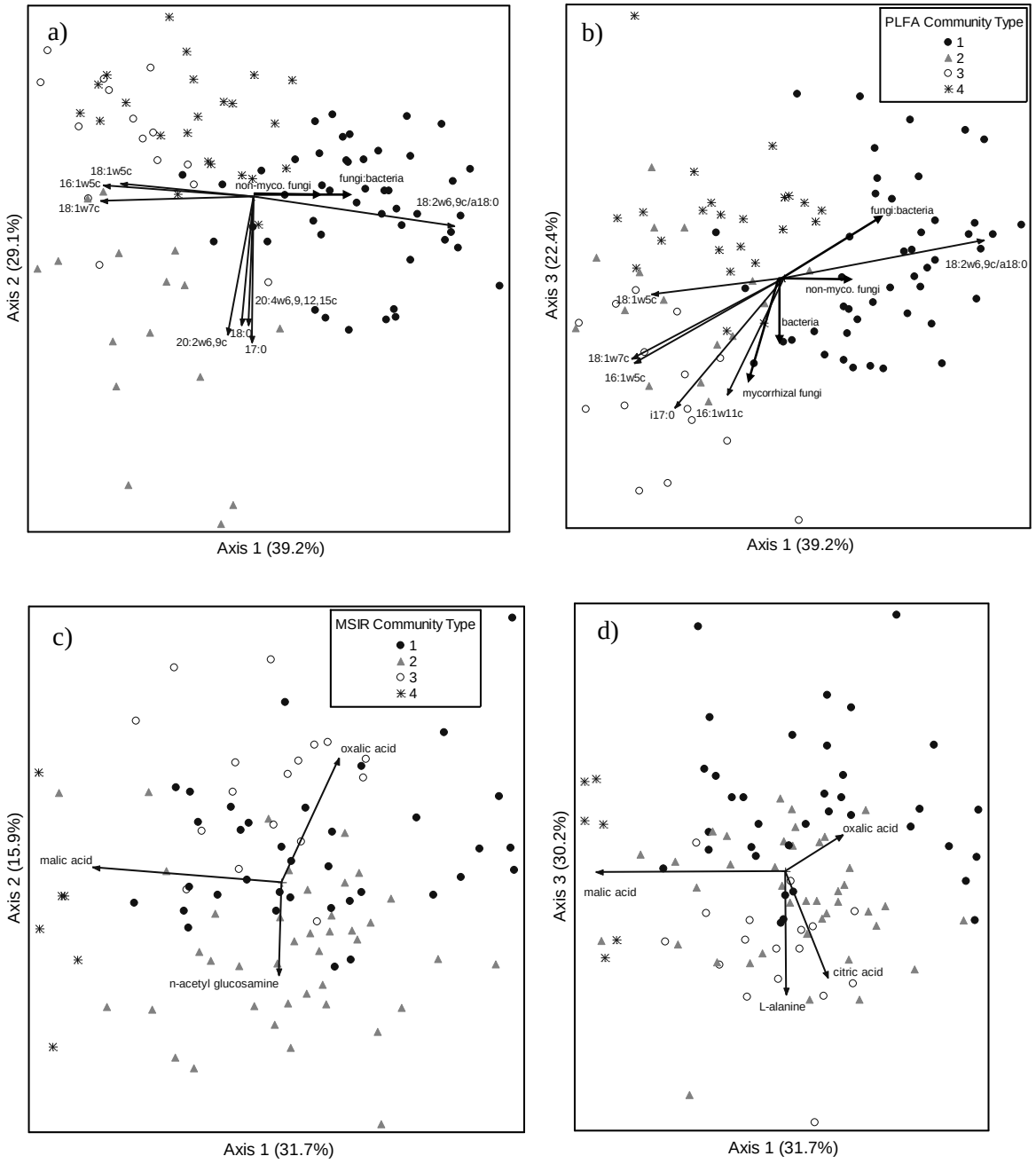

Fig. 3-2. Results of nonmetric multidimensional scaling ordination of a & b) PLFA and c & d) MSIR community composition. The final ordinations were 3-D solutions, so two plots are presented for each ordination. Each symbol in the plots is a subplot, which is coded by PLFA (panels a and b) or MSIR community type (panels c and d) (see Fig. 3-1 and Table 3-2). The amount of variation explained by each axis is included in parentheses. The angles and lengths of the vectors for the individual PLFAs, carbon substrates, and environmental variables (including PLFA biomarker groups) overlain on the ordination vectors indicate direction and strength of associations of them with the ordination axes. The cut-offs for display were: for PLFAs $R^2 > 0.5$ (to improve readability because so many individual PLFAs were highly correlated and obscured interpretation); for carbon substrates and environmental variables $R^2 > 0.25$. No environmental variables had an $R^2 > 0.25$ with the axes of the MSIR ordination.

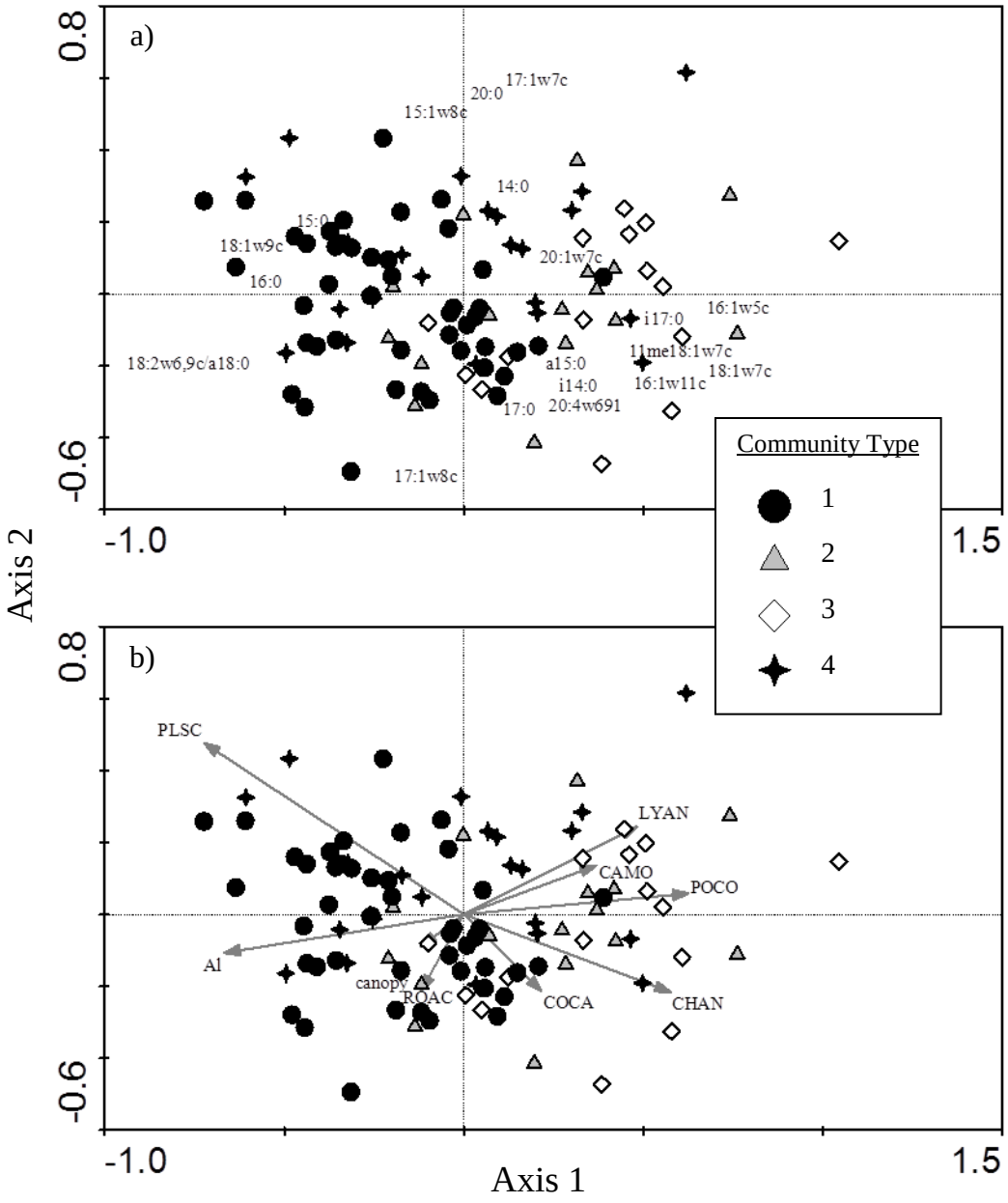

Fig. 3-3. Results of distance-based redundancy analysis of PLFA microbial community composition delineated by the four PLFA community types identified by hierarchical cluster analysis: a) PLFA names indicate the locations of PLFAs which had a Pearson correlation coefficient > 0.4 with either of the first two axes, and b) the direction and length of the vector for environmental variables (description in lowercase letters, see Table 3-4 for details) reflects the direction and strength of correlation for variables that had a Pearson correlation coefficient > 0.4 with either of the first two axes. Each symbol is a subplot, which is coded by PLFA community type (see Fig. 3-2a). The environmental and species scores were scaled up 1.8 and 3.7 times, respectively, to those of sample scores to improve readability.

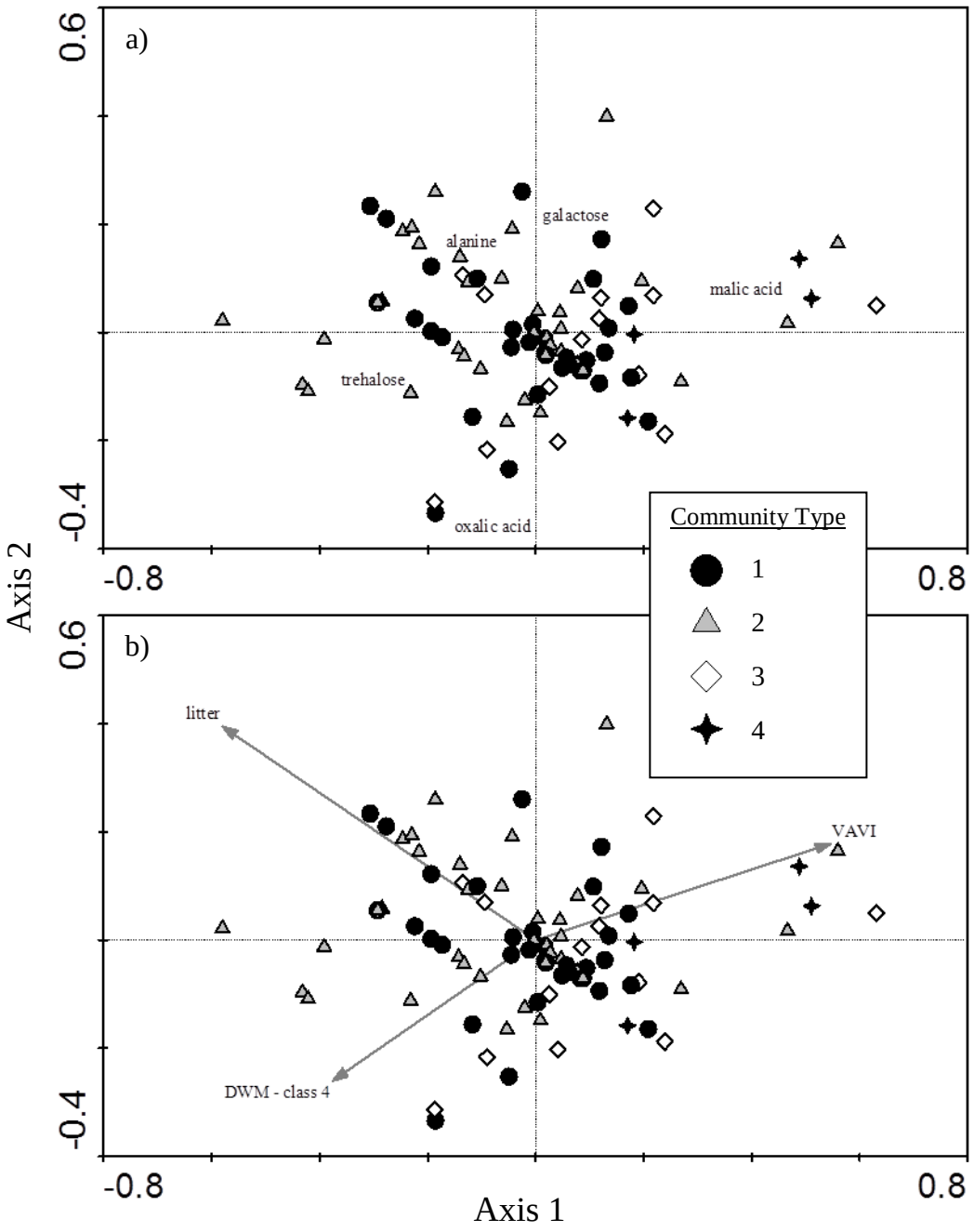

Fig. 3-4. Results of distance-based redundancy analysis of MSIR microbial community composition delineated by the four MSIR community types identified by hierarchical cluster analysis: a) carbon substrate names indicate the locations of carbon substrates which had a Pearson correlation coefficient > 0.4 with either of the first two axes, and b) the direction and length of the vector for environmental variables (description in lowercase letters, see Table 3-4 for details) reflects the direction and strength of correlation for variables that had a Pearson correlation coefficient > 0.4 for either of the first two axes. Each symbol is a subplot, which is coded by MSIR community type (see Fig. 3-2b). The environmental and species scores were scaled up 1.5 and 4.4 times, respectively, to those of sample scores to improve readability.

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3.6. Appendix 3-I. List of phospholipid fatty acids used in this study and the biomarker group (actinomycete, bacteria, fungi, mycorrhizae) they were assigned to (if applicable).

PLFA	Biomarker	PLFA	Biomarker
10:0 2OH	-	i17:0	bacteria
10:0 3OH	bacteria	a17:0	bacteria
12:00	bacteria	17:1 ω8c	-
i13:0	-	17:1 ω7c	-
12:0 2OH	bacteria	17:0 cyclo	bacteria
12:1 3OH	-	17:0	bacteria
12:0 3OH	bacteria	16:1 2OH	-
i14:0	bacteria	16:0 2OH	-
14:1ω5c	-	10me17:0	actinomycete
14:0	bacteria	16:0 3OH	-
15:1 ISO G	-	18:3 ω6c (6,9,12)	fungi
i15:0	bacteria	18:1 ω9c	fungi
a15:0	bacteria	18:1 ω7c	bacteria
15:1 ω8c	-	18:1 ω5c	bacteria
15:0	bacteria	18:2ω6,9c	-
14:0 ISO 3OH	-	a18:0	-
16:1 ISO G	-	18:0	-
i16:0	bacteria	11me18:1 ω7c	-
a16:0	-	10me18:0	actinomycete
16:1 ω11c	-	a19:0	-
16:1 ω5c	mycorrhizae	19:0cycloω8c	-
16:00	-	18:1 2OH	-
15:0 ISO 3OH	-	20:4 ω6,9,12,15c	-
15:0 2OH	-	20:2 ω6,9c	-
10me16:0	actinomycete	20:1 ω9c	fungi
15:0 3OH	-	20:1 ω7c	-
a17:1 ω9c	-	20:00	-

Chapter 4. Mountain pine beetle moves east: short-term resistance of above- and below-ground properties and processes to simulated mountain pine beetle attack in a novel landscape

Abstract **-**Natural forest disturbance regimes are changing, as evidenced by the expansion of the mountain pine beetle (MPB) north and east from British Columbia (BC) into pine forests east of the Canadian Rockies. Thus, research that examines the potential impacts of shifting disturbance regimes on ecosystem properties and processes in these forests is needed. We examined the short-term effects (up to one year after treatment) of four treatments that emulated MPB attack and associated forest management disturbance (i.e., control, moderate intensity MPB attack, high intensity MPB attack, and salvage harvest) on above- and below-ground properties and processes of mature lodgepole pine forests in MPB's recently expanded range east of the Rockies. While the salvage logging treatment showed dramatic effects on the understory plant community and downed woody material with several less dramatic below-ground responses, there were no effects of the moderate MPB attack, and only limited below-ground responses to the high intensity simulated MPB attack. The salvage logged stands had decreased species richness and understory plant cover, increases in small downed wood, litter cover, forest floor pH, and below-ground available soil Ca, Mg, and P, and differences in multiple microbial properties compared with the other treatments. The high intensity simulated MPB attack stands showed some differences in microbial communities - increased respiration rates for three carboxylic acids and one amino acid compared with the salvage treatment. There was considerable variation among years for many below-ground variables (e.g., soil nutrients Al, B, Fe, Mn, NH_4-N, S, Zn, microbial multiple carbon source respiration rates, microbial phospholipid fatty acid (PLFA) biomass), and these were unrelated to treatments. For the majority of below-ground response variables we measured, the differences among study years rather than differences due to the MPB treatments suggest that inter-annual variability in properties exerts a stronger influence than does disturbance effects of MPB attack in the short term. The lack of response to MPB attack in the short-term suggests these forests are resistant to change early after attack, and/or have high ecological inertia. In contrast, salvage logging had immediate and dramatic effects, as expected, indicating lower ecological inertia. We don't yet know how these pine forests will develop under this new disturbance regime of partial canopy disturbance, but it appears likely that salvage logging will push these stands in a potentially very different direction than the modified natural disturbance regime will.

4.1. Introduction

Disturbance, both natural and anthropogenic, is an important ecological driver of successional change in forests. It influences the composition, abundance, and distribution of vegetation by altering the physical environment and the temporal and spatial

distribution of resources (White and Pickett 1985). When disturbance reduces live plant biomass, surviving individuals can use the released resources (i.e., light, space, soil moisture, nutrients) and this will potentially alter the species composition of the remaining forest (Canham and Marks 1985). Depending on the intensity and frequency of disturbance, the structure of the forest, including the distribution of snags and downed woody material, will be altered to varying degrees (e.g., Tinker and Knight 2000, Page and Jenkins 2007). Following disturbance, changes in the below-ground soil nutrient availability (e.g., Thiffault et al. 2007) and microbial communities (e.g., Siira-Pietikäinen et al. 2001, Lindo and Visser 2003, Chatterjee et al. 2009), as well as losses of nutrients from the forests (Vitousek and Melillo 1979) can occur. However, studies have also shown resilience of microbial community structure and function to disturbance (e.g., Hannam et al. 2006), or that topographic position and elevation exert stronger influences than species- or disturbance-related effects on below-ground properties and processes (e.g., Swallow et al. 2009).

Natural disturbance regimes are rapidly changing, and there is a need for ecologists to better understand and anticipate the potential impacts of changing disturbance regimes on ecosystems and associated properties and processes (Turner 2010). While large lightning-caused wildfires have been the predominant natural disturbance throughout much of the boreal and near-boreal forest (e.g., Weber and Flannigan 1997), fire suppression and climate change are leading to increasing importance of other disturbance agents, including insects, disease, and drought (e.g., Ayres and Lombardero 2000, Logan et al. 2003, Soja et al. 2007). For example, the native mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) is a dominant disturbance agent over large areas of lodgepole pine (*Pinus contorta* Douglas ex Loud. var. *latifolia* Engelm.) forests in British Columbia (BC) (Nealis and Peter 2008), and with recent range expansion east across the Rockies

MPB is becoming an increasingly important disturbance agent in pine forests which were previously rarely or never exposed to it. MPB is considered the most destructive forest insect in western North America, with a recent epidemic in BC resulting in mortality of 710 million cubic meters of wood over a cumulative affected area of ~ 18.1 million hectares; the area and impacts of MPB continue to grow (Safranyik et al. 2010, BC 2012). The current MPB infestation in BC is an order of magnitude larger in area and severity than all previously recorded outbreaks (Taylor et al. 2006, Safranyik et al. 2010). As a result of this unprecedented MPB outbreak, western Canadian pine forests formerly functioning as carbon sinks are now functioning as large net carbon sources, and this trend is expected to continue into the foreseeable future (Kurz et al. 2008).

Climate has historically limited the potential for MPB to expand north and east into suitable pine habitat in western Canada (Safranyik 1978, Carroll et al. 2004) but in the last few years, formerly climatically harsh environments have become more favorable, allowing significant expansion of MPB's historical range (Carroll et al. 2004, Nealis and Peter 2008). The availability and connectivity of suitable pine habitat has also contributed to MPB range expansion (Taylor and Carroll 2003). In 2005 MPB moved north and east across the Rocky Mountains and quickly spread through extensive stands of lodgepole pine in Alberta; attack in the novel host jack pine (*Pinus banksiana* Lamb.) has now also been confirmed (Cullingham et al. 2011). While the future course of the MPB attack east of the Canadian Rockies across the boreal is not yet known, MPB is likely to remain east of the Canadian Rockies (Schneider et al. 2010), thus modifying the historic disturbance regimes for lodgepole pine and potentially other boreal pine forests (Nealis and Peter 2008, Coops et al. 2012).

The range expansion of MPB into novel habitats is expected to have large impacts on forest structure, plant community composition, forest floor and below-ground properties and processes, forest regeneration and the future successional trajectory of

stands. MPB differs from other types of disturbance including wildfire, windthrow, and timber harvest because it directly affects the overstory, but with no direct impacts on the understory or soil (Burton 2008). Edburg et al. (2012) suggested that, because of the legacy of living plant material, MPB attacked forests may recover net ecosystem productivity more quickly than after wildfire disturbance. Attacked trees are expected to lose their needles beginning in the second year post-attack, and lose most of their foliage between the second and third years post-attack when the stands transition from the red attack to grey attack stage (Chojnacky et al. 2000); minimal change in the quantity of light transmitted to the forest floor prior to this needle loss is expected. Instead, in the early red attack phase the major abiotic change in MPB-attacked stands is the stand hydrology, with an overall increase in water availability after attack (Knight et al. 1991, Schnorbus 2011). This hydrological change may in turn modify other abiotic and biotic components of the ecosystem, including the understory vegetation and below-ground properties and processes. A conceptual model of ecosystem biogeophysical and biogeochemical responses to MPB attack proposed that in the red attack stage increases in soil moisture would result in increased availability of soil nutrients, in turn influencing growth of surviving trees and the understory in the grey attack stage (Edburg et al. 2012). With the expansion of MPB range, Alberta has implemented a MPB management strategy that includes salvage harvest after MPB attack (ASRD 2007), but the potential impacts of this management practice on forest ecosystem properties and processes in the long run are also unknown.

To our knowledge, no previous studies have addressed the potential effects of MPB on both above- and below-ground ecosystem properties and processes in MPB's expanded range. We need to better understand the potential impacts of the shift in disturbance regime from a fire-dominated regime that dramatically reorganizes the ecosystem structure, to a regime that also includes MPB disturbance that kills trees

without immediate direct impacts to downed woody material, understory vegetation, or below-ground ecosystem components. The main objectives of this study were to examine the effects of three different treatments that emulated mountain pine beetle attack and associated forest management disturbance (i.e., moderate intensity MPB attack, high intensity MPB attack, and salvage harvest) on i) above- and ii) below-ground properties and processes of mature lodgepole pine forests in western Alberta. Given the direct disturbance to the forest floor, we expected immediate significant changes in both above- and below-ground properties to occur in the salvage logged stands. However, we hypothesized that below-ground properties and processes would respond prior to above-ground properties in MPB-attacked stands that experienced canopy mortality without concurrent ground disturbance.

4.2. Methods

Study area

The study area was located in the Upper Foothills natural sub-region of Alberta (Natural Regions Committee 2006) in lodgepole pine forests near Robb, AB. This area is characterized by monoculture serotinous lodgepole pine forests, along with mixed conifer stands of white spruce (*Picea glauca* (Moench) Voss) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). Stand ages in this region are generally younger than 100-120 years old reflecting the regional disturbance regime of relatively frequent stand-initiating wildfire (Beckingham et al. 1996). The climate is temperate continental with mean daily maximum air temperatures during the growing season ranging from a daily maximum of 16.2 ^{0}C in May, to 20.6 ^{o}C in August. Mean monthly precipitation during the growing season is as follows: 57.9 mm (May), 106.7 mm (June), 106.2 mm (July), and 82.2 mm (August), with a mean annual precipitation of 562.4 mm (30 year climate normal 1971-2000). The study stands were approximately 110-120 years old and were located on

brunisolic gray luvisol soils. The study area was classified as ecosite UF e1.1 – Pl/green alder/feather moss (Beckingham et al. 1996). The overstory included only lodgepole pine; there were very few white and black (*Picea mariana* (Mill.)) spruce, trembling aspen (*Populus tremuloides* Michx.), and balsam fir (*Abies balsamea* (L.) Mill) in the lower canopy. Notably, advance regeneration was absent or present in very low numbers (i.e., < 10 seedlings or saplings – ha^{-1} – see Chapter 5). The understory was dominated by feather mosses, including *Pleurozium schreberi* (Brid.) Mitt., *Ptilium crista-castrensis* (Hedw.) De Not., and *Hylocomium splendens* (Hew.) Schimp., and the hair cap moss *Polytrichum commune* Hedw.. Common forbs included *Cornus canadensis* L. and *Linnea borealis* L., common small shrubs included *Rosa acicularis* Lindl. and *Vaccinium myrtilloides* Michx.; *Alnus crispa* (Aiton) Pursh was the dominant tall shrub, and the most common graminoid was *Calamagrostis montanensis* (Michx) Beauv.. Because of the salvage harvested treatments, we selected stands reasonably close to general operating areas already scheduled for harvest in late winter or spring of 2009 in West Fraser Timber Company's forest management area spatial harvest sequence.

Experimental design

This study used a before-after control-impact (Green 1979; Stewart-Oaten et al. 1986, 1992) randomized block design that was carried out over three years. There were three blocks in the study, which ranged in size from 4.8 – 8.8 ha (Table 4-1). Each block contained four experimental units (0.48 ha each), to which the treatments were applied in year two of the study. The four treatments were: i) untreated control (hereafter "Control"), ii) simulated moderate intensity MPB attack (hereafter "50% kill"), iii) simulated high intensity MPB attack (hereafter "100% kill"), and iv) simulated salvage harvested, which were clear-cut harvested to simulate a typical management treatment post MPB attack (hereafter "Salvage"). While MPB selectively kills old and stressed trees

during endemic phases of attack, our research was focused on epidemic levels of MPB, which are currently occurring at unprecedented levels on the landscape. Therefore, we selected targets of 50% kill and 100% kill to capture a gradient of mortality. Experimental units were relatively flat and covered by fairly homogenous mature lodgepole pine forest representative of the dominant forest cover type in this region that is susceptible to MPB attack. Treatments were randomly assigned to the experimental units within blocks, with the restriction that the salvage harvest unit had to be nearest the road to decrease impacts of forest harvest practices on remaining experimental units. The experimental units in all three blocks were 0.48 ha (60-m x 80-m). All experimental units were surrounded by a 20-m (~ one tree height) treated buffer to ensure hydro-climatic uniformity within them. Within each experimental unit we established nine systematically-located nested sample points that were used as the sampling points for measuring the overstory, downed wood, understory, and below-ground (n=3 blocks * 4 experimental units * 9 sample points = 108 sampling points). Sample points were located a minimum of 10-m from the edge of the experimental unit and 20-30 m apart from one another to minimize spatial auto-correlation and were treated as sub-samples in statistical analyses.

Application of treatments

A chemical (glyphosate) was used to kill the trees to simulate MPB attack for the 50% and 100% kill treatments. Glyphosate is a systemic herbicide that kills vegetation by inhibiting the enzyme 5-enolpyruvylshikimate 3-phosphate synthase involved in the synthesis of aromatic amino acids; it rapidly reacts with and is inactivated by most soils (Baylis 2000). When applied at the recommended rate, glyphosate has benign effects on microbial community structure (Ratcliff et al. 2006). EZ-Ject selective injection herbicide capsules (Glyphosate 0.15 grams per capsule, ArborSystems, Omaha, NE

http://www.ezject.com/) were injected at a rate of 1 capsule per 5 cm tree diameter at breast height (dbh) per tree for trees 10– 20 cm dbh, or 1 capsule per 3cm dbh per tree for trees > 20 cm, with capsules equally spaced around the circumference of the tree near the base of the bole. In the 100% kill experimental units, all trees $\geq$ 10 cm dbh (selected as minimum size of trees attacked by MPB - Safranyik and Carroll 2006) were injected. In 50% kill stands, because of possible root-to-root transfer of glyphosate among neighboring trees (M. Mihajlovich, personal communication), every 3rd tree $\geq$ 10 cm dbh was injected with glyphosate to achieve the desired rate of 50% overstory mortality. Chemical girdling was completed in the treatment year from June 15-19, 2009.

Clear-cut 'salvage' harvest operations used a "stump-side processing system" in which a feller-processor unit de-limbed trees at the stump, leaving debris and cones distributed onsite to facilitate regeneration (trees were not herbicided to mimic the effects of MPB prior to being harvested). Harvest operations were completed by West Fraser Timber Company between late July and early August in the treatment year (2009). No site preparation, e.g., scarification or burning, was applied to any of the harvest areas and vegetation was allowed to regenerate naturally for the duration of the study.

Data collection

Data were collected for the growing seasons of three consecutive years in this study unless otherwise noted below: i) pre-treatment year (summer 2008), ii) treatment year (summer 2009), and iii) post-treatment year (summer 2010) at each of the nine sampling points per experimental unit.

The overstory plant community was sampled in 8-m fixed-radius (0.02 ha) circular plots in the pre-treatment year. Standard forest mensuration data were collected for all trees (i.e., with dbh $\geq$ 5 cm and ht > 1.3 m) within each plot (i.e., dbh, height (for a subset of n=2 trees/canopy layer – lower canopy, mid canopy, and upper canopy),

live/dead status, crown vigor (healthy=minimal red needles, moderate=intermediate levels of red needles, poor=mostly/all needles red), and all stems were tagged (except in the stands assigned to be clearcut) to allow for repeated measures at the individual tree level. For a subset of stems (~10) within each circular plot (except for the salvage logged stands) the openness of serotinous cones visible within the canopy was classified (1=all cones open, 2= some cones open, 3=all cones closed, 4=no cones present). Basal area, stem density, and diameter distribution of the stems by live/dead status were calculated. In the post-treatment year tree live/dead status, crown vigor (healthy = no red needles present, moderate = intermediate levels of red needles, and poor = all needles red) and openness of cones were re-assessed. Crown vigor data were used to assess post-treatment survival and mortality rates among the experimental units, quantifying basal area by crown vigor class.

To estimate canopy cover, hemispherical photographs (digital Nikon Coolpix 4500 with FC-E8 fisheye lens) were taken in the middle of each growing season (mid July), with the camera leveled on a tripod ~1.4 m above the forest floor and the bottom of the camera oriented towards North. We analyzed canopy photographs using SLIM (Spot Light Intercept Model v. 3.01), using batch processing to analyze photos with manual color threshold adjustments by experimental unit and year to optimize differences between canopy and sky. The program calculates gap fraction, which measures the area of overhead view (in percent) which constitutes canopy gaps, and we subtracted gap fraction from 100 to provide an estimate of canopy cover at each sample point.

Downed woody material (DWM) was measured using the line intersect method (Brown 1974; Brown et al. 1982; Van Wagner 1968, 1982). Line transects ran from each sampling point out 8 m in a randomly selected direction and the same transects were sampled for three years. The diameter of each DWM piece at the point of intersection with the line transect was measured using calipers and categorized into diameter size

classes as follows: 0-0.5 cm, 0.5-1.0 cm, 1-3 cm, 3-5 cm, 5-7 cm, and > 7 cm (as adopted by the Canadian Forest Service; McRae et al. 1979, Van Wagner 1982). Pieces in diameter size classes 0-0.5, 0.5-1, and 1-3 cm were counted along the first 2 m length of each transect, size classes 3-5 and 5-7 cm along the first 4 m length of each transect, and for all pieces $\geq$ 7 cm we recorded diameter, length, and decay class (i.e., 1-5, based on Table 8.1 in VRI 2007) along the full 8 m. Biomass of DWM (Mg ha^{-1}) for each of the size classes was calculated using the equation and coefficients for Central Alberta foothills lodgepole pine stands (Delisle and Woodard 1988, Nalder et al. 1997). For the large pieces ($\geq$ 7 cm diameter) we also calculated the biomass of sound (i.e., decay classes 1 and 2) and rotten (i.e., decay classes 3-5) wood separately. We calculated the total biomass of DWM by summing the biomass for all size classes. Percent cover of DWM was estimated during assessment of understory communities (see below).

Visual estimates of percent cover to species were made within 1-m x 1-m quadrats for forest floor mosses, forest floor lichens, forbs, graminoids, and small shrubs (see Appendix4- I for detailed list). Nomenclature follows the USDA Plants Database (http://plants.usda.gov/). Cover estimates were also recorded for litter, tree/snag boles, downed woody material (diameter $\geq$ 3 cm), exposed mineral soil, and rock. The thickness of the forest floor (Fibric/Humic layers – i.e., F/H, mm) was measured in each of the four corners of each understory vegetation quadrat. We calculated understory species richness and diversity (i.e., Shannon Index, Magurran 1988) per quadrat. We measured tall shrubs/saplings (i.e., taller than 1.3 m ht but with dbh < 5 cm, e.g., *Alnus crispa*) in 4-m radius circular plots; to estimate their abundance we measured stem basal diameters for shrubs and saplings rooted within the plot and for shrubs that had canopy overhanging the plot. With interest in quantifying the total biodiversity of the experimental units, we also conducted a census at the peak of the growing season during which two people surveyed

each experimental unit for the presence of understory species that had not been found in any of the quadrats within that unit.

Distributed soil moisture measurements using time-domain reflectometry (TDR) were collected at three sampling depths (0-20, 0-40, and 0-60 cm below the mineral soil surface) at two randomly selected sampling points in each experimental unit, with measurements recorded approximately one to two times a month throughout the growing season (mid-June to mid-September). Volumetric soil water content using the empirical relationship for mineral soils proposed by Topp et al. (1980) was calculated and then converted to moisture depth (mm) for each of the three sampling depths. Gross precipitation (mm) was measured in a nearby clear-cut throughout the year using a universal precipitation gauge and a Hobo datalogger (Hobo Event loggers and U12-008, Onset Computer Corporation, MA, USA).

We installed Plant Root Simulator (PRS™) probe ion exchange membranes (Western Ag Innovations, Inc., Saskatoon, SK, Canada) to measure soil nutrient availability. The anion exchange PRS™-probes simultaneously adsorbed all nutrient anions, including NO_3^-, PO_4^{3-}, and SO_4^{2-}. Cation exchange PRS™-probes simultaneously adsorbed nutrient cations such as B^+, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+}. We installed four pairs (pair = 1 cation and 1 anion exchange membrane) of PRS probes vertically at the four corners of each understory quadrat at the interface between the forest floor and mineral soil. Probes were installed for the duration of the growing season each year (mid-June to mid-September), and at the end of the season they were cleaned and shipped to Western Ag for analysis of nutrient supply rates (μg -10 cm^2 -burial $length^{-1}$); the four probe pairs from individual quadrats were pooled prior to elution and analysis. The few samples for which measured values were below the minimum detection limits were still included in analysis, because censoring data below MDL can bias your dataset (Western Ag personal communication). We did not analyze the data for several elements

because their calculated nutrient supply rates were predominately below the minimum detection limits [Cd (n=227/324<MDL), Cu (n=260/325 <MDL), NO_3^- (n=175/324 <MDL), and Pb (n=142/324 <MDL).

To measure rates of decomposition, nylon mesh bags (1.5 mm x 1.5 mm mesh size) with four 90-mm diameter Whatman cellulose filter papers were buried at the forest floor – mineral soil interface for the growing season each year (same time span as PRS probes listed above). Filter papers were oven dried for 1 day (pre-burial) and 3 days (post-burial) at 70°C and weighed before and after being buried. Decomposition rate was calculated as 100 minus the percentage of original filter paper biomass remaining at removal.

Forest floor samples were collected to measure pH, microbial multiple carbon source substrate-induced respiration (MSIR), and phospholipid fatty acid analysis (PLFA) of the below-ground microbial community, as described below. We collected forest floor samples (i.e., the entire depth of litter, F and H layers) from each of the four corners of the understory 1-m^2 quadrats using aseptic techniques (sampling equipment was washed with 70% ethanol between samples) and combined them to form a single homogenous sample (~ 50 g) per quadrat. These were then divided into a portion to be used for pH and MSIR and another for PLFA analysis. Samples were kept cool on ice until transferred back to the lab. Upon arrival at the lab, samples for MSIR and pH were sieved (4 mm) and kept refrigerated (4°C) in bags prior to analysis. PLFA samples were stored at -86°C and then freeze-dried prior to PLFA extraction.

Forest floor pH was measured potentiometrically in a saturated paste in equilibrium with a soil suspension of a 1:4 soil:liquid mixture. We used 0.01 M $CaCl_2$ in place of water following the instructions for measuring pH of field-moist organic samples described in Kalra and Maynard (1991).

Multiple carbon source substrate-induced respiration (MSIR) was used to examine the functional composition of microbial communities related to the activity of the soil microflora, particularly in the carbon cycle using the MicroResp™ method (Campbell et al. 2003, Chapman et al. 2007). We prepared detection agar plates containing a gel-based bicarbonate buffer with indicator dye that responded to the pH change within the gel resulting from carbon dioxide evolved from the soil. The plates were stored in a closed desiccation chamber in the dark when not being used for analysis.

Each MSIR substrate was prepared as 30 mg of substrate per gram of water (Cameron 2008); a separate set of substrates was prepared for each of the three blocks (2008) or treatments (2009, 2010) because of differences in forest floor moisture content among them. To estimate mean forest floor moisture content within each study unit (in 2008) and within treatments (in 2009 and 2010), we sub-sampled ~1 g of each field-moist sieved forest floor MSIR sample and then combined all 36 samples (9 sampling points * 4 experimental units) within each block (2008) or all 27 samples (9 sampling points * 3 blocks) within each treatment (2009, 2010) into a single sample. Each of the pooled samples were weighed (fresh) and then dried for 48 hours at 65° C and reweighed (dry). Percent dry weight was calculated ((dry/fresh)*100), and soil moisture content was calculated as 100 – percent dry weight; this moisture content was then used for calculating substrate concentrations. Fifteen substrates commonly used in MSIR analysis and thought to be associated with plant root exudates (e.g., Garland and Mills 1991, Stevenson et al. 2004) were used: five aminos acids (L-alanine, L-arginine, glutamine, L-lysine, γ aminobutyric acid), six carbohydrates (n-acetyl glucosamine, L-(+)-arabinose, D-(+)-galactose, glucose, mannose, trehalose), four carboxylic acids (citric acid, L-malic acid, oxalic acid, 3,4-dihydroxybenzoic acid), and water as a control to measure basal respiration. Substrates at desired concentrations were stored at 4° C for the duration of the respiration analysis.

Field-moist forest floor samples were incubated in a dark chamber at 25°C for ~24 hours prior to MSIR analysis. Forest floor samples were added to the 96-well microtiter deep well plates after 30 µl of each substrate was dispensed (three replicate substrate wells per sample, two samples per deep well plate). The deep well plate was then hermetically sealed with a gasket, face-to-face, with the detection plate, such that each well of the deep-well plate interacted with the opposite well of the detection plate. The two plates were incubated in the dark at 25° C for six hours. The color change in the detection plate was then read on a standard laboratory microplate reader (detection plate read before and after 6 hrs of incubation, absorbance = 570 nm). A maximum of 16 samples could be analyzed in a day, so samples were randomly selected each day to reduce bias associated with differences in time since collection and all analyses were completed within two weeks of sample collection. Respiration rates (µg CO_2-C/g/hr) for individual substrates were compared among treatments and time as µg CO_2-C/g/hr. Respiration rates for the 15 substrates were normalized to basal respiration (respiration rates were divided by basal respiration) and compared among treatments and time using multivariate analyses. Six samples had five or more carbon substrate respiration rates below basal respiration and were excluded from analysis. Catabolic evenness of respiration rates was calculated using the Simpson-Yule index ($1/\sum p_i^2$, Magurran 1988), where p_i was the respiration response for individual substrates as a proportion of total respiration rates from all substrates for a forest floor sample (Degens et al. 2000).

Microbial phospholipid fatty acid analysis (PLFA) produces a lipid profile of microbial communities. We transferred 0.30 g of each freeze dried forest floor sample to muffled test tubes and then analyzed them for PLFAs following the detailed methods described in Hannam et al. (2006). To summarize, we analyzed forest floor samples by extraction with a single-phase chloroform mixture, lipid fractionation on a solid-phase-extraction Si column and then subjected them to a mild methanolysis. The resulting fatty

acid methyl esters were then analyzed using an Agilent 6890 Series capillary gas chromatograph (GC; Agilent Technologies, Wilmington, DE) equipped with a 25 m Ultra 2 (5%-phenyl)-methylpolysiloxane column. The MIDI peak identification software (MIDI, Inc., Newark, DE) was used to identify individual fatty acids. Fatty acids were designated X:YωZ, where X represents the number of carbon atoms, Y represents the number of double bonds, and Z indicates the position of the first double bond from the aliphatic (ω) end of the molecule. The suffixes c and t indicate cis and trans geometric isomers. The prefixes 'a' and 'i' refer to anteiso and iso branching and Me and OH specify methyl groups and hydroxyl groups, respectively. PLFAs that were present in 5% or less of the samples were excluded from analysis. PLFAs for 16:1ω9c and 16:1ω11c were combined and 18:2ω6,9c and a18:0 were combined for analysis as they could not be distinguished by the GC. We excluded seven samples with <85% peak matching from analysis. There were a total of 59 PLFAs included in the final analysis. PLFAs used as biomarkers for functional groups (i.e., fungi, bacteria, actinomycetes, and arbuscular mycorrhizae) were quantified on a mol percent basis to standardize for differences in the amounts of forest floor PLFAs among samples. The fungal PLFAs 18:1ω9c, 20:1ω9c, and 18:3ω6c were used to estimate the contribution of fungi (Myers et al. 2001, Hamman et al. 2007), and 16:1ω5c was used to estimate arbuscular mycorrhizae (Frostegård and Bååth 1996, Olsson 1999). Bacterial PLFAs included 10:0 3OH, 12:0, 12:0 2OH, 12:0 3OH, 14:0, i14:0, 15:0, a15:0, i15:0, i16:0, i17:0, a17:0, 17:0, cy17:0, 18:1 ω5c, 18:1ω7c (Bååth et al. 1992, Frostegård and Bååth 1996, Olsson and Alstrom 2000, Myers et al. 2001, Hassett and Zak 2005). The ratio of fungal to bacterial PLFAs was used to estimate the relative contributions of fungi and bacteria. The 10-methyl branched fatty acids (10me16:0, 10me17:0 and 10me18:0) were used to measure actinomycetes (Kroppenstedt 1985, Brennan 1988). Total phospholipid fatty acids were compared among treatments

and time as nmol g^{-1} forest floor. Mol percent of 56 PLFAs present in >5% of samples were used in multivariate analyses.

We calculated the microbial metabolic quotient for each forest floor sample as the ratio of soil basal respiration to microbial biomass (qCO_2 - Anderson and Domsch 1985).

Statistical analyses

For univariate analyses, we first determined whether each variable met the assumptions for analysis of variance (ANOVA) and transformed response variables when necessary. Repeated measures ANOVAs were used to test for significant ($\alpha = 0.05$) differences in the response of individual variables (e.g., DWM, total Nitrogen, mean total cover, Shannon Diversity, pH) to the treatments and to differences in treatments within years, as well as for significant interactions ($\alpha = 0.10$) between treatment and year (Proc Mixed, SAS Institute Inc., Version 9.2 (32-bit), Cary, NC, USA: SAS Institute Inc., 2008). For overstory data, we compared among years and treatments, excluding the salvage harvested stands from our analyses because they did not have stems present for assessment post-treatment. To calculate species richness we looked at both species richness per 1 m^2 quadrat, as well as species richness per experimental unit, based on the total species list from sampling of the understory quadrats and combined with the species census. When the treatment effect was significant we used post-hoc linear contrasts to compare among treatments within each year separately to assess whether there were differences among the experimental units prior to the treatments being applied, in the treatment year, and the post-treatment years. When treatment was not significant but time was significant and there was no significant interaction, we compared among years, combining data for all treatments within each year. When there was a significant treatment by time interaction we compared among treatments for each year separately and

among years for each treatment separately. For all of these post-hoc comparisons we used Bonferroni-adjusted α-values (family-wise α=0.05) as follows: comparisons among treatments α= 0.008 (i.e., 0.05/6); comparisons among years α = 0.0167 (i.e. 0.05/3) (Proc Mixed, SAS Institute Inc., Version 9.2 (32-bit), Cary, NC, USA: SAS Institute Inc., 2008).

For multivariate analysis, we used data for 34 understory species/taxa that occurred in 5% or more of the experimental units (see Appendix 4-I for detailed species/taxa list). The uncommon/infrequent species were excluded from multivariate analysis because their sample sizes were too small to analyze patterns in their relative abundance among the experimental units over time.

Multivariate patterns among treatments and years were examined using nonmetric multidimensional scaling (NMS) ordination (McCune and Grace 2002) for the understory plant community, soil nutrient availability, and forest floor microbial communities (MSIR and PLFA). Ordinations used PC-ORD (Version 5 MjM Software Design, Gleneden Beach, OR), with Sørenson as the distance measure, 100 runs with real data and 100 Monte Carlo randomized runs, starting with a six-dimensional solution and stepping down to a one-dimensional solution. We determined the number of dimensions of our final solution by evaluating the scree plot and the reduction in stress with step-down in dimensionality of the preliminary runs (McCune and Grace 2002). Stability of the solution (stability criterion = 0.00005) was assessed by plotting stress versus iteration. After the preliminary runs we ran a final NMS with the optimal number of dimensions, using the starting configuration that worked best in our preliminary runs, and omitting the Monte Carlo test. We then calculated the Pearson correlation coefficients of the vegetation and forest floor descriptive variables (e.g., pH, soil moisture at each of the three depths, downed wood biomass, understory cover by growth form) with the NMS ordination axes and overlaid variables with correlation (R^2>0.25) on the ordination plots.

We used permutation-based MANOVAs (perMANOVA, PC-ORD version 5 MJM Software Design, Gleneden Beach, Ore, USA) to test for statistically significant differences in understory plant community composition, below-ground nutrient supply rates, microbial (MSIR and PLFA) community composition among the treatments over time. For the perMANOVA, 4999 randomizations were used and significance was based on the proportion of randomized trials with a response value greater than or equal to the observed response value. The initial perMANOVA comparing the treatments over time was followed up by pairwise comparisons among treatments and years following the same procedure as for the ANOVAs and similarly using alpha-values that were Bonferroni-adjusted so the family-wise $\alpha = 0.05$.

4.3. Results

Above-ground

There was a decrease in healthy basal area in both the 50% kill and 100% kill stands in the post-treatment year, but not in the control, and the three treatments differed from one another only in the post-treatment year (Table 4-2, Fig. 4-1; recall the salvage logged treatment was not included in analysis for basal area). There were no significant differences among treatments or years for canopy cover or the openness of cones (Table 4-2).

Biomass of the three smallest downed wood size classes increased over time in salvage stands, which had increased biomass in the treatment and post-treatment years compared with the pre-treatment year (Tables 4-2, 4-3). Further, in both the treatment and post-treatment years, salvaged stands had greater biomass of the three smallest size classes of DWM than the other treatments did (Tables 4-2, 4-3). There was a significant effect of treatment on biomass of DWM in the 3-5 cm size class (Table 4-2); post-hoc comparisons showed no differences among treatments in individual years (Table 4-3).

With all years combined, biomass of this size class was significantly higher in the salvage stands compared with the 50% kill stands (Table 4-3). Biomass of DWM in the 5-7 and > 7 cm size classes showed no effects of treatment or year (Table 4-2). Total DWM biomass was significantly greater in the post-treatment year compared with the pre-treatment year, with intermediate levels in the treatment year, independent of treatment (all treatments combined; Tables 4-2, 4-4).

There was an increase in litter in each year in the salvage stands, and between the pre-treatment year compared with the treatment and post-treatment year for the 50% kill stands (Tables 4-2, 4-3). There was higher litter cover in the salvage stands compared with the control and 50% kill stands in the pre-treatment year, compared with the control in the treatment year, and compared with all three treatments in the post-treatment year (Tables 4-2, 4-3).

There was a decrease in understory species richness per quadrat for the salvage stands in the treatment and post-treatment years compared with the pre-treatment year (Table 4-2, Fig. 4-2a). In the post-treatment year, the salvage stands had lower quadrat-level species richness than the 100% kill stands with intermediate levels in the control and 50% kill stands (Table 4-2, Fig. 4-2a). For experimental-unit level species richness, the salvage stands had lower species richness compared with the 50% kill stands in the treatment year, and than the 50% and 100% kills stands in the post-treatment year, but no differences with the control stands in any of the years (Table 4-2, Fig. 4-2b).

There were decreases in total, forb, and shrub cover for the salvage stands in the treatment and post-treatment years compared with the pre-treatment year, but no difference for the other three treatments among years (Table 4-2, Fig. 4-3abc). Bryophyte cover decreased for the salvage stands in the post-treatment year compared with the pre-treatment year, with intermediate levels in the treatment year (Table 4-2, Fig. 4-3d). Total understory cover was lower in both the treatment and post-treatment years for the salvage

stands compared with the other treatments (Fig. 4-3a). Shrub cover was lower in the salvage stands compared with the 50% kill and 100% kill stands in the treatment year, and compared with all the other treatments in the post-treatment year (Table 4-2, Fig. 4-3b). The salvage stands had lower forb cover compared with the control and 100% kill stands in the treatment year, and compared with all three other treatments in the post-treatment year (Table 4-2, Fig. 4-3c). In the salvage stands there was also lower bryophyte cover compared with the control and 50% kill stands in the treatment year, and compared with all three other treatments in the post-treatment year (Table 4-2, Fig. 4-3d).

The NMS two-dimensional solution (final stress = 11.9 after 29 iterations) explained 91.9% of the variation in the understory vegetation community and visualized the separation between the salvage stands post-treatment, and the other treatments; the separation of the salvage stands was associated with decreased vegetation cover and increased litter cover and pH, with the greatest separation occurring one year post-treatment (Fig. 4-4). There were significant differences in community composition among the treatments (P=0.0002); salvage logged stands differed from all three other treatments (salvage vs control P= 0.003; salvage vs 50% kill P= 0.0004, salvage vs 100% kill P= 0.0004). PerMANOVA of the understory vegetation community showed no significant differences among years (P=0.15) and no interaction between treatment and year (P=0.49). For Shannon diversity of the understory plant community, graminoid cover, and basal area of tall shrubs, no significant differences among treatments or year were detected (Table 4-2).

Below-ground

The forest floor was deeper in the pre-treatment year compared with the treatment and post-treatment years for the 50% kill stands, and compared with the treatment year in the control stands, with no inter-annual differences for the other two treatments (Tables 4-2,

4-3). There were only differences in forest floor thickness among treatments in the pre-treatment year (Tables 4-2, 4-3).

Soil moisture increased over time for all three soil depth increments, independent of treatment (Tables 4-2, 4-4). Soil moisture at both 20 cm and 40 cm was significantly lower in the pre-treatment year compared with both the treatment and post-treatment years, while at 60 cm depth it increased in each year (Table 4-4).

The control and 50% kill stands had lower forest floor pH than the salvage stands, with intermediate pH in the 100% kill stands in the treatment year, with the same pattern in the post-treatment year except that 50% kill had pH intermediate between the control and salvage stands (Tables 4-2, 4-3). The pre-treatment year pH was lower than the post-treatment year, independent of treatment (Tables 4-2, 4-4).

There was no consistent pattern in supply rates of the nutrients (Tables 4-2 - 4-4). Calcium and Mg supply rates differed among treatments only in the treatment year; the control and 100% kill stand rates were lower than the salvage, with intermediate rates in the 50% kill stands (Tables 4-2, 4-3). Phosphorus rates differed among treatments only in the post-treatment year; salvage stand rates were higher than for the control, with intermediate rates in the MPB treatments (Tables 4-2, 4-3). In the control stands, Ca rates significantly increased in the post-treatment year compared with the previous two years, whereas Mg rates increased in the post-treatment year compared with the pre-treatment year, with intermediate levels in the treatment year (Table 4-3). There were no differences in Ca, Mg, and P rates among years for the 50% kill stands (Table 4-3). In the 100% kill stands, Ca and Mg increased in the post-treatment year compared with the previous two years, whereas P increased in the post-treatment year compared with the treatment year, but with intermediate levels in the pre-treatment year (Table 4-3). For the salvage stands, both Ca and Mg significantly increased in the treatment and post-treatment years compared with the pre-treatment year, whereas P increased only in the

post-treatment year compared with the previous two years (Table 4-3). For the nutrient supply rates of Al, B, Fe, Mn, NH_4-N, S, and Zn there were only differences among years, independent of treatment and there was no consistent pattern in rates among years for these nutrients (Tables 4-2, 4-4). The NMS two-dimensional solution (final stress = 10.0 after 35 iterations) explained 95.0% of the variation in the nutrient supply rate profiles and showed separation among the years; the annual increases in soil moisture at 60 cm depth and catabolic evenness and decrease in the ratio of PLFA fungi:bacteria ratio appeared to be consistent among treatments (Fig. 4-5). Consistent with findings for individual nutrients, there were differences in nutrient supply rate profiles among years (PerMANOVA P=0.0002); post-treatment nutrient supply profiles differed from both pre- (P=0.0002) and treatment year (P=0.00006) profiles. There were no differences in nutrient supply rates among treatments (PerMANOVA P=0.07) and no interaction between treatment and year (PerMANOVA P=0.42).

The carbon respiration substrates aminobutyric acid, 3,4-dihydroxybenzoic acid, lysine, and oxalic acid only differed among treatments in the post-treatment year; aminobutyric acid respiration rates were higher in the 100% kill stands than in the control and salvage stands, whereas 3,4-dihydroxybenzoic acid, lysine, and oxalic acid respiration rates were also higher in the 100% kill stands, but only significantly higher than in the salvage stands (Tables 4-2, 4-3). The differences among years within each treatment for aminobutyric acid, 3,4-dihydroxybenzoic acid, lysine, and oxalic acid generally showed a pattern of having the lowest respiration rates in the treatment year (Table 4-3). The only differences for basal respiration and MSIR of the remaining 12 carbon substrates were among years, independent of treatment; their respiration rates decreased in the treatment year, and then subsequently increased in the post-treatment year, compared with the pre-treatment year (Tables 2, 4). Pre-treatment year catabolic evenness was significantly lower than both the treatment and post-treatment years,

independent of treatment (Tables 4-2, 4-4). The NMS two-dimensional solution (final stress = 10.1 after 48 iterations) explained 94.8% of the variation in the multiple-carbon-source respiration profiles and illustrated separation among the years, independent of treatment (Fig. 4-6 - vectors connecting experimental units among years were excluded because they showed similar patterns among all treatments). Pre-treatment year respiration profiles were associated with higher values for gross precipitation, treatment year respiration profiles were positively associated with Al and catabolic evenness, post-treatment year profiles were negatively associated with B and Fe (Fig. 4-6). There were respiration differences among years (perMANOVA P=0.004); pre-treatment respiration profiles differed from treatment year profiles (P=0.005), but there were no differences among treatments (P=0.90) or interaction between treatment and year (PerMANOVA P=0.75).

Examining patterns in PLFAs, for bacterial and actinomycete PLFAs in the 50% kill stands, PLFAs increased in the post-treatment year compared with the treatment year (Tables 4-2, 4-3). For the bacterial PLFAs there were also increases in the 100% kill stands in the post-treatment year compared with the pre-treatment and treatment years, and increases in the salvage stands in the post-treatment year compared with the treatment year. Comparing treatments among individual years did not reveal any differences but combining data for all years for each treatment showed higher PLFA mol percent in the salvage stands, compared with the control and 50% kill stands for bacterial PLFAs, and compared with the 100% kill stands for actinomycete PLFAs. The fungi:bacteria ratio was lower in the post-treatment year, compared with the pre-treatment and treatment years for the salvage stands (Tables 4-2, 4-3). There were no differences among treatments within years, but combining data for all years, there was a lower ratio of fungi:bacteria in the salvage stands compared with the 100% kill stands, with intermediate levels in the other treatments (Tables 4-2, 4-3). There were only differences

among years, independent of treatment, for total PLFA biomass, fungal PLFAs, arbuscular mycorrhizae PLFAs, and metabolic quotient, and there was no consistent pattern among years for these PLFA variables (Tables 4-2, 4-4). The NMS two-dimensional solution (final stress = 9.88 after 37 iterations) explained 93.9% of the variation in the PLFA dataset, and illustrated the separation of the stands among years, independent of treatment (Fig. 4-7 - vectors connecting experimental units among years were excluded because they showed similar patterns among all treatments). Pre-treatment year PLFA communities were most positively associated with gross precipitation, treatment year PLFA communities were positively associated with B, Fe, S, and the PLFA ratio of fungi:bacteria, post-treatment year PLFA communities were positively associated with below-ground variables including Mg, soil moisture, basal respiration, actinomycete PLFAs, and litter cover. PLFA community structure in all three years differed (perMANOVA P=0.0002; post-hoc comparisons all had P=0.0002), but there were no differences among treatments (perMANOVA P=0.29) or their interaction with year (perMANOVA P=0.99). There were no differences in decomposition or K among treatment or years (Table 4-2).

4.4. Discussion

To our knowledge, this is the first experimental study that provides an evaluation of the potential impacts of mountain pine beetle early attack and associated forest management on downed woody material, vegetation composition, and below-ground responses to attack, rather than using a chronosequence to substitute space for time. The gradient of decreased basal area of healthy trees in the 50% and 100% kill stands supports that our treatments were effective in capturing a gradient of overstory tree mortality associated with simulated MPB attack, and these findings were also supported by evidence of decreased sapflow conductivity in killed trees in a paired hydrology study that was

conducted on our study sites (Piña 2012). Despite the canopy mortality resulting from simulated MPB attack, we saw no effects of the MPB treatments on other above- and below-ground properties and processes in these forests, except for increased microbial respiration of four carbon substrates. Our results differ with Edburg et al.'s (2012) conceptual model of MPB ecosystem impacts that proposed biogeochemical ecosystem responses will occur during the red attack stage. As we hypothesized, we saw more immediate changes in the above-ground plant community, DWM, and several below-ground properties for the salvage logged stands than in the MPB treatments. Our findings suggest ecological resistance in the early attack stage of MPB, and that the ecological inertia (i.e., the lag in time before the plant community and associated ecosystem properties and processes respond to disturbance – Jentsch and Beierkuhnlein 2008) is higher for the MPB-disturbed stands compared with the salvage logged stands.

We saw responses in the three smallest DWM size classes (<0.5, 0.5-1, and 1-3 cm diameter) in the salvage treatment compared with the other treatments in the treatment and post-treatment year. The lack of response of DWM to the MPB treatments is likely a result of the lag between the trees being attacked and the needles dropping and the trees falling. Our findings are in agreement with previous studies which also showed minimal changes in DWM biomass associated with MPB red attack. Previous research in ponderosa pine forests showed that MPB-attacked trees lost most of their foliage between the second and third year post-attack (Chojnacky et al. 2000). In Oregon lodgepole pine snags began falling three years after MPB attack, and 80% of trees fell within 10 years (Mitchell and Preisler 1998). Lewis and Thompson (2011) found that for pine trees killed by MPB in central BC, most dead trees did not start to fall until eight years after they died. Interestingly, a study in Colorado found there were no differences in fine or coarse DWM loads between unattacked stands and stands with current or recent (up to 7 years prior) MPB attack (Klutsch et al. 2009). Simard et al. (2011) compared surface fuel loads

for multiple size categories for unattacked, red, and grey stages of MPB attack and also found no differences among them. Rather than changes in biomass of DWM in the red attack stage, Simard et al. (2011) concluded that there were changes in the moisture content of DWM, which we did not measure. In the harvested stands it was surprising that we did not detect increases in biomass across all size classes compared with the other treatments, as the stump-side processing system leaves debris on site. Instead we observed increases in total biomass of downed wood in the treatment and post-treatment years across all treatments. This overall increase is likely a function of both natural mortality associated with the mature successional stage of these stands, and blow-down resulting from windthrow. There were multiple extreme wind events recorded in the study area in both the treatment and post-treatment years that could have contributed to the increases we observed.

Previous studies have shown significant effects of harvest on the understory vegetation of boreal forests after harvest, with a gradient of effects dependent on the intensity of harvest/thinning (e.g., Bergstedt and Milberg 2001). Thus, with mortality and loss of the overstory canopy as a result of disturbance, we expected associated changes in the understory plant community in the salvage logged stands. Changes in the salvage logged stands are likely a result of the direct impact of harvesting on the understory and forest floor, including trampling of the vegetation by the harvesting machinery and increased deposition of litter. The impact of mechanical disturbance to the forest floor is great, and is the primary difference between disturbance by MPB – and other insects – versus fire or any kind of harvesting, which translates to more immediate effects on the understory plant community. The increased litter that was associated with the salvage logged stands is likely a consequence of the slash and the mortality in the understory that occurred after harvest in these stands. While harvesting frequently increases overall understory species richness relative to unharvested forests by increasing the number of

early-successional shrub and herb species while decreasing the number of late-successional lichens, mosses and herbs (e.g., Battles et al. 2001, Haeussler et al. 2002), it takes time post-disturbance for these changes to occur. One-year post treatment is likely too early to see these patterns and instead we likely saw decreases in plant cover and richness because of the machinery damage, and exposure of remaining plants to higher levels of solar radiation, compared with the other treatments that did not directly affect the forest floor.

Past research on the response of the understory community to MPB attack has primarily been focused on tree regeneration with little attention to the understory composition and biodiversity after MPB attack. Kovacic et al. (1985) studied ponderosa pine after MPB attack and found that understory biomass peaked five years post MPB attack. In the red stage of MPB attack, Griffin et al. (2011) found significantly higher total and forb cover compared with undisturbed stands, but no differences in shrubs and graminoids. Stone and Wolfe (1996) found changes in understory biomass depended upon the mortality rate of lodgepole pine in the overstory of stands in Utah that were attacked within the previous 10 years, with exponentially increasing understory biomass with increasing overstory mortality, but species richness peaked at intermediate levels of MPB attack. A redistribution of biomass production from overstory trees to understory vegetation in lodgepole pine ecosystems was still seen in Waterton Lakes National Park 25 years after the disturbance (Dykstra and Braumandl 2006). Our findings suggest that while one year after simulated MPB treatment changes in the understory have not yet occurred, we expect changes to occur in the future. This is supported by Klutsch et al. (2009) who compared MPB-infested and uninfested lodgepole pine stands both 0-3 years and 4-7 years post-infestation in Colorado and also found no difference in percent cover among understory vegetation groups in the short term. Given that attacked trees do not lose most of their foliage until between the second and third year post-attack, perhaps

changes in the forest understory plant community will not occur until the needles have dropped and in turn have altered other ecosystem properties and processes, such as nutrient cycling. Thus, we expect that as the stands transition from red attack to grey attack and drop their needles, increases in light transmission to the understory and increased litter inputs will alter forest floor nutrient cycling properties and processes, which are likely to modify plant growth in the understory. However, for the understory vegetation variables we studied, the lack of differences among the MPB treatments and control stands suggest that the understory plant community is resistant to changes in the forest associated with the early red-attack stage of MPB.

For most below-ground response variables we saw differences among the years across treatments rather than differences among the treatments. Our findings of no significant differences in microbial biomass between the control and clearcut stands are consistent with Entry et al. (1986), who also found no differences in microbial biomass between control stands and clearcut lodgepole pine stands in Montana that had both residue removed and residue left on-site. Further, Entry et al. (1986) found that for soil temperatures above 5º C, microbial biomass was positively correlated with soil moisture. In a comparison of seven different forest types in BC, including lodgepole pine, Brockett et al. (2012) found the properties most closely related to microbial community characteristics were soil moisture and organic matter, which is consistent with our observations of the inter-annual patterns of soil moisture at 60 cm depth that were associated with the separation of microbial PLFA communities. The lack of difference in MSIR among stands post-salvage is supported by Siira-Pietikäinen et al. (2001), who also did not find differences in respiration one year post harvest for Norway spruce (*Picea abies* (L.) Karst.) - Scots Pine (*Pinus sylvestris* L.)- *Betula* spp. stands in central Finland. However, Siira-Pietikäinen et al. (2001) did find differences two years after clear-cut harvest, suggesting that changes are more likely to occur in these stands as time post-

disturbance advances and nutrient cycling dynamics change in response to additions of litter and changes in soil moisture and light. The increased microbial respiration rates in the 100% kill stands for the three carboxylic acids and lysine in the post-treatment year compared with the salvage treatment suggest that below-ground changes are beginning to occur in the MPB-attacked stands, with a shift to a microbial community that differs in its respiration patterns compared to the salvage microbial community, although this was not illustrated in the MSIR ordination.

Even though we did not observe short-term responses of PLFA biomass to harvesting, other studies have shown that responses of PLFAs to harvesting do eventually occur (e.g., Mummey et al. 2010), and can in fact be very long lasting (E.g., Chatterjee et al. 2008). We did see signs of differences in the relative composition of the PLFA microbial community among treatments suggesting that structure of the PLFA community may be more sensitive to salvage than is PLFA biomass, but this trend couldn't be separated out within individual years (although there seemed to be a trend in which the fungi:bacteria PLFA ratio was shifting towards an increasing proportion of bacterial PLFAs in the clearcut stands). The microbial community is impacted by soil pH, and it is generally accepted that fungi are favored over bacteria at low pH (Alexander 1977). This pattern has been shown across large pH ranges (e.g., Högberg et al. 2003), but also within narrow ranges of soil pH (e.g., Pennanen et al. 1999) such as the increased pH we observed in the salvage logged stands in the treatment and post-treatment years compared with the control stands. This variation in pH and the microbial community composition, in turn, will influence biogeochemical cycles among the treatments, including potentially affecting the soil concentrations of plant root exudates and nutrients. However, our findings suggest that in the immediately post-disturbance time period, patterns of increased availability of nutrients are not yet evident in the MPB-attacked stands or in the salvage, except for Ca, Mg, and P. Our findings correspond with Bock

and Van Rees (2002) who also found an increase in exchangeable Ca in boreal mixedwood forests after harvest compared with uncut forests. These findings contrast with marginally significant decreases of Ca found under newly MPB-killed trees compared with live trees in mature pine forests in Colorado (Xiong et al. 2011). Our findings also differ with Griffin et al. (2011) who found significantly higher Ca in Yellowstone red attacked lodgepole pine stands compared with unattacked stands, along with higher rates of N mineralization and nitrification compared with undisturbed forests. Hynes and Germida (2012) showed higher levels of ammonia and nitrate in lodgepole pine stands 2-19 years after clear-cutting in western Alberta, with ammonia peaking four years post clearcut. Our results suggest the assart effect is just beginning one year after disturbance in our stands, and should peak in the coming years as decomposition processes respond to the treatments. Overall, our dominant finding of differences among study years rather than differences among the treatments suggest that inter-annual variability at the landscape scale exerts a stronger influence on most forest floor properties and processes than do treatment effects of MPB or associated forest management in the short term (i.e., one year post treatment).

In the salvage logged stands we saw immediate changes in the understory plant community, DWM, and in several below-ground properties. However, we found short-term resistance to simulated MPB at the early red-attack stage for DWM, understory, and the majority of below-ground properties and processes in these forests. Thus, it remains uncertain what successional trajectory lodgepole pine forests in Alberta will follow post-MPB attack in the longer term, but our research demonstrates that the successional trajectory of stands that are salvage logged will be very different from that of stands that are attacked by MPB and left unmanaged. Research on MPB in other regions that has been carried out over longer periods suggests that as these MPB-attacked stands transition from red to grey attack they will begin to show more significant responses

compared with the undisturbed stands, however when and the magnitude of these delayed responses to this novel disturbance agent remain unknown. Longer-term research that continues to follow MPB-attacked stands temporally post-disturbance is needed to better understand how MPB-attacked stands will continue to respond to this novel disturbance agent and shifting disturbance regimes from stand-replacing disturbances such as fire and clear-cut harvesting, to the addition of partial canopy disturbances that result from MPB attack.

Table 4-1. Summary of site characteristics of lodgepole pine (*Pinus contorta*) in Upper Foothills of Alberta for the three study units before the treatments were applied (2008). Given are the means for all trees > 5cm dbh; the minimum and maximum values for subplots are in parentheses.

Study Unit	Latitude/Longitude	Basal area (m^2 ha^{-1})	Density (trees ha^{-1})	Dbh (cm)	Canopy cover (%)
1	53.2248/116.8094	39.6 (26.7-56.2)	1420 (950-1900)	18.3 (5-34.7)	63.9 (56.2-86.9)
2	53.24129/116.8288	37.3 (21.6-55.1)	978 (550-1350)	21.5 (6.6-43.3)	59.2 (51.4-70.7)
3	53.22647/116.8212	40.3 (27.1-54.0)	1182 (450-1850)	20.1 (8.0-38.3)	62.1 (54.9-77.4)

Table 4-2. Results (P values) for repeated measures ANOVAs testing for the effect of treatment, year (pre- to post-treatment) and the interaction between treatment and year for overstory, downed woody material (DWM), understory, and below-ground variables for lodgepole pine study units. Significant P-values highlighted in bold.

Component	Variable	Treatment	Year	Treatment*Year
Overstory[i]	Healthy basal area	**0.002**	**<0.0001**	**<0.0001**
	Canopy cover (%)	0.37	0.44	0.98
	Openness of cones	0.26	0.88	0.52
DWM biomass	Class 1: < 0.5cm[ii]	**<0.0001**	**<0.0001**	**0.02**
	Class 2: 0.5-1 cm[ii]	**0.0005**	**<0.0001**	**0.014**
	Class 3: 1-3 cm	**<0.0001**	**0.03**	**0.03**
	Class 4: 3-5 cm[iii]	**0.03**	0.45	0.67
	Class 5: 5-7 cm[iii]	0.10	0.37	0.99
	Class 6: > 7 cm[ii]	0.17	0.05	0.97
	Total biomass[ii]	0.49	**0.03**	1.0
Understory	Understory vegetation richness per quadrat	**0.04**	0.28	**0.03**
	Understory vegetation richness per experimental unit	**<0.0001**	0.43	0.14
	Total cover	**<.0001**	0.78	**0.0004**
	Forb cover[ii]	**0.0007**	0.86	**0.01**
	Shrub cover[ii]	**0.0023**	0.26	**0.01**
	Graminoid cover	0.16	0.96	0.33
	Bryophyte cover	**<0.0001**	0.91	**0.06**
	Shannon diversity per quadrat	0.10	0.23	0.26
	Litter cover	**<0.0001**	**<0.0001**	0.07
	Large shrub basal area	0.17	0.81	0.52
Below-ground	Forest floor depth[iii]	**0.02**	**<0.0001**	0.51
	Soil moisture: 20 cm depth	0.42	**0.0011**	0.99
	Soil moisture: 40 cm depth[ii]	0.55	**0.0004**	0.99
	Soil moisture: 60 cm depth	0.39	<**0.0001**	0.95
	Forest floor pH	**<0.0001**	**0.02**	0.97
	Nutrient supply: Al[iii]	0.32	**0.001**	0.99
	Nutrient supply: B[iii]	0.96	**<0.0001**	0.94
	Nutrient supply: Ca	0.12	**<0.0001**	**0.03**
	Nutrient supply: Fe[iii]	0.78	**<0.0001**	0.69
	Nutrient supply: K[ii]	0.27	0.34	0.99
	Nutrient supply: Mg	**0.005**	**<0.0001**	0.31
	Nutrient supply: Mn[ii]	0.81	**0.02**	0.31
	Nutrient supply: NH_4-N[ii]	0.74	**0.03**	0.74
	Nutrient supply: P	**0.04**	**0.0008**	**0.03**
	Nutrient supply: S[iii]	0.20	**0.0018**	0.79
	Nutrient supply: Zn	0.10	**<0.0001**	0.63
	Decomposition	0.25	0.85	0.90
	MSIR: N-acetyl glucosamine[iii]	0.82	**<0.0001**	0.31
	MSIR: L-alanine	0.29	**<0.0001**	0.20
	MSIR: Aminobutyric acid	0.19	**<0.0001**	**0.01**
	MSIR: Arabinose	0.55	**<0.0001**	0.18
	MSIR: L-arginine	0.33	**<0.0001**	0.27
	MSIR: Citric acid[iii]	0.59	**<0.0001**	0.15
	MSIR: 3,4-Dihydroxybenzoic acid[iii]	0.59	**<0.0001**	0.08
	MSIR: Galactose[iii]	0.36	**<0.0001**	0.19
	MSIR: Glucose[iii]	0.49	**<0.0001**	0.73
	MSIR: Glutamic acid[iii]	0.32	**<0.0001**	0.22
	MSIR: L-lysine[iii]	0.30	**<0.0001**	0.07

Component	Variable	Treatment	Year	Treatment*Year
	MSIR: Malic acid[iii]	0.51	**<0.0001**	0.13
	MSIR: Mannose	0.54	**<0.0001**	0.26
	MSIR: Oxalic acid	0.11	**<0.0001**	**0.03**
	MSIR: Trehalose	0.95	**<0.0001**	0.75
	MSIR: Basal (water)[iii]	0.49	**<0.0001**	0.22
	Catabolic evenness	0.73	**<0.0001**	0.97
	PLFA Total biomass	0.63	**0.002**	0.97
	Bacterial PLFA mol%	**0.02**	**<0.0001**	0.62
	Fungal (excl. myco) PLFA mol%	0.14	**0.02**	0.82
	Arbuscular mycorrhizae PLFA mol%	0.57	**0.005**	0.30
	Actinomycetes PLFA mol%	**0.045**	**0.002**	0.93
	Fungi:bacteria ratio	**0.02**	**0.0005**	0.81
	Metabolic quotient[ii]	0.55	**<0.0001**	0.30

[i] Only comparing among the three treatments of control, 50% kill, and 100% kill because overstory stems were all removed in the salvage logged treatment.

[ii] Square-root transformed for analysis

[iii] Log transformed for analysis

Table 4-3. Mean and 95% confidence intervals for measured variables separated by treatment within each year. Years with a different lower case letter (x,y,z) within a given treatment were significantly different for that variable. Treatments with different capital letters (A,B,C,D) within a year were significantly different for that variable. These were based on post-hoc lsmeans comparisons for variables that showed significant treatment, treatment and year, and/or treatment-year interaction effects in the ANOVA (see Table 4-2).

Variable	Year	Control	50% Kill	100% kill	Salvage
DWM (Mg ha^{-1})					
DWM (0-0.5 cm)	Pre-treatment	0.16 (0.07-0.26)	0.16 (0.07-0.26)	0.19 (0.09-0.28)	0.17 (0.07-0.26)x
	Treatment	0.26 (0.17-0.35)A	0.27 (0.18-0.36)A	0.27 (0.18-0.37)A	0.56 (0.47-0.65)By
	Post-treatment	0.24 (0.14-0.33)A	0.20 (0.10-0.29)A	0.21 (0.11-0.30)A	0.57 (0.48-0.66)By
DWM (0.5-1 cm)	Pre-treatment	0.25 (0.09-0.42)	0.30 (0.14-0.46)	0.37 (0.21-0.54)	0.32 (0.16-0.48)x
	Treatment	0.45 (0.29-0.61)A	0.35 (0.19-0.52)A	0.45 (0.29-0.62)A	0.99 (0.83-1.16)By
	Post-treatment	0.48 (0.32-0.65)A	0.45 (0.29-0.61)A	0.38 (0.22-0.55)A	0.89 (0.73-1.06)By
DWM (1-3 cm)	Pre-treatment	1.03 (-0.22-2.28)	0.99 (-0.26-2.24)	1.43 (0.18-2.68)	1.21 (-0.04-2.46)x
	Treatment	1.06 (-0.19-2.31)A	1.06 (-0.19-2.31)A	1.32 (0.07-2.60)A	4.47 (3.22-5.72)By
	Post-treatment	1.58 (0.33-2.83)A	1.32 (0.07-2.57)A	1.36 (0.11-2.61)A	4.51 (3.26-5.76)By
DWM (3-5 cm)	Pre-treatment	1.93 (0.66-3.20)	1.05 (-0.22-2.32)	2.28 (1.01-3.55)	2.02 (0.75-3.29)
	Treatment	1.84 (0.57-3.11)	1.14 (-0.13-2.41)	2.11 (0.84-3.36)	4.04 (2.77-5.31)
	Post-treatment	2.11 (0.84-3.38)	1.23 (-0.04-2.50)	2.63 (1.36-3.90)	4.39 (3.12-5.66)
	All years combined[i]	1.96 (1.16-2.76)AB	1.14 (0.34-1.94)A	2.34 (1.54-3.14)AB	3.48 (2.68-4.28)B
UNDERSTORY					
Litter cover (%)	Pre-treatment	42.3 (34.9-49.7)A	43.7 (36.4-51.0)Ax	50.2 (42.7-57.6)AB	59.5 (52.0-66.9)Bx
	Treatment	51.7 (44.3-59.1)A	59.8 (52.5-67.1)ABy	61.9 (54.5-69.4)AB	69.8 (62.4-77.3)By
	Post-treatment	55.4 (48.0-62.9)A	57.0 (49.7-64.4)Ay	62.5 (55.1-70.0)A	89.1 (81.7-96.6)Bz
BELOW-GROUND					
Forest floor depth	Pre-treatment	0.90 (0.76-1.04)ABy	1.05 (0.91-1.18)By	0.89 (0.75-1.03)AB	0.82 (0.68-0.95)A
	Treatment	0.63 (0.49-0.77)x	0.71 (0.57-0.85)x	0.74 (0.60-0.88)	0.66 (0.53-0.80)
	Post-treatment	0.76 (0.62-0.90)xy	0.79 (0.65-0.92)x	0.82 (0.69-0.96)	0.64 (0.50-0.78)
Forest floor pH	Pre-treatment	3.52 (3.33-3.71)	3.56 (3.37-3.75)	3.64 (3.45-3.82)	3.68 (3.49-3.87)
	Treatment	3.51 (3.33-3.70)A	3.53 (3.34-3.72)A	3.66 (3.47-3.85)AB	3.74 (3.55-3.92)B
	Post-treatment	3.56 (3.38-3.75)A	3.63 (3.45-3.82)AB	3.76 (3.57-3.95)AB	3.82 (3.63-4.01)B

Variable	Year	Control	50% Kill	100% kill	Salvage
Nutrient supply rates (micro grams/10cm^2/summer burial length)					
Ca	Pre-treatment	1192 (1070-1314)x	1362 (1241-1484)	1394 (1272-1516)x	1302 (1174-1430)x
	Treatment	1275 (1154-1397)Ax	1357 (1235-1478)AB	1281 (1160-1403)Ax	1529 (1401-1657)By
	Post-treatment	1522 (1400-1643)y	1493 (1371-1615)	1648 (1526.-1770)y	1554 (1426-1682)y
Mg	Pre-treatment	234 (200-269)x	269 (234-304)	246 (212-281)x	279 (243-315)x
	Treatment	272 (238-307)Axy	292 (257-327)AB	256 (221-291)Ax	338 (302-373)By
	Post-treatment	316 (281-350)y	313 (278-348)	320 (285-354)y	333 (297-369)y
P	Pre-treatment	13.2 (8.1-18.3)	16.6 (11.5-21.7)	19.2 (14.1-24.4)xy	13.4 (8.1-18.6)x
	Treatment	16.9 (11.8-22.0)	17.4 (12.3-22.5)	15.2 (10.1-20.3)x	17.8 (12.6-23.1)x
	Post-treatment	15.4 (10.3-20.5)A	20.1 (15.0-25.3)AB	23.1 (18.0-28.2)ABy	27.9 (22.6-33.1)By
MSIR respiration rates (µg CO_2-C/g/hr)					
Aminobutyric acid	Pre-treatment	21.5 (18.8-24.1)y	19.7 (17.1-22.4)x	19.4 (16.8-22.1)y	21.1 (18.5-23.8)y
	Treatment	16.8 (14.2-19.5)x	16.9 (14.3-19.6)x	14.9 (12.3-17.5)x	14.8 (12.1-17.4)x
	Post-treatment	23.9 (21.3-26.6)Ay	25.4 (22.7-28.0)ABy	29.4 (26.8-32.1)Bz	21.5 (18.9-24.2)Ay
3,4-	Pre-treatment	20.3 (17.7-22.8)y	19.3 (16.7-21.8)xy	19.1 (16.5-21.6)y	20.4 (17.8-23.0)y
Dihydroxybenzoic	Treatment	16.4 (13.9-19.0)x	16.6 (14.0-19.1)x	14.7 (12.1-17.2)x	14.7 (12.2-17.3)x
acid[ii]	Post-treatment	21.7 (19.2-24.3)yAB	24.3 (21.7-26.8)ABy	28.8 (26.3-31.4)Bz	21.8 (19.2-24.4)Ay
L-lysine[ii]	Pre-treatment	15.8 (13.6-18.0)xy	14.8 (12.7-17.0)x	14.0 (11.9-16.2)x	14.9(12.8-17.1)y
	Treatment	13.2 (11.0-15.3)x	12.8 (10.6-15.0)x	11.4 (9.2-13.6)x	11.2 (9.0-13.4)x
	Post-treatment	17.3 (15.1-19.5)ABy	19.2 (17.1-21.4)ABy	22.4 (20.2-24.5)By	16.0 (13.8-18.1)Ay
Oxalic acid[ii]	Pre-treatment	25.4 (22.4-28.5)y	25.4 (22.4-28.5)xy	24.9 (21.9-28.0)y	26.7 (23.6-29.7)y
	Treatment	19.8 (16.8-22.9)x	20.8 (17.8-23.9)x	18.2 (15.2-21.3)x	17.6 (14.5-20.6)x
	Post-treatment	28.9 (25.8-31.9)ABy	29.5 (26.5-32.6)ABy	34.0 (31.0-37.1)Bz	24.6 (21.5-27.7)Ay
Microbial PLFA (mol %)					
Bacterial	Pre-treatment	27.6 (25.6-29.5)	27.9 (26.0-29.9)xy	27.6 (25.6-29.5)x	29.7 (27.7-31.7)xy
	Treatment	26.9 (24.9-28.8)	26.5 (24.5-28.4)x	27.0 (25.1-29.0)x	27.5 (25.6-29.5)x
	Post-treatment	28.6 (26.6-30.5)	28.9 (27.0-30.9)y	30.0 (28.0-31.9)y	30.6 (28.6-32.5)y
	All years combined	27.7 (26.0-29.3)A	27.8 (26.1-29.4)A	28.2 (26.5-29.9)AB	29.3 (27.6-30.9)B
Actinomycetes	Pre-treatment	4.69 (4.08-5.30)	4.79 (4.19-5.40)xy	4.54 (3.93-5.14)	5.06 (4.44-5.67)
	Treatment	4.44 (3.83-5.05)	4.30 (3.69-4.91)x	4.49 (3.88-5.10)	4.80 (4.19-5.41)
	Post-treatment	5.07 (4.46-5.68)	5.13 (4.52-5.74)y	4.87 (4.27-5.48)	5.64 (5.03-6.25)
	All years combined[1]	4.73 (4.27-5.20)AB	4.74 (4.27-5.21)AB	4.63 (4.16-5.10)A	5.17 (4.70-5.64)B

Variable	Year	Control	50% Kill	100% kill	Salvage
Fungi:bacteria ratio	Pre-treatment	0.78 (0.72-0.83)	0.78 (0.72-0.83)	0.77 (0.72-0.83)	0.72 (0.67-0.78)x
	Treatment	0.74 (0.69-0.79)	0.74 (0.69-0.80)	0.76 (0.70-0.81)	0.73 (0.67-0.78)x
	Post-treatment	0.71 (0.65-0.76)	0.71 (0.67-0.77)	0.72 (0.66-0.77)	0.64 (0.58-0.69)y
	All years combined[1]	0.74 (0.70-0.78)AB	0.74 (0.71-0.78)AB	0.75 (0.71-0.79)B	0.70 (0.66-0.73)A

[i] All years combined is also reported because pairwise comparisons of treatments within each of the three years did not show any significant differences.

Table 4-4. Mean and 95% confidence intervals in each year of the study; years with a different lower case letter (x, y, z) were significantly different for that variable. These were based on post-hoc lsmeans comparisons for variables that showed significant year effects, but for which there was no significant interaction between treatment and time in the ANOVA (see Table 4-2).

Variable	Pre-Treatment Year	Treatment Year	Post-treatment Year
Total DWM (Mg ha^{-1})	37.0 (23.0-50.9)x	43.4 (29.4-57.3)xy	50.7 (36.7-64.6)y
Soil moisture (mm)			
Soil moisture (0-20 cm)	48.2 (42.5-54.0)x	57.8 (52.1-63.6)y	59.5 (53.7-65.2)y
Soil moisture (0-40 cm)	112.6 (104.6-120.7)x	123.3 (115.2-131.4)y	132.4 (124.4-140.5)y
Soil moisture (0-60 cm)	163.6 (154.1-173.2)x	178.5 (168.8-188.1)y	208.4 (198.8-217.9)z
Nutrient supply rates (micro grams/10cm^2/burial length)			
Al	69.1 (60.3-77.9)x	88.2 (79.3-97.0)y	83.0 (74.2-91.9)y
B	0.90 (0.78-1.02)x	1.00 (0.88-1.12)x	0.49 (0.38-0.61)y
Fe	69.1 (60.3-78.0)x	88.2 (79.3-97.0)y	83.1 (74.2-91.9)z
Mn	53.1 (40.3-65.8)x	59.8 (47.1-72.6)xy	67.9 (55.2-80.7)y
NH_4-N	3.90 (2.58-5.22)x	6.20 (4.89-7.52)y	5.58 (4.26-6.89)xy
S	73.8 (26.7-120.9)x	96.3 (49.2-143.4)y	87.6 (40.5-134.7)y
Zn	3.57 (2.57-4.56)x	4.22 (3.22-5.22)x	5.28 (4.29-6.28)y
pH	3.60 (3.43-3.76)x	3.61 (3.45-3.77)xy	3.70 (3.53-3.86)y
Multi carbon source SIR (µg CO_2-C/g/hr)			
Basal (water)	13.1 (12.1-14.1)x	10.8 (9.8-11.7)y	16.5 (15.5-17.5)z
N-acetyl glucosamine[ii]	17.7 (16.3-19.1)x	14.4 (13.0-15.8)y	22.7 (21.3-24.1)z
L-alanine	19.6 (18.4-20.9)x	14.9 (13.6-16.1)y	23.7 (22.5-24.9)z
Arabinose	22.0 (20.7-23.4)x	16.8 (15.4-18.2)y	26.0 (24.6-27.3)z
L-arginine	15.3 (14.1-16.5)x	12.1 (10.9-13.3)y	19.6 (18.3-20.8)z
Citric acid[ii]	22.0 (20.5-23.5)x	17.9 (16.4-19.3)y	26.4 (24.9-27.9)z
Galactose[ii]	21.2 (19.8-22.6)x	16.5 (15.1-18.0)y	26.5 (25.0-27.9)z
Glucose[ii]	24.9 (23.1-26.7)x	18.7 (17.0-20.5)y	28.7 (26.9-30.5)z
Glutamic acid[ii]	23.6 (22.0-25.2)x	17.4 (15.8-19.0)y	26.2 (24.6-27.8)z
Malic acid[ii]	29.2 (27.1-31.3)x	23.3 (21.1-25.4)y	32.4 (30.2-34.5)z
Mannose	23.3 (21.5-25.1)x	17.8 (16.1-19.6)y	27.7 (26.0-29.5)z
Trehalose	21.9 (20.2-23.7)x	16.6 (14.8-18.4)y	26.2 (24.5-28.0)z
Catabolic evenness	15.6 (15.5-15.6)x	15.8 (15.7-15.9)y	15.8 (15.7-15.9)y
Total PLFA biomass (nmol g^{-1})	1198 (1121-1275)x	1414 (1338-1491)y	1305 (1229-1382)xy
Fungal (excl. myco) mol %	15.9 (15.3-16.4)x	15.3 (14.7-15.8)xy	15.0 (14.5-15.6)y
Arbuscular mycorrhizae mol %	2.70 (2.34-3.05)x	2.27 (1.92-2.63)y	2.63 (2.28-2.99)x
Metabolic quotient	0.011 (0.011-0.012)y	0.008 (0.007-0.009)x	0.013 (0.012-0.014)y

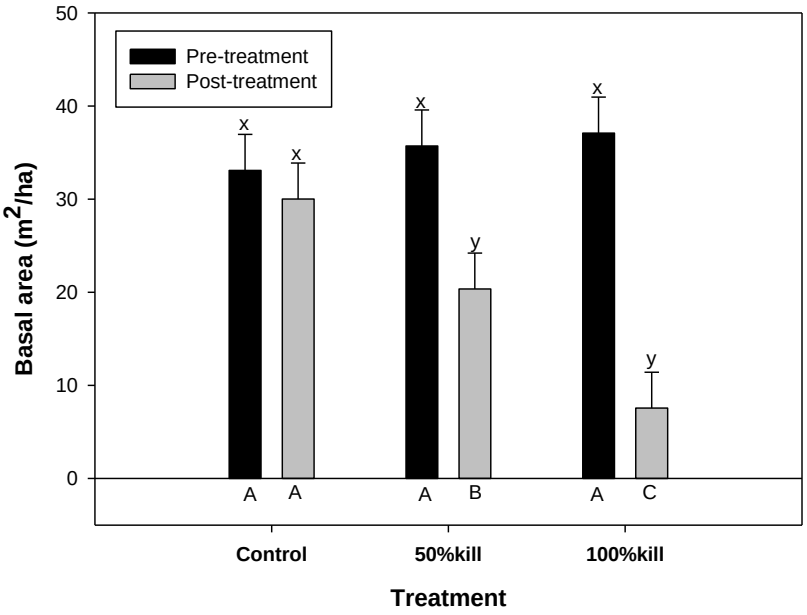

Fig. 4-1. Mean basal area for trees with healthy crown vigor by treatment and year (± 95% confidence interval). Years with different letters (x, y) within a treatment were significantly different. Treatments with different letters (A, B, C) within a year were significantly different.

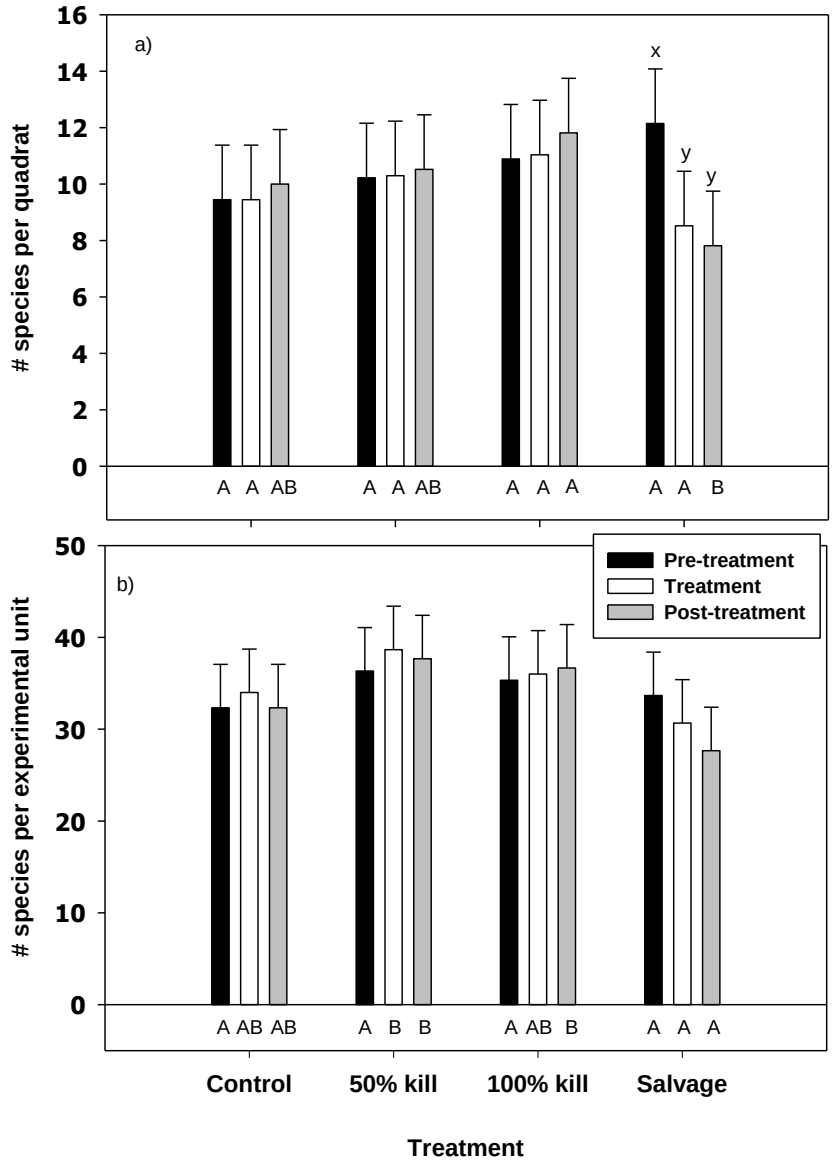

Fig. 4-2. Mean understory vegetation species richness at the scale of a) 1-m^2 quadrat, and b) 0.48 ha experimental unit, by treatment and year (± 95% confidence interval). Years with different letters (x, y) within a treatment were significantly different. Treatments with different letters (A, B, C) within a year were significantly different.

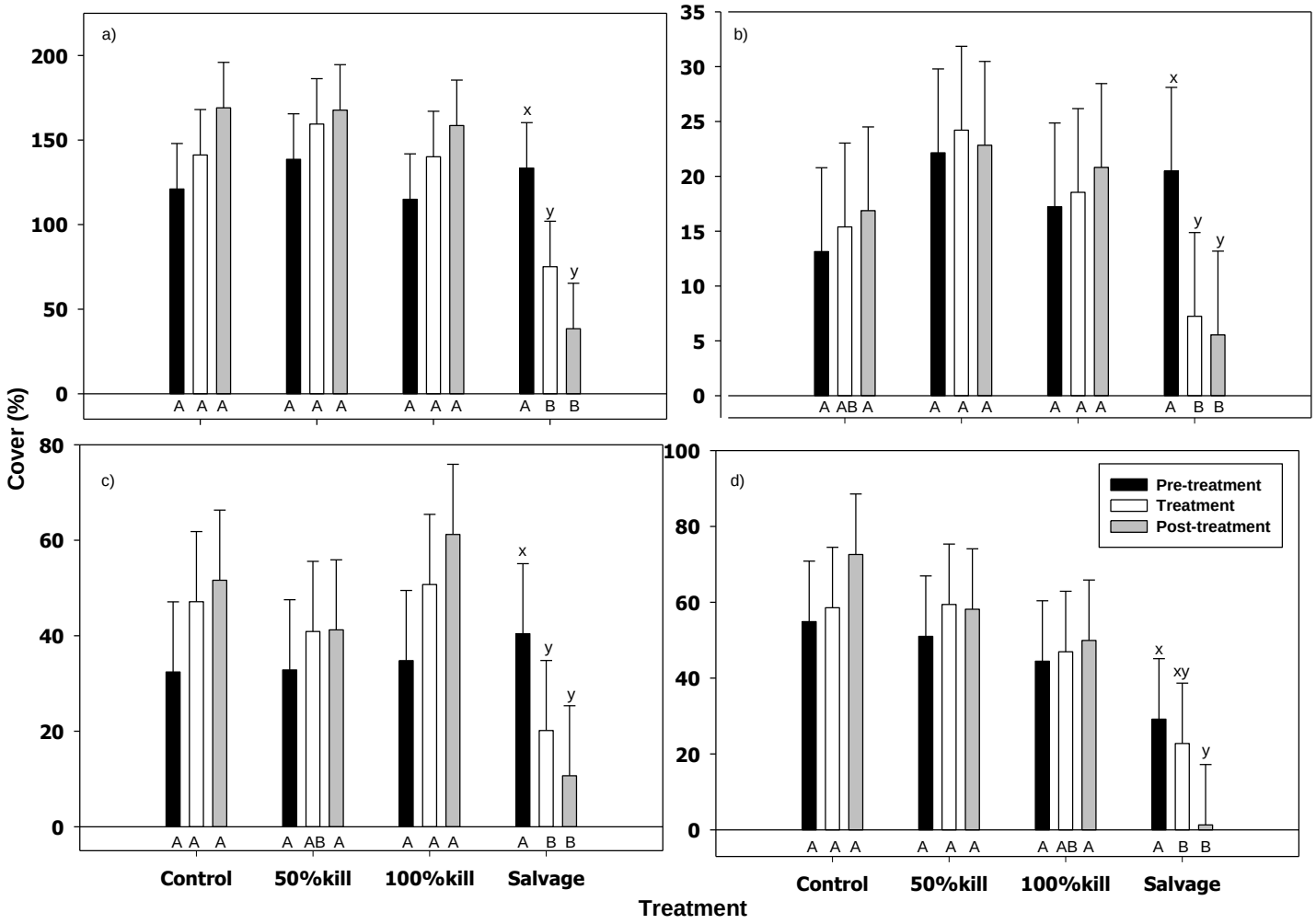

Fig. 4-3. Mean percent cover for a) total understory, b) bryophytes, c) forbs, and d) shrubs by treatment and year (± 95% confidence interval). Years with different letters (x, y) within a treatment were significantly different. Treatments with different letters (A, B, C) within a year were significantly different.

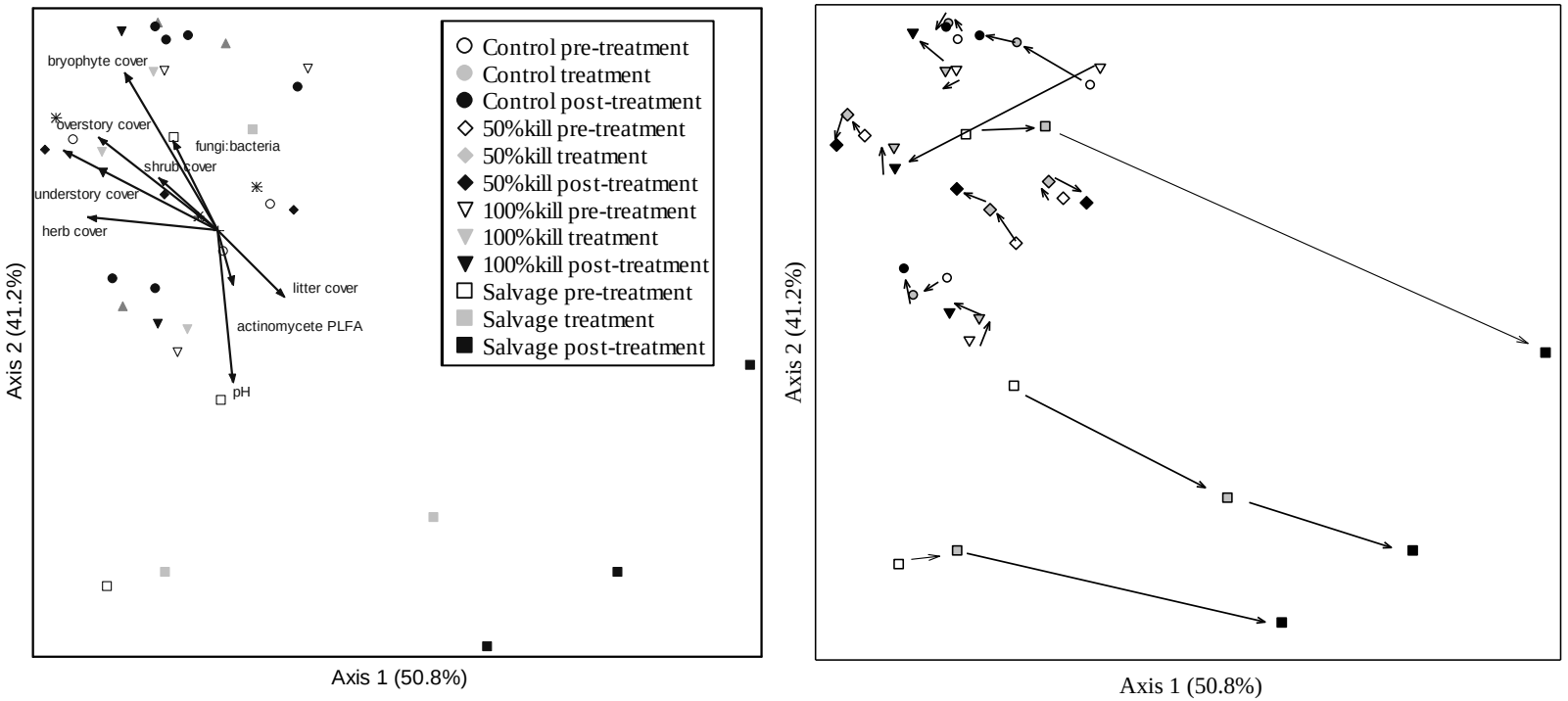

Fig. 4-4. Results of nonmetric multidimensional scaling ordination for the understory vegetation community delineated by treatment and year presented with: a) the angles and lengths of the vectors which indicate direction and strength of relationships of the variables (see Table 4-2 for information on the variables) with the ordination axes (cut-off for displayed variables was $R^2 > 0.25$, and vector scaling was set to 65% so that vectors could be displayed without going beyond the ordination plot); and b) vectors that connect each experimental unit among the pre-treatment, treatment, and post-treatment years. The amount of variation explained by each axis is included in parentheses.

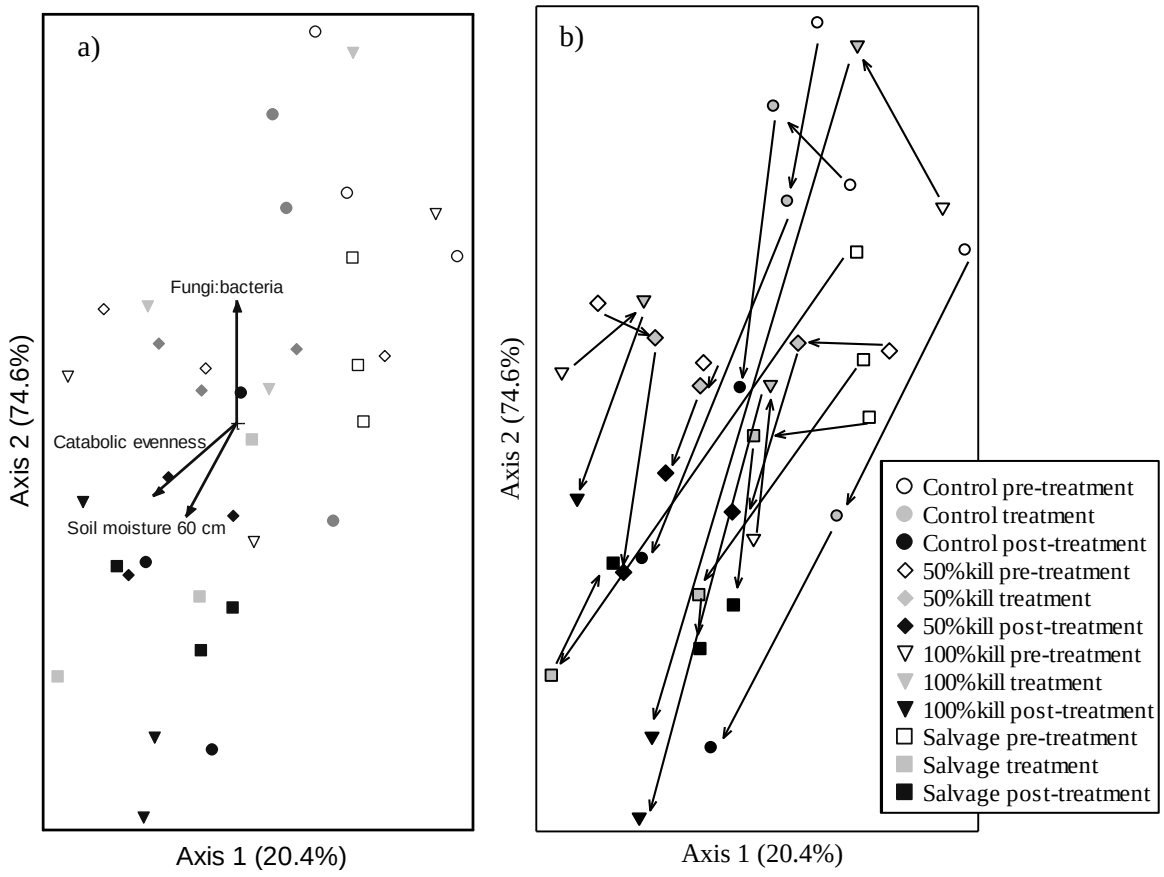

Fig. 4-5. Results of nonmetric multidimensional scaling ordination for the nutrient supply rates delineated by treatment and year presented with: a) the angles and lengths of the vectors which indicate direction and strength of relationships of the variables (see Table 4-2 for information on the variables) with the ordination axes (cut-off for displayed variables was $R^2 > 0.25$); and b) vectors that connect each experimental unit among the pre-treatment, treatment, and post-treatment years. The amount of variation explained by each axis is included in parentheses.

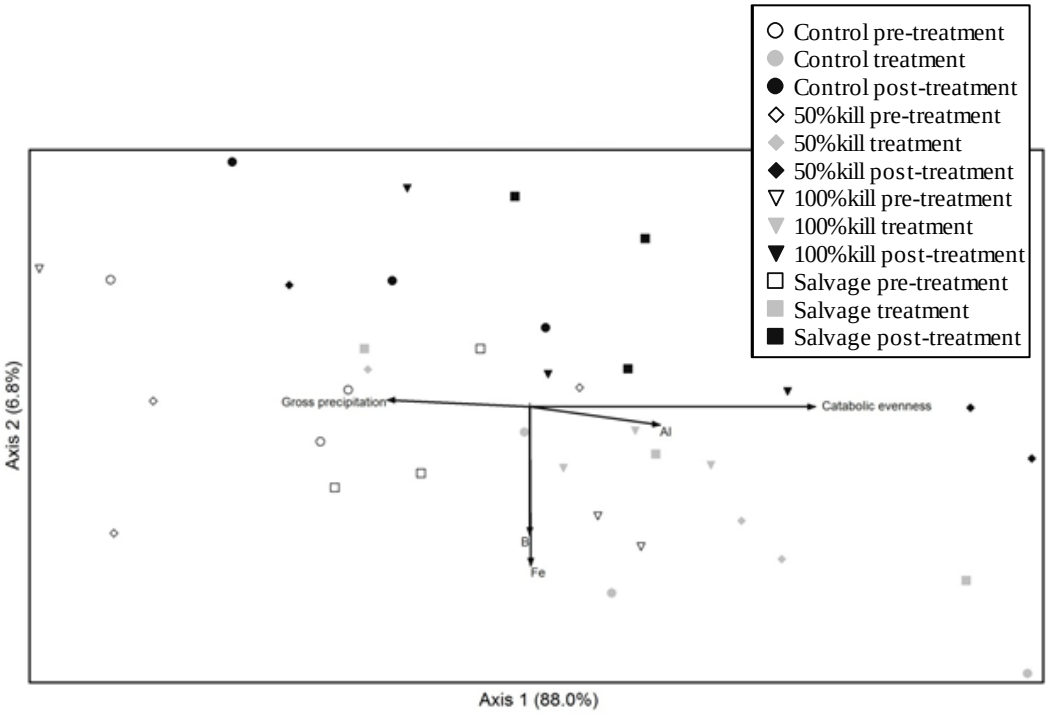

Fig. 4-6. Results of nonmetric multidimensional scaling ordination for the MSIR delineated by treatment and year. The angles and lengths of the vectors indicate direction and strength of relationships of the variables (see Table 4-2 for information on the variables) with the ordination axes (cut-off for displayed variables was $R^2 > 0.25$). The amount of variation explained by each axis is included in parentheses.

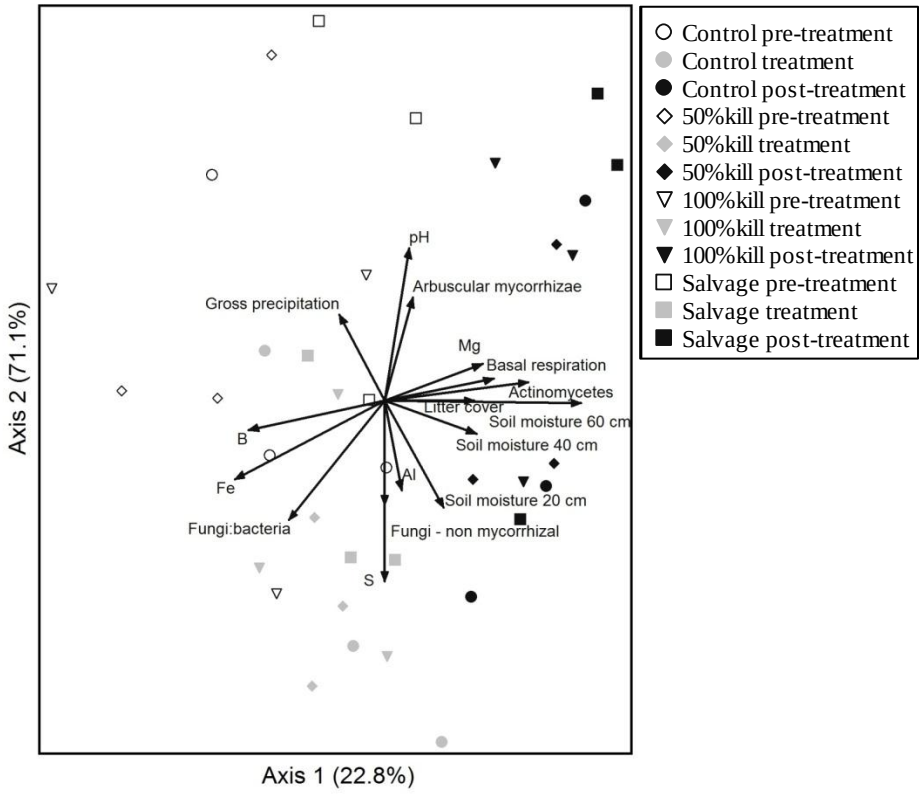

Fig. 4-7. Results of nonmetric multidimensional scaling ordination for the phospholipid fatty acid profiles delineated by treatment and year. The angles and lengths of the vectors indicate direction and strength of relationships of the variables (see Table 4-2 for information on the variables) with the ordination axes (cut-off for displayed variables was $R2 > 0.25$). The amount of variation explained by each axis is included in parentheses.

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4.6. Appendix 4-1. Plant species identified in the study, grouped by growth form.

Code	Genus	Species	Authority	Growth form
CLspp.	*Cladina*	spp.		lichen
PEAP	*Peltigera*	*aphthosa*	(L.) Willd.	lichen
BRST	*Brachythecium*	*starkei*	(Brid.) Schimp.	bryophyte
DIPO	*Dicranum*	*polysetum*	Sw.	bryophyte
HYSP	*Hylocomium*	*splendens*	(Hedw.) Schimp.	bryophyte
PLSC	*Pleurozium*	*schreberi*	(Brid.) Mitt.	bryophyte
POCO	*Polytrichum*	*commune*	Hedw.	bryophyte
PTCR	*Ptilium*	*crista-castrensis*	(Hedw.) De Not.	bryophyte
LYAN	*Lycopodium*	*annotinum*	L.	club-moss
LYCL	*Lycopodium*	*clavatum*	L.	club-moss
LYCO	*Lycopodium*	*complanatum*	L.	club-moss
DRAU	*Dryopteris*	*austriaca*	(Jacq.) Woynar ex Schinz & Thellung	fern
GYDR	*Gymnocarpium*	*dryopteris*	(L.) Newm.	fern
CACA	*Calamagrostis*	*canadensis*	(Michx.) Beauv.	grass
CAMO[i]	*Calamagrostis*	*montanensis*	Scribn. ex Vasey	grass
CILA	*Cinna*	*latifolia*	(Trevir. ex Göpp.) Griseb.	grass
ELIN	*Elymus*	*innovatus*	(Beal) Pilg.	grass
PHPR	*Phleum*	*pratense*	L.	grass
POPA	*Poa*	*palustris*	L.	grass
CAAE	*Carex*	*Aenea*	Fern.	sedge
ARNU	*Aralia*	*nudicaulis*	L.	herb
ARCO	*Arnica*	*cordifolia*	Hook.	herb
CHAN	*Chamerion*	*angustifolium*	L.	herb
COCA	*Cornus*	*canadensis*	L.	herb
EQSY	*Equisetum*	*sylvaticum*	L.	herb
FRVI	*Fragaria*	*virginiana*	Duchesne	herb
GABO	*Galium*	*boreale*	L.	herb
GOOB	*Goodyera*	*oblongifolia*	Raf.	herb
LIBO	*Linnaea*	*borealis*	L.	herb
LICO	*Listera*	*cordata*	(L.) R. Br.	herb
MACA	*Maianthemum*	*canadense*	Desf.	herb
MEPA	*Mertensia*	*paniculata*	(Ait.) G. Don	herb
MINU	*Mitella*	*nuda*	L.	herb
PEPA	*Petasites*	*palmatus*	(Aiton) A. Gray	herb
PYAS	*Pyrola*	*asarifolia*	Michx.	herb
PYSE	*Pyrola*	*secunda*	L.	herb
PYVI	*Pyrola*	*virens*	Schreb.	herb
SMTR	*Smilacina*	*trifolia*	(L.) Desf.	herb

Code	Genus	Species	Authority	Growth form
STAM	*Streptopus*	*amplexifolius*	(L.) DC	herb
TRHY	*Trifolium*	*hybridum*	L.	herb
TRPR	*Trifolium*	*pratense*	L.	herb
VIRE	*Viola*	*renifolia*	A. Gray	herb
ALCR	*Alnus*	*crispa*	(Aiton) Pursh	shrub
LOIN	*Lonicera*	*involucrata*	(Richards.) Banks ex Spreng.	shrub
OPHO	*Oplopanax*	*horridus*	(Sm.) Miq.	shrub
RHGR	*Rhododendron*	*groenlandicum*	(Oeder) Kron & Judd	shrub
RILA	*Ribes*	*lacustre*	(Pers.) Poir.	shrub
ROAC	*Rosa*	*acicularis*	Lindl.	shrub
RUID	*Rubus*	*idaeus*	L.	shrub
RUPE	*Rubus*	*pedatus*	J. E. Smith	shrub
RUPU	*Rubus*	*pubescens*	Raf.	shrub
SOSC	*Sorbus*	*scopulina*	Greene	shrub
SPBE	*Spirea*	*betulifolia*	Pallas	shrub
VACA[ii]	*Vaccinimum*	*caespitosum*	Michx.	shrub
VAMY[ii]	*Vaccinium*	*myrtilloides*	Michx.	shrub
VAVI	*Vaccinium*	*vitis-idaea*	L.	shrub
VIED	*Viburnum*	*edule*	(Michx.) Raf.	shrub
ABBA	*Abies*	*balsamea*	(L.) Mill.	tree
PIGL	*Picea*	*glauca*	(Moench) Voss	tree
PIMA	*Picea*	*mariana*	(Mill.) Britton, Sterns & Poggenb.	tree
PICO	*Pinus*	*contorta*	Douglas ex Loudon	tree
POTR	*Populus*	*tremuloides*	Michx.	tree

[i] *Calamagrostis montanensis* did not flower in our sites, so a small number of individuals may have been misidentified as *C. montanensis* that were in fact other graminoid species.

[ii] *Vaccinium caespitosum* and *Vaccinium myrtilloides* were combined for analysis because they were only identified to genus in the field.

Chapter 5. Potential for lodgepole pine regeneration after mountain pine beetle attack in newly invaded Alberta stands

Abstract **-** The range expansion of mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) from the focal region of the recent major epidemic in British Columbia, Canada to the north and east into Alberta's lodgepole pine forests may alter the future successional pathway of these forests. Thus, there is a need for an improved understanding of recruitment dynamics of lodgepole pine in both healthy and MPB-attacked stands arising from this shift from a fire-driven to MPB-driven disturbance regime. We evaluated the potential impacts of MPB attack and associated forest management on the future regeneration potential of lodgepole pine forests in western Alberta. We quantified seedbed availability and advance regeneration, and compared lodgepole pine recruitment of sown seed on five different seedbed types (i.e., moss, shallow organic, deep organic, decayed wood, and mineral soil) within four different stand treatment types that simulated MPB attack and forest management disturbance (i.e., control, moderate MPB attack, high intensity MPB attack, and salvage harvest) in the Upper Foothills of western Alberta. Recruitment from sown seed 1-3 years after MPB attack was poor (median 0%, 0-2% 5^{th}- 95^{th} percentiles) across stand treatment types for moss and both organic seedbed types. Decayed wood and mineral soil were the best seedbed types, with higher recruitment rates than the organic and moss seedbeds, although recruitment was still relatively low (median 0%, 0-6% 5^{th}- 95^{th} percentiles). Recruitment rates of seedlings on decayed wood and mineral soil seedbed types increased with increasing levels of disturbance; recruitment was lowest in control stands, higher in the simulated MPB-attack treatments, and highest in the salvage logged stands. However, these favorable seedbeds were scarce among all stand treatment types. Given the extremely low levels of advance regeneration and lack of natural regeneration we observed initially after MPB attack in our study sites, we anticipate that future stand development will be hampered by a lack of lodgepole pine recruitment, at least in the short term. If the goal is a re-stocked forest, significant silvicultural intervention will be required for lodgepole pine forests attacked by MPB within novel habitat ranges in western Alberta.

5.1. Introduction

Lodgepole pine (*Pinus contorta* Douglas ex Loud. var. *latifolia* Engelm.) forests have historically initiated as even-aged pioneer forests after stand-replacing wildfire. However, this historical disturbance regime is currently being altered, with increased impacts of disturbance by mountain pine beetle (MPB; *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), which is actively expanding its range in western

Canadian pine forests (Carroll et al. 2004, Nealis and Peter 2008). MPB is considered the most destructive forest insect in western North America with a recent epidemic in British Columbia resulting in mortality of 710 million cubic meters of timber over a cumulative affected area of ~ 18.1 million hectares and the area and impacts continue to grow (Furniss and Carolin 1977, Safranyik and Carroll 2006, BC 2012). In 2005 MPB moved east across the Rocky Mountains and quickly spread through the extensive stands of boreal lodgepole pine in Alberta; attack in the novel host jack pine (*Pinus banksiana* Lamb.) has now also been confirmed (Cullingham et al. 2011). Continued expansion of MPB further east and north across the boreal is possible, although this will depend on suitable climate and host availability. The long-term implications of the expanding range of this disturbance agent are uncertain, but MPB is likely to remain in Alberta (Schneider et al. 2010).

MPB differs from other disturbance agents, including wildfire, windthrow, and timber harvest, in that it directly affects the overstory without disturbance to the understory, forest floor or soil (Burton 2008). Severity of MPB mortality can range widely (0-100%) with environmental conditions (e.g., elevation, climate, topography), but in British Columbia (BC), Canada MPB has resulted in mean overstory tree mortality in the range of 25 - 50% of the pine trees per stand (Shore et al. 2006). Serotiny provides a long-term canopy seed bank available for dispersal after disturbance. This seed can remain viable for an extended period of time in the cones (e.g., Ackerman 1966, Teste et al. 2011a), with viability >30% possible even after 20+ years (Aoki et al. 2011). While we would expect cone serotiny to be detrimental to pine regeneration in the absence of fire, a study in BC showed 45% of the canopy seedbank was released via cone opening, breakage of cone-bearing limbs, and squirrel predation within six years of MPB attack, with a sustained seedbank release even nine years after attack (Teste et al. 2011b). Thus, we do not expect the seed availability and viability to be limiting factors in the

regeneration of lodgepole pine after MPB attack. However, the sustained longer-term release of seed in MPB disturbed stands, as compared to the synchronous release of seed from serotinous cones which occurs after fire disturbance, will have important implications for the post-disturbance development of these forests. More importantly, seedbed limitations and environmental conditions in the understory will likely be dominant factors influencing regeneration.

A better understanding of the availability of seedbeds favorable to lodgepole pine recruitment in MPB attacked stands, compared with both undisturbed and managed lodgepole pine forests is required (Dhar and Hawkins 2011). Teste et al. (2011b) suggested that after MPB attack, either fire or anthropogenic disturbance would be needed for normal levels of pine regeneration to occur. Favorable germination microsites will depend on the availability of both suitable seedbeds for germination and survival of germinants, and the associated environmental conditions, including light availability (Wright et al. 2000), and soil moisture status (Despain 2001). While lodgepole pine can regenerate on a wide range of seedbeds, it generally prefers mineral soil, decayed wood or disturbed organic material (Lotan 1964, Nyland 1998), which are unlikely to be dominant seedbeds in MPB attacked stands that have a predominately undisturbed forest floor (Astrup et al. 2008). Thus, unsalvaged beetle-killed stands may lack the ground disturbance needed to create a suitable seedbed for natural regeneration of lodgepole pine, and are likely to regenerate slowly with low densities (Mitchell 2005).

Research in multiple regions has suggested that the pre-disturbance composition and dynamics of the advance regeneration seedling/sapling bank will play an important role in determining the future structure and dynamics of these forests after MPB attack (e.g., Collins et al. 2011). In some lodgepole pine forest types, advance regeneration includes sufficient lodgepole pine to meet stocking density guidelines for a new forest (e.g., FPBSR 2007 and Nigh et al. 2008 in BC, Diskin et al. 2011 in CO, Kayes and

Tinker 2012 in WY). However, in other lodgepole pine forest types, including those with mixed species composition, the advance regeneration often favors a shift towards later-seral shade-tolerant species such as spruce and fir (e.g., Vyse et al. 2009, see review by Dhar and Hawkins 2011). Yet other studies have shown limited regeneration of any species after MPB attack (e.g., Astrup et al. 2008). For lodgepole pine forests without a seedling bank the future successional pathway is uncertain.

Management for MPB can range from individual tree removal to post-attack salvage harvest to managing for other objectives, such as watershed protection and wildfire fuel management, in areas where infestation levels are uncontrollable (ASRD 2007). In salvage logged stands, serotinous cones on or near the ground open and release their seed when exposed to warm soil surface temperatures (Lotan 1964), thus creating a short-term seed bank for natural regeneration (Ackerman 1966) that can contribute to regeneration of fully-stocked lodgepole pine stands (e.g., Collins et al. 2010). But given the magnitude of MPB attack and associated dead timber on the landscape, there may also be a large portion of the MPB-attacked landscape left unmanaged to undergo natural successional processes (Mitchell 2005). For example, < 15% of beetle-killed forests in Colorado are likely to be actively managed (Collins et al. 2011). Thus, we need to better understand the consequences to stand regeneration of leaving a portion of the MPB attacked landscape unmanaged.

We are unaware of any previous studies that have compared the effects of MPB and associated forest management on lodgepole pine recruitment experimentally in situ using a direct seeding experiment, and this the first study we are aware of that examines the potential for regeneration in newly invaded stands in MPB's expanded range east of the Canadian Rockies. The overall goal of this study was to examine the regeneration potential of lodgepole pine stands post MPB attack, focusing on a region where MPB is a novel disturbance agent. Our main objectives were to: i) quantify the availability of

seedbed types for germination; ii) measure the abundance of advance regeneration; and iii) compare lodgepole pine germination and survival (i.e., recruitment) on five seedbed types (moss, shallow organic, deep organic, decayed wood, and mineral soil). We addressed these objectives among three different stand treatment types that simulated MPB attack and associated forest management disturbance (i.e., control, moderate intensity MPB attack, high intensity MPB attack, and salvage harvest) in the Upper Foothills of western Alberta.

5.2. Methods

Study area

The study area was located in the Upper Foothills natural sub-region of Alberta (Natural Regions Committee 2006) in lodgepole pine forests near Robb, AB. This area is characterized by pure lodgepole pine forests that are highly serotinous, along with mixed conifer stands of white spruce (*Picea glauca* (Moench) Voss) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.). Stand ages in this region are generally younger than 100-120 years old reflecting the regional disturbance regime of relatively frequent stand-initiating wildfire (Beckingham et al. 1996). The climate is temperate continental with mean daily maximum air temperatures during the growing season ranging from a daily maximum of 16.2 ^{0}C in May, to 20.6 ^{o}C in August. Snow usually covers the frozen ground from the end of October to late April. Mean monthly precipitation during the growing season is as follows: 57.9 mm (May), 106.7 mm (June), 106.2 mm (July), and 82.2 mm (August), with a mean annual precipitation of 562.4 mm, of which approximately 75% falls as rain (30 year climate normal 1971-2000). The study stands were approximately 110-120 years old, and were located on brunisolic gray luvisolic soils. The study area was classified as ecosite UF e1.1 – Pl/green alder/feather moss (Beckingham et al. 1996). The overstory included only lodgepole pine; there were a very few white spruce (*Picea glauca*

(Moench) Voss), black spruce (*Picea mariana* (Mill.)), trembling aspen (*Populus tremuloides* Michx.), and balsam fir (*Abies balsamea* (L.) Mill) in the lower canopy. The understory was dominated by feather mosses, including *Pleurozium schreberi* (Brid.) Mitt., *Polytrichum commune* Hedw., *Ptilium crista-castrensis* (Hedw.) De Not., and *Hylocomium splendens* (Hedw.) Schimp. Common forbs included *Cornus canadensis* L. and *Linnea borealis* L., common small shrubs included *Rosa acicularis* Lindl. and *Vaccinium myrtilloides* Michx.; *Alnus crispa* (Aiton) Pursh was the dominant tall shrub, and the common graminoid was *Calamagrostis montanensis* (Michx) Beauv..

Experimental design

We used a randomized block split-split-plot design to study the influence of four stand-level simulated MPB and forest management treatment types, five seedbed types, and three years on germination and early survival of sowed lodgepole pine seeds. There were three blocks in the study (~ 5 – 10 ha each) and a total of 12 experimental units (n=3 replicate blocks * 4 experimental units per block). The four treatments were each applied to one experimental unit (i.e., stand = 0.48 ha) within each block in early summer 2009 using a randomized complete block design: i) untreated control (hereafter "Control"), ii) simulated moderate intensity MPB attack (hereafter "50% kill"), iii) simulated high intensity MPB attack (hereafter "100% kill"), and iv) clear-cut – salvage harvested to simulate a typical management treatment post MPB attack (hereafter "Salvage"). While MPB selectively kills larger and older trees in an endemic setting, our research was focused on epidemic levels of MPB, which are occurring at unprecedented levels on the landscape. Therefore, we selected targets of 50% kill and 100% kill to capture a gradient of mortality. A 10-m x 10-m grid was overlaid on each stand to facilitate placement of a subset of nine systematically-located points for sampling the overstory, forest floor thickness, saplings, seedbed availability, natural regeneration, and recruitment rates for

the spring-sown seed (minimum distance 20 m apart), and an additional 9 randomly selected sampling points on our grid were used to sample fall-sown seed recruitment rates (minimum distance 10 m apart).

Application of treatments

We used stem injection application of glyphosate herbicide to chemically girdle individual stems to simulate MPB attack in these treated stands. Glyphosate is a systemic herbicide that kills vegetation by inhibiting the enzyme 5-enolpyruvylshikimate 3-phosphate synthase involved in the synthesis of aromatic amino acids, it rapidly reacts with and is inactivated by most soils (Baylis 2000). EZ-Ject selective injection herbicide capsules (Glyphosate 0.15 grams per capsule, ArborSystems, Omaha, NE http://www.ezject.com/**)** were injected at a rate of 1 capsule per 5 cm tree dbh per tree for trees 10– 20 cm dbh, or 1 capsule per 3cm dbh per tree for trees > 20cm, with capsules equally spaced around the circumference of the tree near the base of the bole. In the 100% kill experimental units, all trees $\geq$ 10 cm dbh (selected as minimum size of trees attacked by MPB - Safranyik and Carroll 2006) were injected. In 50% kill stands, because of root-to-root transfer of glyphosate among neighboring trees, every 3rd tree $\geq$ 10 cm dbh was injected with glyphosate to achieve the desired rate of 50% overstory mortality. Chemical girdling was completed in the treatment year from June 15-19, 2009.

Salvage harvest operations used a "stump-side processing system" in which a feller-processor unit dropped the tree and then limbed and cut it in sections and laid it on the ground. These stands were not herbicided to mimick mountain pine beetle prior to salvage. A tracked forwarder moved the logs out of the experimental unit. This system leaves debris and cones distributed onsite to facilitate regeneration and is preferable to feller/buncher/skidder operations for small harvest units, where the latter approach leaves a massive pile of debris in the middle of the unit. Harvest operations were completed by

West Fraser Timber Company, with one block being cut at a time between late July and early August in the treatment year (2009). No site preparation, e.g., scarification or burning, was applied to any of the harvest areas and vegetation was allowed to regenerate naturally for the duration of the study.

Data collection

In summer 2010, we sampled the overstory plant community in 8-m fixed-radius (0.02 ha) circular subplots centered at each of the nine systematic sample points within each stand. Standard forest mensuration data were collected for all trees (i.e., with dbh $\geq$ 5 cm and ht > 1.3 m) within each subplot, including species, dbh, and live/dead status. Live status of trees was determined based on a visual assessment of tree crowns: healthy trees had green full crowns, dying trees had crowns with moderate to significant quantities of red needles, and dead trees lacked needles, or if needles were still present they were all red. These data were used to calculate basal area and stem density, separated by live/dead status (healthy, dying, dead). To estimate canopy cover, hemispherical photographs were taken in the middle of the growing season (mid-July) at each of the nine systematically located sample points using a digital Nikon Coolpix 4500 with FC-E8 fisheye lens. Hemispherical photographs were taken approximately 1.4 m above the forest floor, with the camera leveled on a tripod and the bottom of the camera oriented towards North. We analyzed canopy photographs using SLIM (Spot Light Intercept Model v. 3.01), using batch processing to analyze photos with manual color threshold adjustments by plot to optimize differences between canopy and sky. The program calculated gap fraction, which measures the area of overhead view (in percent) which constitutes canopy gaps, and we subtracted gap fraction from 100 to provide an estimate of canopy cover at each sample point. The thickness of the forest floor (excluding the recent litter fall, or L layer, but including both Fibric and Humic layers – i.e., F/H, mm) was measured in each of the

four corners of the nine seedling quadrats (quadrats described below). A summary of these forest attributes for the study units is provided in Table 5-1.

We estimated availability of different seedbed types and quantified advance regeneration for the 12 stands using 9 1-m^2 quadrats centered on the systematically-located sample points. To estimate relative seedbed availability, we estimated percent cover (0-100) of moss, exposed mineral soil, downed dead wood (any decay class), exposed rock, and organic matter/litter within each quadrat. Total cover estimates exceeded 100 % because of overlap among the litter recorded within the organic matter seedbed and the other seedbed elements. We counted the number of live tree seedlings (i.e., < 1.3 m height) by species. We also tallied live tree saplings (i.e., taller than 1.3 m ht and dbh < 5 cm) by species within 4-m radius circular plots centered at the same nine sampling points. We also conducted an additional census for advance regeneration within each stand; four people surveyed each stand for an hour and tallied the total number of pine seedlings found.

We sowed 100 lodgepole pine seeds at five subplots (one for each seedbed type) at each of nine sample points within each of the twelve stands for each of two seasons: i) a set sowed at randomly-selected sample points in the fall before snowfall in 2009 (the year of treatment application) (hereafter 'fall sowed'), and ii) a set sowed at the systematic sample points that was cold-stratified and sown in the spring of 2010 after the snow had melted (hereafter 'spring sown')(n=1080 seed subplots). We seeded five forest floor seedbed types : i) exposed mineral soil; ii) mineral soil covered by a Fibric/Humic (FH) layer < 2.5 cm depth (hereafter 'shallow organic'); iii) mineral soil covered by a FH layer > 2.5 cm depth (hereafter 'deep organic'); iv) feather moss; and v) decayed wood (i.e., considerably decomposed wood that was incorporated into the forest floor). When we could not locate naturally-occurring mineral and/or decayed wood seedbed types close to a sample point, we carefully removed the forest floor to expose mineral and decayed

wood to create these seedbed types. From each sampling point, the nearest location (usually within 5 meters) of each seedbed type comprising an area of 10-cm by 10-cm (100 cm^2) was chosen for seeding. Seeds were sprinkled on the surface to mimic the effects of natural wind dispersal that would occur after cones opened and were not caged to exclude predators because we wanted to mimic the natural conditions for recruitment within these stands. The lodgepole pine seed we used was collected from the seed zone of the study area by the Alberta Tree Improvement and Seed Centre, Smoky Lake, AB, and originated from seed zone UF1.4 and had a stratified germination rate of 65% (D. Palamarek personal communication). Unseeded control subplots were also established on whatever seedbed was present at each of the nine systematically located sampling point centers in each stand to assess any background recruitment occurring from natural seed rain in the area. We counted the number of lodgepole pine germinants within each seedbed type and control subplot bi-weekly throughout the summer of 2010. In June 2011 and July 2012 we revisited the plots once and counted the number of germinants within each seedbed type and control subplot.

Statistical analyses

To assess germination and survival rates (hereafter 'recruitment' rates), we calculated percent recruitment as the number of germinants (surviving at a given point in time) divided by the number of sown seeds within each seedbed type subplot. We used a three-way repeated measures ANOVA (Proc Mixed) to test for significant (α= 0.05) effects of treatment type, seedbed type, year, and their interactions on recruitment using data on the % surviving germinants from the last visit of the growing season in 2010 and from the single visits in 2011 and 2012. This was done for the fall- and spring-sown sets separately. Analysis of residuals and normal probability plots revealed non-constant variance and lack of normality for juvenile recruitment, so these data were logit

transformed prior to analysis. When significant main effects were detected, we used post-hoc linear contrasts to make pairwise comparisons among seedbed types within stand treatment types, between stand treatment types for a given seedbed type, and compared them within and among years, using Bonferroni-adjusted P-values (family-wise α=0.05) (Proc Mixed). SAS software (version 9.2 (32 bit); SAS Institute Inc., Cary, North Carolina. ©2000) was used for all analysis.

5.3. Results

Seedbed composition

The forest floor seedbed was dominated by feather mosses and litter for all the stand treatment types except for the salvage harvested areas, which were dominated only by litter (Table 5-2). All four stand treatment types had much lower cover of downed wood compared with feather moss and litter (Table 5-2). Exposed mineral soil cover was very low in all stand treatment types, although the salvage logged stands had the highest cover of mineral soil (Table 5-2).

Advance regeneration

There was minimal natural advance regeneration in the study sites, with very low quantities of seedlings. There was a single lodgepole pine seedling located on a squirrel midden in one quadrat in a 100% kill stand (Table 5-3). We only counted four juvenile aspen within quadrats: three in 100% kill stands and one in a 50% kill stand. No statistical analysis could be conducted on the seedling data because of low sample sizes. Saplings of aspen, balsam fir, and white spruce existed in some of the stand treatment types, but mean sapling counts were very low in all stand treatment types (Table 5-3). There were no lodgepole pine saplings in any of the 4-m^2 radius sapling plots and in the

pine seedling/sapling census we saw a range of only 0-5 seedlings/saplings per stand, which translates to a density of 0-10.4 understory pine seedlings/saplings per ha.

Recruitment from fall-sown seeds

Recruitment rates differed among the stand treatment types and among the three years for the fall-sown seeds, but the only significant interaction was between seedbed type and stand treatment type (Table 5-4). Comparing among the five seedbed types within stand treatment types, there were no significant differences in recruitment rates within the control treatment type, but there were in the other three stand treatment types (Fig. 5-1a). In the 50% kill stand treatment type, moss and deep organic seedbed types had significantly lower recruitment rates than the mineral soil, shallow organic, and decayed wood seedbed types (Fig. 5-1a). In the 100% kill stand treatment type, moss had the lowest recruitment rate, but it did not significantly differ from deep organic or decayed wood (Fig. 5-1a). The mineral soil seedbed type had the highest recruitment rate in the 100% kill stand treatment, but was not significantly different than decayed wood. In the salvage logged stand treatment type, mineral soil had the highest recruitment rate differing from all the other seedbed types (Fig. 5-1a). Comparing among years for all seedbed types combined, the percent of seed sown in fall 2009 that germinated and survived was highest in 2010, intermediate in 2011, and decreased to the lowest rates in the third year of the study (Fig. 5-1b). No tree seedlings naturally recruited into the control (i.e., unsowed) subplots.

Recruitment from spring-sown seeds

Patterns in recruitment rates of germinants from the spring-sown seeds differed among the stand treatment types and among the years, with significant interactions between seedbed and both stand treatment type and year (Table 5-4). There were significant differences among the seedbed types for all four stand treatment types (Fig. 5-2a). In the

control stand type, moss had the lowest recruitment rate, although it was not significantly different than the rate for the deep organic seedbed; decayed wood had the highest recruitment rate, although it was not significantly different than the mineral soil rate (Fig. 5-2a). In the 50% kill stand treatment, moss again had the lowest recruitment rate, although it was not significantly different than the shallow organic seedbed type (Fig. 5-2a). Mineral soil had the highest recruitment rate, but this was not significantly different from the recruitment on the decayed wood seedbed type (Fig. 5-2a). In the 100% kill stand treatment type there was a clear separation into two recruitment groups; moss and the two organic seedbeds had significantly lower recruitment rates than the decayed wood and mineral soil seedbed types (Fig. 5-2a). In the salvage logged stands, there was also a clear pattern of the highest recruitment rates on decayed wood and mineral soil, but with the two organic seedbed types having intermediate recruitment rates that were significantly higher than for the moss seedbed type (Fig. 5-2a).

Comparing each of the five seedbed types across stand treatment types, there were only significant differences in the decayed wood and mineral soil seedbed type recruitment rates (Fig. 5-2a). For decayed wood, recruitment rate was significantly higher in the salvage logged stands compared with intermediate rates in the 100% kill stands, and the lowest recruitment rates in the control and 50% kill stand types (Fig. 5-2a). Mineral soil seedbed recruitment was significantly higher in the salvage logged stand treatment type compared with the other three stand treatment types (Fig. 5-2a).

There were significant differences among years for the seedbed types for the spring-sown seeds (Fig. 5-2b). In all three years, the decayed wood and mineral soil seedbed types had the highest recruitment rates (Fig. 5-2b). The percent of sown seed that germinated and survived to the end of 2010 and then 2011 was intermediate on the organic seedbed types and lowest on the moss seedbed, although not significantly lower than the deep organic seedbed type in 2010. Survival to the end of 2012 was similarly

low for the moss and two organic seedbed types. Comparing among years for the seed originally sown in 2009, there were only significant differences in recruitment rates for the decayed wood and mineral soil seedbed types, which were both significantly lower in 2011 and 2012 compared with 2010 (Fig. 5-2b).

5.4. Discussion

Our finding of no or very low densities of advance regeneration in these forest stands contrasts with previous studies that have found abundant advance lodgepole pine regeneration after MPB attack, with the potential to produce reasonably well stocked stands or at least provide a significant contribution to future stand stocking in other regions including southern BC (Nigh et al. 2008), Colorado (Collins et al. 2011), and Wyoming (Kayes and Tinker 2012). Our study also differs with studies that have shown abundant advance regeneration of other tree species that could contribute to a successional shift to more shade-tolerant species composition of the future overstory (Dhar and Hawkins 2011). Our findings suggest that, given the minimal advance regeneration in these monoculture pine forests, their future regeneration will require recruitment of newly released seed and/or active management.

We did not observe natural recruitment of seedlings for any of the stand treatment types. Our findings are consistent with Astrup et al. (2008) who found limited natural regeneration even ten years after MPB attack in BC, which they attributed to the intact feather moss-dominated seedbed. An intact feather moss layer in our stands is likely contributing to the low levels of recruitment we observed. Our results differ with Collins et al. (2011) who found that during an MPB outbreak in Colorado, seedlings established in nearly all of the attacked stands they sampled and contributed to the future stand development. However, the seedbed in those sites was comprised of a relatively thin forest floor with <25% herbaceous cover and no moss, which likely contributed to

the relatively high seedling recruitment (Collins et al. 2011). We also found no natural recruitment in the salvage logged stands where many of the cones on the ground had opened (personal observation); this suggests that seedbed availability is likely a limiting factor for natural regeneration.

Our direct seeding experiment allowed us to compare regeneration potential among stand treatment and seedbed types over time without seed rain being a limiting factor. The fall- and spring-sown seeds showed similar patterns in terms of relative differences in recruitment rates over time; germination peaked in the first year (2010) and there was a decline in the survival of these germinants over the 3 year time period. In general, we found that recruitment rates for the fall-sown seeds were much lower across seedbed and stand treatment types compared with the spring-sown seeds. This could be due to predation, as suggested by Wright et al. (1998) who found the same decline in recruitment rates for overwintered seeds. However, Radvanyi (1971) found increased seed predation in the summer compared with in the winter in lodgepole pine clearcuts in central Alberta, suggesting that summer predation also contributed to the low germination rates in our study for both sets of sown seed. The decreased recruitment may also have resulted from seed movement from the recruitment subplots prior to recruitment, as the snow melting in the spring could have shifted the seeds, especially on the mineral and decayed wood seedbeds, which had relatively smooth surfaces compared with the other three seedbed types. However, this seems unlikely as we did not observe any germinants in areas adjacent to our recruitment subplots. Our findings suggest that if seeding was to be used as a reforestation strategy in these lodgepole pine stands, that it would be best to disperse seed in the spring rather than the fall, even though the latter is more representative of natural dispersal patterns after fire (Astrup et al. 2008).

While the absolute recruitment rates for the spring-sown seeds were higher than for the fall-sown seeds, both showed similar patterns in terms of relative differences in

recruitment rates among seedbed types; we consistently found that decayed wood and mineral soil seedbed types had higher recruitment rates than the organic and moss seedbed types did. While forest floor feather mosses have been shown to be positively associated with recruitment of boreal conifers (e.g., Parker et al. 1997), our study showed the opposite for lodgepole pine; the moss seedbed type had consistently no or low recruitment across treatment types. Thus, the potential benefits that mosses can confer to seed, such as decreased vascular plant competition and maintaining a consistent moisture supply (Munier et al. 2010), did not appear to be beneficial to pine regeneration in these stands. In a lodgepole pine seeding experiment in BC, organic seedbeds had higher recruitment rates than moss, but they did not compare these seedbeds with mineral soil (Wright et al. 1998). Given the high rates of germination on organic seedbeds, Wright et al. (1998) suggested mineral soil was not required for good regeneration levels, but that a moderate level of ground disturbance to remove the moss layer would greatly improve seedling establishment. Other studies have also suggested that shallower organic layers were more favorable than deeper organic seedbeds (Greene et al. 2007); however, we found little evidence of this, except for higher recruitment of fall-sown seed for the shallow organic versus the deep organic and moss seedbed types in the 50% kill stand treatment type. Our finding that decayed wood and mineral soil were the most favorable seedbeds is consistent with other studies that have shown these to be the most favorable seedbeds for lodgeople pine germination (Lotan and Perry 1983, Landhäusser 2009). Thus, given the generally low abundance of both exposed mineral soil and decayed wood, ground disturbance that exposes mineral soil and/or decayed wood would likely greatly improve recruitment rates in these stands.

Both the fall- and spring-sown seeds also showed similar patterns in terms of relative differences in recruitment rates among stand treatment types; we observed the lowest rates of recruitment in control stands, intermediate recruitment rates in the

simulated MPB attack stands and highest rates in the salvage logged stands, particularly for the most favorable seedbeds, mineral soil and decayed wood. In a study in Colorado, new seedling recruitment was four times higher in salvage logged stands, compared with unsalvaged MPB attacked stands (Collins et al. 2011); this was attributed to a decreased density of competing herbaceous vegetation and reduced organic depth. Greene et al. (2009) showed broad consensus that initial survivorship in forest gaps for boreal tree species was highest on mineral soil, humus, and well-rotted logs, and suggested similar patterns for intact forests. This pattern is consistent with our findings of highest recruitment levels on the decayed wood and mineral seedbeds in the salvage logged stands, with a gradient of decreasing recruitment on these seedbeds in the MPB and control stand treatment types. In contrast to our findings, Lepage et al. (2000) found regeneration of lodgepole pine in partially-disturbed stands was best on rotten wood, and interestingly, regeneration on mineral soil was very poor; however they had very low sample sizes for lodgepole pine. Our findings suggest that recruitment of lodgepole pine will be higher in stands that are salvage logged, compared with MPB-attacked stands that are left unsalvaged.

Our findings of differences in recruitment rates for decayed wood and mineral soil among stand treatment types suggests that other factors beyond what we measured are also contributing to the variable recruitment rates among stand treatment types. Microclimatic differences among the stand treatment types may also be contributing to these patterns. We expect that as the attacked stands drop their needles and branches and transition to grey attack, the increase in light may lead to an increased pulse in shade-intolerant pine regeneration, as has been proposed for regeneration in other regions (Axelson et al. 2009, Kayes and Tinker 2012). Interestingly, other studies have suggested that light is not the primary factor determining regeneration (e.g., Stuart et al. 1989, Williams et al. 1999). Rather than light limitation, drought has been identified as a

primary factor leading to mortality of pine seedlings (e.g., Despain 2001), with soil moisture and microclimate considered the limiting factors for lodgepole pine natural regeneration (Stuart et al. 1989). A companion study adjacent to our research sites examined patterns in soil moisture (at 5 cm depth in the mineral soil) among the stand treatment types in the summer of 2010 and found a gradient of moisture from the driest soil moisture conditions in the control stand treatment type, with a trend of increasing soil moisture in the 50% kill (10% greater than the control) and 100% kill (13% greater than the control) stand treatment types, with the salvage logged stands being the wettest (33% greater than the control; Piña 2012). This gradient of increasing soil moisture associated with increasing intensity of disturbance could also be contributing to the overall increase in recruitment in the most favorable seedbed types, especially given that the canopy cover was relatively consistent between the MPB and control stand treatment types. Thus, the patterns of increasing recruitment levels in the salvage logged stands for the mineral and decayed wood seedbed types suggests that both seedbed and additional factors that we did not explicitly study, such as light and soil moisture, are important factors in the regeneration of lodgepole pine in these stands.

Regardless of changes in environmental factors influencing regeneration, an increase in availability of favorable seedbeds is critical for successful recruitment in these forests. Across stand treatment types, there was a scarcity of favorable seedbeds for lodgepole pine recruitment. Even in the salvage logged stands where we had the most soil disturbance occur, mineral soil covered < 2% of the available seedbed, and there was a maximum of 14% wood, much of which was not yet decayed (personal observation). Thus, unsalvaged MPB killed stands may lack the ground disturbance required for natural regeneration of lodgepole pine (Mitchell 2005). As the stand breaks up and trees drop to the ground, soil disturbance should increase, creating additional mineral seedbeds. However, in a Colorado MPB attack, there were no differences between fine or coarse

DWM loads in uninfested stands and stands with current or recent (up to 7 years prior) MPB attack (Klutsch et al. 2009). Simard et al. (2011) found no differences in surface fuel biomass among unattacked, red, and grey stages of MPB attack. There will be an as yet unknown time lag between when trees are killed and they fall and disturb the soil; MPB-killed trees in central BC did not start to fall until eight years after they died (Lewis and Thompson 2011). It seems unlikely that there will be a pulse of decayed wood or mineral soil available as seedbeds in the short term after MPB attack.

In conclusion, our findings of minimal advance regeneration, no natural recruitment post attack, and the highest recruitment rates on mineral soil and decayed wood, which were scarce across all four stand treatment types and declined over time, suggest that significant silvicultural intervention will be required to supplement regeneration if the goal is a stocked future forest, in particular for the MPB attacked stands. For attacked forests similar to those in our study that are left unmanaged, which may comprise a large portion of the landscape depending on the magnitude of MPB outbreak in Alberta, it appears that regeneration towards a replacement forest will be slow, owing in part to the lack of both advance regeneration and low availability of suitable seedbed types and associated environmental factors. Another potential concern may be that by the time favorable seedbed types are more available for germination, seed may have already been released from the cones, although Teste et al. (2011b) showed a sustained seed rain even nine years after MPB attack. Future research is needed that monitors recruitment and longer-term survival of seedlings in attacked forests as they transition to grey attack and eventually fall to the ground in order to better predict the future successional pathway of MPB-disturbed stands that lack additional anthropogenic or natural wildfire disturbances.

Table 5-1. Mean (± SE) values for lodgepole pine stand attributes for the four treatments measured in 2010, which was the first growing season after the treatments were applied and the year in which the first set of germinant measurements were recorded.

Stand treatment	Basal area (m^2 ha^{-1})			Trees ha^{-1}			Canopy cover	Forest floor(FH) thickness
	Healthy	Dying	Dead	Healthy	Dying	Dead	(%)	(mm)
Control	30.0	5.1	16.2	970	172	987	64.1	76.6
	(1.6)	(0.8)	(2.9)	(101)	(15)	(227)	(1.3)	(3.1)
50%kill	20.4	12.8	16.1	574	331	894	61.1	78.7
	(2.7)	(1.6)	(0.9)	(114)	(15)	(93)	(2.0)	(4.3)
100%kill	7.6	20.9	22.9	202	585	1220	60.0	84.7
	(2.8)	(0.7)	(3.3)	(46)	(44)	(267)	(1.5)	(3.9)
Salvage	0	0	0	0	0	0	0	64.6 (3.8)

Table 5-2. Mean (± SE) seedbed availability (% of ground surface) for the four treatment types, measured in 2010, which was the first growing season after the treatments were applied and the year in which the first set of germinant measurements were recorded.

Treatment	Litter (%)	Mineral (%)	Moss (%)	Wood (%)[i]
Control	55.4 (2.9)	0.1 (0.1)	72.6 (4.7)	10.5 (1.6)
50%kill	57.0 (3.4)	0 (0)	58.2 (4.3)	13.9 (3.0)
100%kill	63.7 (3.7)	0 (0)	49.9 (5.7)	8.9 (1.5)
Salvage	89.2 (2.1)	1.6 (1.1)	1.3 (0.5)	8.4 (1.1)

[i] This includes both non-decayed and decayed wood.

Table 5-3. Mean number of saplings (taller than 1.3 m ht and dbh < 5 cm) per 50 m^2 plot within each treatment type (range of counts provided in parentheses).

	Sapling count[i]				
Treatment	ABBA	PICO	PIGL	POTR	Total
Control	0	0	0.07 (0-2)	0	0.07 (0-2)
50%kill	0.04 (0-1)	0	0.07 (0-1)	0.41 (0-9)	0.52 (0-9)
100%kill	0.11 (0-2)	0	0	1.89 (1-33)	2 (0-33)
Salvage	0	0	0	0	0

[i] ABBA is balsam fir, PICO is lodgepole pine, PIGL is white spruce, POTR is quaking aspen

Table 5-4. Results (P values) of repeated measures ANOVAs testing for the effects of seedbed type, stand treatment type, year, and the interactions among them on recruitment rates for fall- and spring- sown seed. Significant P-values are highlighted in bold.

Season sowed	Seedbed	Treatment	Year	Seedbed*Treatment	Seedbed*Year	Treatment*Year	Seedbed*Treatment*Year
Fall	**<0.0001**	**0.01**	**0.02**	**<0.0001**	0.16	0.70	1.0
Spring	**<0.0001**	**0.004**	**0.0004**	**<0.0001**	**0.0009**	0.31	1.0

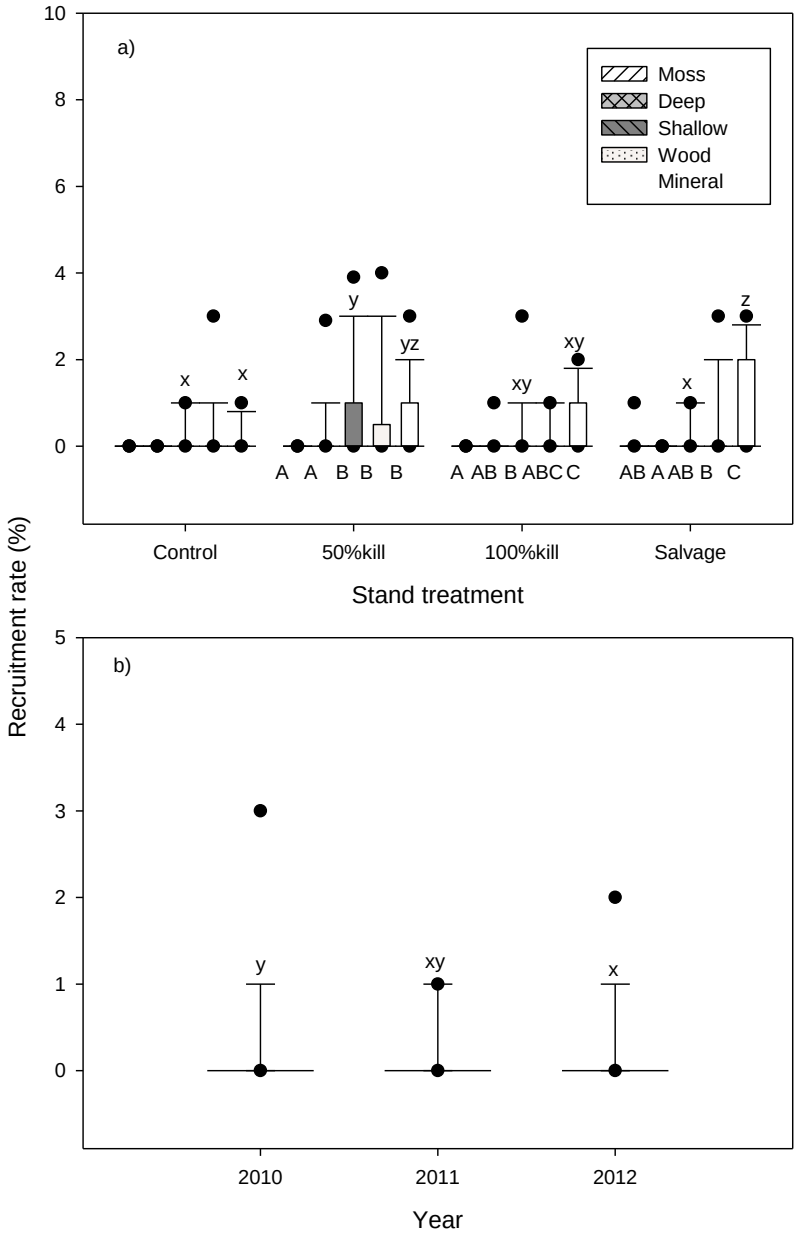

Fig. 5-1. Recruitment rates (%) for fall-sown seed for a) mean % of sown seeds surviving to the end of each year (averaged for 2010, 2011, 2012) for each of the five seedbed types (ordered from left to right within each treatment as follows: moss, deep organic, shallow organic, decayed wood, and mineral soil) and b) averaged across seedbed types for each year separately. The boundaries of the box are the 25th and 75th percentiles and the line in the middle of the box is the median. Whiskers above and below the box indicate the 90th and 10th percentiles and the circles below and above indicate the 5th and 95th percentiles. Capitalized letters (A,B,C) directly below boxplots indicate significant differences among seedbed types within a given treatment; lower case letters (x, y, z) above the boxes indicate significant differences a) among treatment types for each seedbed type separately or b) among years for all seedbed types combined.

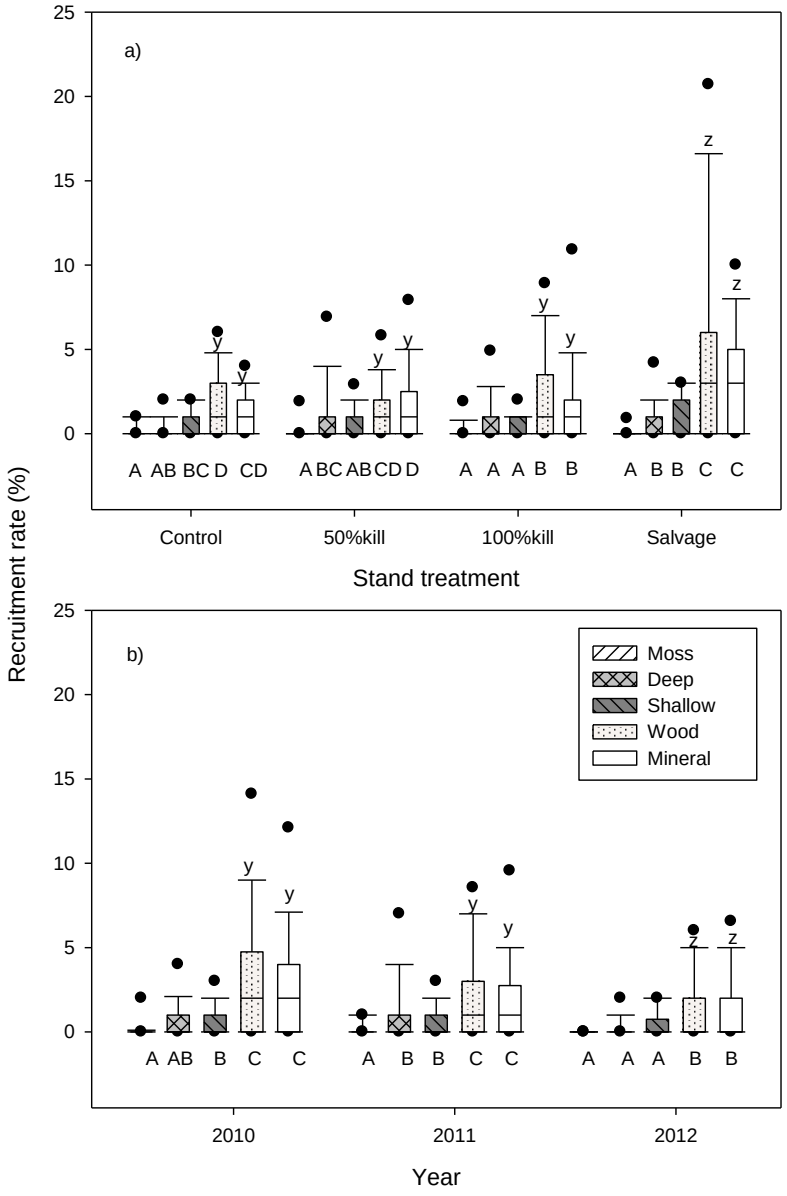

Fig. 5-2. Recruitment rates (%) for spring-sown seed for a) mean % of sown seeds surviving to the end of each year (averaged for 2010, 2011, 2012) for each of the five seedbed types (ordered from left to right within each treatment as follows: moss, deep organic, shallow organic, decayed wood, and mineral soil) and b) averaged across seedbed types for each year separately. The boundaries of the box are the 25th and 75th percentiles and the line in the middle of the box is the median. Whiskers above and below the box indicate the 90 th and 10 th percentiles and the circles below and above indicate the 5th and 95th percentiles. Capitalized letters (A,B,C) directly below boxplots indicate significant differences among seedbed types within a given treatment; lower case letters (x, y, z) above the boxes indicate significant differences for a) treatment types or b) years.

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Chapter 6. Tree species versus regional controls on ecosystem properties and processes: an example using introduced *Pinus contorta* in Swedish boreal forests[1]

Abstract - When species are introduced into new regions, there is great uncertainty whether the trait differences of the introduced species or regional factors, such as climate or edaphic properties, will serve as the dominant control of ecosystem properties or processes. In this study, we examined whether the introduction of *Pinus contorta* into Sweden has altered forest floor properties and processes or whether these properties are more strongly controlled by regional factors. We compared forest floor pH, potential N mineralization rates, bulk density, litter and forest floor depths, C and N concentrations and pool sizes, C:N ratios, and soil microbial communities using substrate-induced respiration and phospholipid fatty acid analysis among stands of introduced *P. contorta* (SwPc), native Swedish *Pinus sylvestris* L. (SwPs), and native Canadian *P. contorta* (CaPc). For most forest floor properties (pH, net NH_4^+ mineralization, bulk density, N mass, and the microbial phospholipid fatty acid community structure), SwPc sites were more similar to SwPs than to CaPc, whereas litter and forest floor depth were significantly higher in SwPc than the two other forest types. Our findings suggest that regional factors exerted a stronger control on most forest floor properties and processes than did species differences between the two *Pinus* species for the regions we studied.

6.1. Introduction

Humans frequently distribute species outside their native ranges, and sometimes these introductions adversely affect the composition and diversity of native communities; indeed, non-native species invasions are considered one of the greatest threats to biodiversity globally (Pejchar and Mooney 2009). One way that exotic species can adversely affect native communities is by altering critical ecosystem processes or properties (hereafter referred to as ecosystem-level change; Ehrenfeld 2003), which can in turn affect the performance of native species (Vitousek 1986). Introduced species can sometimes strongly affect the storage and release of soil carbon (C), nitrogen (N), and other elements, and have been shown to be of equal or greater importance in controlling these properties than some other more frequently studied ecosystem drivers, such as plant

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species diversity or herbivore abundance (Ehrenfeld 2003). Despite the important effects that introduced species can have on native ecosystems, including through below-ground interactions with soil biota in the introduced ecosystem (Nuñez et al. 2009), relatively few studies have evaluated their impacts on below-ground properties or processes (Levine et al. 2003). Thus, an increased understanding of the below-ground ecosystem-level changes arising from non-native species introductions is needed.

Most studies evaluating ecosystem-level change following non-native species introductions suggest that observed changes are primarily due to invader abundance (Strayer et al. 2006) or functional differences between the introduced species and those of the native community (Vitousek 1990, Ehrenfeld 2003). For instance, if the introduced species is abundant and possesses different traits than the native species - such as differences in litter quality - these can lead to significant changes in ecosystem processes (e.g., decomposition and nutrient cycling rates) or ecosystem properties (e.g., soil C pool sizes)(Wardle et al. 2011). Even introduced species that are functionally similar to native species may drive changes in ecosystem properties and processes if they have different growth and C input rates in their introduced environments. Herein, we consider three possible outcomes for ecosystem change following a species introduction: 1) ecosystem change results from functional differences between the introduced species and the native community (e.g., Vitousek 1990); 2) there is no effect of the introduction because regional factors in the introduced range have stronger ecosystem-level effects than does the introduced species; and 3) despite functional similarity of the introduced species relative to the native species, ecosystem-level change occurs due to positive interactions between the introduced species and the biotic or abiotic attributes of the introduced ecosystem (e.g., increased growth of the introduced species relative to its native range or relative to native species in the introduced range, possibly due to escape from antagonistic biotic interactions of the introduced species within its native range). Which

of these three outcomes occurs is likely to vary depending on the ecosystem property or process being measured, the species or introduced region evaluated, or time since introduction (Strayer et al. 2006). Few studies have been able to evaluate these alternative outcomes because they lack explicit comparisons of the introduced species in both native and introduced ranges (Hierro et al. 2005).

In this study we evaluated the effects of the introduction of the North American tree species *Pinus contorta* Douglas ex Loud. var. *latifolia* Engelm. into northern Sweden on forest floor properties and processes. *Pinus contorta* is a commercially-valuable timber species that has been shown to be a strong invader in many parts of the world where it has been introduced, particularly in Southern Hemisphere ecosystems (Ledgard 2001, Langdon et al. 2010), where it has been shown to alter community and ecosystem-level properties (Simberloff et al. 2010). However, relatively little is known about the ecological consequences of its introduction in European forest ecosystems (Engelmark et al. 2001). Nearly 600 000 ha of *P. contorta* have been planted in Sweden, with its widespread introduction beginning approximately 50 years ago (Elfving et al. 2001). *Pinus contorta* produces approximately 36% more total volume relative to the phylogenetically similar native Swedish species, *Pinus sylvestris* L., irrespective of site conditions (Elfving et al. 2001); further, it exhibits higher growth rates in Sweden relative to its native range (B. Elfving Personal Communication). Thus, interactions between *P. contorta* and biotic or abiotic conditions in the introduced range may play an important role in determining its effects on forest floor properties or processes as compared with the native *P. sylvestris*.

Forest floor properties and processes are primarily influenced by litter inputs (i.e. quality and quantity of litter); and regional factors such as climate, site fertility, or pH, that influence rates of decomposition (Fisher and Binkley 2000). Boreal forests are considered N limited, and differences in litter quality or quantity between *P. contorta* and

P. sylvestris are likely to influence decomposition and N mineralization rates, as well as the long term accumulation of C and N in the forest floor. Studies have shown that *P. sylvestris* produces less litter, has higher initial litter decomposition rates, and has lower lignin concentrations compared to *P. contorta* in Swedish forests (Berg and Lundmark 1987, Norgren 1995, Ågren and Knecht 2001), suggesting that differences in forest floor properties between *P. contorta* and *P. sylvestris* may occur. Differences in litter properties may also alter soil microbial properties such as microbial biomass or community composition (Wolfe and Klironomos 2005). Whether or not forest floors are altered in response to *P. contorta* introduction should depend on the relative importance of species differences versus regional factors in controlling ecosystem properties or processes.

Using an explicit comparison of *P. contorta* in both its native and introduced ranges, we tested the following hypotheses: 1) In these N-limited ecosystems several forest floor properties relevant to N cycling, including N availability and potential mineralization rates, would be controlled to a greater extent by canopy species than by regional factors (i.e. regional abiotic or biotic controls); 2) Accumulation rates of forest floor organic matter, C, and N would be higher in Swedish *P. contorta* stands than in Canadian *P. contorta* or Swedish *P. sylvestris*, due to the higher productivity that introduced *P. contorta* is reported to achieve (Elfving et al. 2001); 3) Both *P. contorta* forest types would have different microbial communities than *P. sylvestris* stands, and microbial biomass would be higher in the Swedish *P. contorta* stands due to their higher productivity and greater litter production. Collectively, these three hypotheses provide a rare evaluation of the potential outcomes for ecosystem-level change following the introduction of a non-native species.

6.2. Methods

Study area

We selected study sites consisting of *P. contorta* forest in Alberta, Canada (15-57 yrs), and two *Pinus* forest types in northern Sweden: similarly-aged stands of *P. contorta* and *P. sylvestris* (17 – 47 yrs), which are representative of the age range of *P. contorta* in the boreal forest of northern Sweden (Table 6-1). For each forest type we sampled eight replicate stands, yielding a total of 24 stands sampled (3 forest types x 8 replicates). Each stand was a minimum size 0.5 ha. We determined the age of each stand by evaluating stand records or by aging trees using increment cores, calculated basal area of each stand using a basal area factor prism for stands > 3 m tall, and used a clinometer and tape to measure tree heights.

The Canadian study sites (hereafter referred to as CaPc) were located in the Upper Foothills sub-region of Alberta in *P. contorta* forests near Robb, AB (53°10'N to 53°20'N; 116°44'W to 117°29'W; Table 6-1). The climate is continental subhumid (Dfc) under the Köppen classification system (National Atlas of Canada 1974). The annual mean air temperature is 2ºC, and the annual mean summer (June-August) temperature is 13.6ºC (1971–2000). Snow usually covers the frozen ground from the end of October to late April. Mean annual precipitation is approximately 560 mm (1971–2000), of which approximately 75% falls as rain. The underlying geology of the Upper Foothills is shale and sandstone overlain by medium textured, weakly calcareous glacial till, with gray luvisol soils characteristic of the region. The Swedish study sites were located in Västerbotten County, Sweden (63°56'N to 64°17'N; 19°27'E to 20°35'E; Table 6-1). The annual mean air temperature is 1ºC, and the annual mean summer (June-August) temperature is 13.0ºC (1961–2010). Snow usually covers the frozen ground from early December to late April. Mean annual precipitation is approximately 600 mm (1980–

1999), of which half falls as rain and half as snow. The underlying geology of the Swedish study area is fine to medium textured glacial till weathered from granitic bed rock, with brown podzols characteristic of the region. Swedish *P. contorta* stands consisted of different provenances derived from the Canadian Rocky Mountains. At each of the eight Swedish sites we selected similarly-aged paired stands of *P. contorta* and *P. sylvestris* (hereafter referred to as SwPc and SwPs), no greater than 1 km from each other (usually directly adjacent to each other), so that slope, aspect, elevation, and underlying geology were held constant between the paired stands (paired stands were identical in age, except for one site where the SwPs was three years older than SwPc). Sites we chose were relatively flat, and therefore did not have much variation in slope and aspect, and with our paired sampling design moisture status was considered equal for paired Swedish stands. Our sample size was restricted to eight replicates because of the limited availability of Swedish *P. contorta* stands with adjacent similarly-aged *P. sylvestris* stands.

Forest floor sampling and measurements

Within each of the 24 stands we collected five forest floor sub-samples (F and H horizon combined; each sample was ~ 10 g) for phospholipid fatty acid (PLFA) analysis using aseptic techniques, and 10 intact forest floor cores (10 cm diameter) for characterization of other forest floor properties and processes. These were collected a minimum distance of 10 m from the stand edge and at random intervals of 5-10 m along transects, but if the sampling point fell next to a tree base, it was moved 50 cm away from the tree base. For five of the intact cores, we measured the depths of the litter layer and F-H organic layer, and measured bulk density of the F-H layer. Forest floor sub-samples and cores were kept in a cooler and transferred back to the lab. The samples collected for PLFA analysis were immediately placed in a sterile Whirlpak™ bag, stored in a freezer (-20°C) and then

freeze-dried prior to PLFA extraction. Five of the forest floor cores were sieved (4 mm) and kept refrigerated (4°C) in polyethylene bags for up to five weeks prior to several analyses requiring field moist soil. In order to report forest floor response variables on a dry weight basis, we used a portion of the five sieved cores to measure the field-moist and oven-dried (48 hours at 65°C) mass, which allowed for calculation of soil moisture content. We also oven-dried (65 ºC) the remaining un-sieved five cores for three days, weighed them, and calculated bulk density as mass per core volume. The mass of these samples was also used to report total C and N data on an area basis.

The sieved forest floor cores were analyzed for pH, total C and N, extractable ammonia, potential N mineralization, and substrate-induced respiration (SIR) as a measure of active microbial biomass, as described below. Oven-dried samples (65 ºC) were used to measure pH, and total C and N content. Soil pH was measured potentiometrically in a saturated paste that was in equilibrium with a soil suspension of a 1:10 soil:0.01M $CaCl_2$ liquid mixture. Total percent C and N were measured on ground samples using a NC Soil Analyzer (Thermo Electron Corporation, Thermo Fisher Scientific, Bremen, Germany).

Potential N mineralization rates were measured on each sieved core using an aerobic laboratory incubation (Hart et al. 1994, Gundale et al. 2005). Briefly, this method involves creating two parallel incubation tubes from each forest floor sub-sample, one which is extracted immediately, and one which is dark-incubated (22 ºC), and extracted one month later. Differences in inorganic N concentrations between the beginning and end of the incubation indicate net mineralization, which can be positive or negative (indicating net immobilization occurred). Each sample was prepared by placing 5 g sieved forest floor (wet weight) into a 100 ml glass nalgene bottle. For incubated samples, the moisture content during the incubation period was maintained by checking weekly whether addition of water to maintain the initial jar and sample weight was

needed. Inorganic N extraction was done on the two sets of samples by placing 50 ml of 1 M KCl in each bottle, shaking for one hour, and then filtering through Whatman #42 filter paper. Extracts were used for determination of NH_4^+ -N and NO_3^- -N using standard colorimetric methods, on an Auto-Analyzer III (Omni Process, Solna SE). There was no detectable nitrate measured in the majority of extractions, therefore only NH_4^+ -N data are reported.

Field moist sieved cores were also used to measure substrate-induced respiration (SIR), which is a measure of the relative active microbial biomass responsible for regulating the supply of plant-available nutrients from the soil (Anderson and Domsch 1978). SIR was measured by placing 5 g (dry wt equivalent) from each sub-sample into a 100 ml glass bottle, and then adjusting the moisture content to 250% (dry wt basis). After adjusting the water content, samples were placed in a dark incubator (25ºC) for 24 hours to equilibrate. The following day 2.5 ml of glucose solution (40 g l^{-1}) was pipetted into each bottle, which increased the water content of the soil to 275%, and added a quantity of glucose C equivalent to 2% soil dry weight. The jar was then sealed, and we determined evolution of CO_2 between 1 h and 4 hrs following glucose addition by injecting 0.2 ml subsamples of headspace gas into an infrared gas analyser, which allowed for estimation of SIR. Forest floor samples too wet after moisture content adjustment to allow for meaningful SIR readings (23 of 120 samples) were excluded from analysis; the numbers of excluded samples was evenly distributed among forest types and thus should not bias our results.

Soil microbial community structure was analyzed using phospholipid fatty acid (PLFA) analysis. PLFA profiles were analyzed for each sub-sample by freeze-drying and extracting each forest floor sample (0.3 g) using a modified Bligh and Dyer method, which included extraction with a single-phase chloroform mixture, lipid fractionation on a solid-phase-extraction Si column, followed by a mild methanolysis (Bligh and Dyer

1959, King et al. 1977, White et al. 1979, Kates 1986, Frostegård et al. 1991). The fatty acid methyl esters were then analyzed by capillary gas chromatography (Perkin Elmer Clarus 500GC; Waltham, MA, USA), and chromatograms interpreted using Perkin Elmer Total Chrome peak identification software. A total of twenty-seven individual fatty acids were detected. Excluding the internal standard (19:0) and one peak that was only present in a few PLFA samples, 25 PLFAs were included in the analysis of total PLFA biomass. Fatty acids were designated X:YωZ, where X represents the number of C atoms, Y represents the number of double bonds, and Z indicates the position of the first double bond from the aliphatic (ω) end of the molecule. Total phospholipid fatty acids were quantified as nmol g^{-1} forest floor. In addition, PLFAs used as biomarkers for functional groups (i.e., fungi, bacteria, gram positive bacteria, gram negative bacteria, actinomycetes, and arbuscular mycorrhizae) were quantified on a mol percent basis. The fungal PLFA 18:2ω6 was used to estimate the contribution of fungi (Frostegard and Bååth 1996), while 16:1ω5 was used to estimate arbuscular mycorrhizae (Frostegard and Bååth 1996, Olsson 1999). Bacterial PLFAs included 14:0, i14:0, 15:0, a15:0, i15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7, cy19:0 (Bååth et al. 1992, Frostegard and Bååth 1996, Myers et al. 2001). The ratio of 18:2ω6 to the bacterial PLFAs was used to estimate the relative contributions of fungi and bacteria. The 10-methyl branched fatty acids (10me16:0, 10me17:0 and 10me18:0) were used to measure actinomycetes (Kroppenstedt 1985, Brennan 1988).

Statistical analyses

We first determined whether each variable met the assumptions for analysis of variance (ANOVA) and transformed response variables when necessary. One-way ANOVAs were used to test for significant (α= 0.05) differences in the response of individual variables among the three forest types (i.e. CaPc, SwPc, and SwPs; Proc Mixed). For data that

could not be transformed to meet the assumptions non-parametric Kruskal-Wallis tests were used (Proc Npar1way). When significant differences were detected, we used post-hoc linear contrasts with Bonferroni-adjusted P-values (family-wise α=0.05) to identify significant pairwise differences for ANOVAs (Proc Mixed) and we used a permutation test to do pairwise comparisons after Kruskal-Wallis tests (Proc Multtest). SAS (SAS Institute Inc., Version 9.2, Cary, NC: SAS Institute Inc., 2000) was used for ANOVA, Kruskal Wallis tests, permutations and linear contrasts.

Multivariate patterns of microbial community structure using individual forest floor PLFAs (mol%) among forest types were examined using nonmetric multidimensional scaling (NMS) ordination (McCune and Grace 2002). We used PC-ORD (Version 5 MjM Software Design, Gleneden Beach, OR), with Sørenson as the distance measure, and completed 100 runs with real data and 100 Monte Carlo randomized runs, starting with a six-dimensional solution and stepping down to a one-dimensional solution. We determined the number of dimensions of our final solution by evaluating the scree plot and the reduction in stress with step-down in dimensionality of the preliminary runs (McCune and Grace 2002). Stability of the solution (stability criterion = 0.00005) was assessed by plotting stress versus iteration for each of our iterations. After checking the optimal number of dimensions and best solution from the preliminary runs, we ran a final NMS with the number of dimensions determined from the preliminary runs (n=2), using the starting configuration that worked best in our preliminary runs and omitting the Monte Carlo test. We then calculated the Pearson correlation coefficients for stand and forest floor descriptive variables (e.g., PLFA functional groups, bulk density, total C and N) with the NMS ordination axes. We used the multi-response permutation procedure (MRPP) to test for statistically significant differences in PLFA community profiles among the three forest types. MRPP is a nonparametric multivariate procedure for testing the null hypothesis of no difference

between two or more groups of entities (Zimmerman et al. 1985). The initial MRPP comparing the three forest types was followed up by pairwise comparisons among forest types; P-values were Bonferroni-adjusted so the family-wise Type I error rate remained 0.05.

6.3. Results

For several of the measured forest floor properties and processes we found that SwPc was similar to SwPs and both were different from CaPc. There were significant differences in pH among forest types, with the pH of CaPc significantly higher than for both Swedish forest types (Table 6-2). Although the Kruskal-Wallis test indicated there were significant differences in initial NH_4^+-N concentrations (P=0.03) among the three, none of the pair-wise comparisons were significant (Fig. 6-1). NH_4^+ -N concentrations for samples incubated for one month were not significantly different among forest types (ANOVA P=0.12, Fig. 6-1); however, potential net mineralization rates during the one-month incubation were similar for both SwPc and SwPs, but significantly higher compared with the CaPc stands, which showed net immobilization (Kruskal Wallis P=0.0009, Fig. 6-1). The bulk density of the forest floor in the CaPc stands was significantly higher than for both Swedish forest types (ANOVA P=0.004, Fig. 6-2). Both litter and forest floor depths were significantly deeper in SwPc than both CaPc and SwPs stands (Kruskal-Wallis tests for litter P=0.0002 and forest floor P<0.0001, Fig. 6-2). The CaPc stands had significantly higher N mass in the forest floor than the Swedish forest types, although there were no significant differences in C mass or the C and N concentrations (Table 6-2). Forest floor C to N ratios did not significantly differ among the stand types (Table 6-2).

There was no significant difference in active microbial biomass as measured by SIR among the forest types (Table 6-2), or in total PLFA biomass among the forest types (Table 6-2). There was also no significant difference in PLFA mol percent for any of the

PLFA functional groups among the three forest types (Table 6-2). The fungi to bacteria ratio did not significantly differ among the forest types (Table 6-2).

The NMS two-dimensional solution (final stress = 7.62 after 36 iterations) explained 96.1% of the variation in the dataset (Fig. 6-3). MRPP analysis of PLFA profiles showed significant differences among the three forest types (A=0.14, P=0.0002); the CaPc stands were different from the Swedish forest types, whereas the Swedish forest types did not significantly differ from each other (CaPc vs SwPc A=0.15 P=0.002, CaPc vs SwPs A=0.16 P=0.002, SwPc vs SwPs A=-0.02 P=1.0). Overlaying the other stand and forest floor descriptive variables on the NMS ordination of individual PLFAs showed correlations of several variables with the two ordination axes (Fig. 6-3). CaPc stands were located towards the lower end of axis one and wide spread across axis two, and based on their relative locations in the plot were correlated with higher values for pH (axis 1 r= -0.86, axis 2 r= 0.24), mol% of fungal PLFAs (axis 1 r= -0.63, axis 2 r= -0.87), and time 0 ammonia (axis 1 r= -0.40, axis 2 r= 0.48). PLFA profiles for the two Swedish forest types were overlapping, both loaded toward the upper end of axis one with spread across axis two. Their relative locations on the ordination were positively correlated with the greater depth of forest floor (axis 1 r= 0.46, axis 2 r= 0.00), bacteria:fungi ratio (axis 1 r= 0.47, axis 2 r= 0.92), bacteria (axis 1 r= 0.16, axis 2 r= 0.93), gram positive bacteria (axis 1 r= 0.45, axis 2 r= 0.53), gram negative bacteria (axis 1 r= 0.04, axis 2 r= 0.89), C:N ratio (axis 1 r= 0.15, axis 2 r = -0.49), and arbuscular mycorrhizae (axis 1 r= 0.13, axis 2 r= 0.73). Correlations for other variables (e.g., stand age, total N, total C, Net mineralization) with the ordination axes were low (axis 1 R^2 ranged from 0.0-0.16, and axis 2 R^2 ranged from 0 to 0.17) and thus were not considered in further detail.

6.4. Discussion

This study provides a rare evaluation of whether species differences versus regional factors control ecosystem processes and properties following the introduction of a non-native species. The differences among the Canadian and Swedish forest types we observed suggest that regional factors that differ between the native and introduced range appear to exert a stronger influence on most forest floor properties and processes than do species differences; whereas some forest floor properties, notably litter and forest floor depth, appear to be driven by a positive interaction between *P. contorta* and its introduced location. Contradicting our first hypothesis, that canopy species would exert a stronger control than regional factors on N cycling attributes, we alternatively found significantly lower potential net mineralization rates for CaPc than SwPc and SwPs, and SwPc and SwPs did not differ. This analysis showed that CaPc stands exhibited net immobilization, whereas both Swedish forest types exhibited positive net mineralization rates. Nitrogen mineralization from decomposing plant litter is a major source of N for forest trees (Fisher and Binkley 2000). Typical of most boreal forests, all three forest types had relatively high forest floor C:N ratios, which generally stimulates net immobilization. One factor that might explain the differences in mineralization we saw among regions is that *in situ* decomposition rates may be faster for CaPc than for the Swedish forest types. If decomposition rates were higher for CaPc, then we would expect initial NH_4^+ -N concentrations to be higher, and lower concentrations of labile organic N left to mineralize (i.e. because it has already mineralized), leading to very small changes in NH_4^+ -N concentrations during the controlled incubation. Although we did not directly measure decomposition rates, our findings of higher bulk density levels in the CaPc stands than in both Swedish forest types, and a shallower F,H layer in the CaPc forest floor relative to the Swedish forest floors further suggest that decomposition rates are

higher in CaPc compared with the Swedish forest types. The Swedish *Pinus* forests are likely to have a poorer decomposition environment than the Canadian stands, due to their colder summer temperatures and the significantly more acidic forest floor. These conditions likely result in lower *in situ* decomposition rates, resulting in a greater accumulation of labile organic N in the forest floor, which would likely produce lower initial NH_4^+ -N concentrations, and larger increases under the ideal conditions of the controlled incubation. The more acidic forest floors of the Swedish *Pinus* forest floors compared with CaPc described above is likely a function of the underlying geology of the two study areas; in the Swedish stands the underlying geology is granite, which typically weathers into low pH soils, whereas the underlying geology of the Alberta foothills is shale and sandstone overlain by medium textured, weakly calcareous glacial till, which weathers into less acidic soils. The assertion that abiotic regional factors are affecting pH more than species differences is further supported by a study which compared 27-yr-old Swedish stands of *P. contorta* and *P. sylvestris* and found no significant difference in soil pH, despite observing differences in the leaf chemistry for these two species (Alriksson and Eriksson 1997).

Our second hypothesis was that SwPc would cause organic matter to accumulate faster relative to SwPs and CaPc due to its higher productivity in Sweden than in Canada and relative to SwPs, which would in turn lead to higher accumulation rates of C and N. In support of this hypothesis, our results showed significantly higher litter and forest floor depths for SwPc, which is consistent with other studies that found *P. sylvestris* has a lower production of litter than *P. contorta* in Swedish forests (Berg and Lundmark 1987, Ågren and Knecht 2001). Our higher litter depth for the SwPc stands is also consistent with Nilsson et al. (2008), who found that *P. contorta* had more than three times greater ground litter cover percentage compared with *P. sylvestris* stands. However, inconsistent with our second hypothesis, for soil N mass we found differences only between CaPc and

the Swedish forest types and we did not find differences in total mass per ha of C, or percent C or N among any of the forest types. Regional factors contributing to N mass differences between Canada and Sweden could include the warmer summer conditions in the Canadian stands, potentially causing N-fixation rates to be higher (Gundale et al. 2011, Gundale et al. 2012). Berg (2000) examined litter from *P. sylvestris* and *P. contorta* across a large geographical region (i.e., across Scandinavia) and found that the species formed distinct groups with respect to their litter chemical composition; *P. sylvestris* needle litter was characterized by relatively low concentrations of both lignin and N, while *P. contorta* also showed relatively low N but with higher lignin concentrations. Despite these differences, Ågren and Knecht (2001), using modeled relationships examining future storage potential of C, N, and other soil nutrients for Swedish *P. contorta* and *P. sylvestris*, found it unlikely that the small inter-specific differences in litter concentrations of elements would lead to any major changes in soil stores of N. Likewise, in a study of pine forests (8 species, primarily focused on *P. sylvestris* and excluding *P. contorta*), from 31 ºN to 71 ºN, Berg et al. (1993) showed that climate (annual actual evapotranspiration) was the dominant rate-regulating factor for litter mass loss, while none of the species substrate-quality factors were significant, which is consistent with our findings of regional similarities in N mass properties for SwPc and SwPs. One reason for the similarity among all forest types for C mass and percent C and N could be that these stands are all relatively young, so perhaps differences in soil C pools have not yet had time to emerge. Forest floor C in these boreal stands could be largely residual C from prior to stand establishment. Therefore, the litter of these relatively young stands may be only a minor contribution to the total forest floor C pool at this successional stage. However, the thicker litter and FH horizons of the SwPc stands we measured could indicate that the C forest floor properties are starting to diverge, as evidenced by the lower (albeit non-significant) C mass of SwPs compared with SwPc.

With our third hypothesis, we predicted that differences in both forest floor chemical composition and total quantity of litter inputs among the two *Pinus* species would cause greater differences in the microbial biomass and community structure compared with regional factors; however, our findings were inconsistent with this prediction. With respect to the active total microbial biomass, the lack of difference in SIR or of PLFA biomass among all three forest types suggests that the total pool of microbes regulating the supply of plant-available nutrients from the soil do not differ among forest types. Carbon is generally the primary control of soil microbial biomass, so the lack of difference in percent or mass of C among the forest types likely explains the lack of difference in microbial biomass among the forest types. In contrast, our data showed significant differences in microbial community structure among the three forest types, with different microbial community composition occurring in CaPc relative to both Swedish forest types. Given the reported differences in C quality between the two pine species (Berg and Lundmark 1987, Berg 2000), we expected soil microbial communities to differ beneath the two pine species. However, from the pattern of individual stands in the NMS ordination and the highly correlated vectors overlaid on the ordination, it is clear that non-species factors had a much greater influence on soil microbial community composition than did any differences between the two tree species. As shown in the ordination plot, the differentiation between CaPc and the Swedish forest types appears to be most strongly associated with pH, which may be associated with other regionally-driven factors that we did not measure in this study, such as precipitation, age of soil development, or underlying parent materials. Additionally, regional differences in understory plant species composition may also contribute to the regional differences in soil pH or in the quality of C inputs, especially given that understory litterfall can make a large contribution (up to 50% of total) to total litterfall in *Pinus* forests (e.g., Stendahl et al. 2010).

Pinus contorta is an ideal species to investigate the relative control of species traits versus regional factors on ecosystem properties and processes following introduction because of its controlled and documented introduction in Sweden during the last half century, and its growth in monocultures both in its native and introduced ranges. Based on a survey of existing literature, Ehrenfeld (2003) found that when a new species is introduced, its' impacts on nutrient cycling depends on how different it is from the constellation of traits present within the existing plant community. While a number of studies have found that soil properties change in response to the introduction of new traits and new functional groups, few studies have explicitly compared the effect of these traits in both a species' native and introduced range (Hierro et al. 2005). Similarities in Swedish forest floor properties and processes among stands of the functionally and phylogenetically similar *Pinus* species evaluated in this study, contrasting with numerous significant differences in the properties of *P. contorta* stands between Canada and Sweden, signals that forest floor properties appear to be more driven by regional ecosystem factors rather than by species-specific properties for these species and regions. Our findings of limited differences between native and introduced Swedish *Pinus* species indicate that changes in forest floor properties may be minor relative to the major changes associated with *P. contorta* introduction and invasion documented in other parts of the world, where *P. contorta* has vastly different litter properties than the native vegetation (Simberloff et al. 2010).

Understanding the ecosystem-level consequences of introduced and invasive organisms has great societal importance. Our study provides a rare evaluation of the effect of non-native species on ecosystem-level properties by comparing a species' effects on ecosystem properties and processes in both its native and introduced range, and by making comparisons to the comparable native species in the region of introduction. Our results suggest that the impact of species introductions on ecosystem processes will

be functions of regional influences and ecological differences between the introduced and comparable native species. An introduced species that is functionally similar to a native species may have minor ecosystem-level effects, but we currently lack an understanding of how functionally different an introduced species must be to cause ecosystem-level change. These concepts could be explored in the future by studying the impacts following introduction of a species into a range of ecosystem types and regions. Further, the regional influence by species interaction could be explored through studies involving reciprocal introductions of comparable species between regions.

Table 6-1. Properties of the stands for each of the three forest types, Canadian *Pinus contorta* (CaPc), Swedish *Pinus contorta* (SwPc), and Swedish *Pinus sylvestris* (SwPs).

Forest Type	Age	Origin	Basal area[i] (m^2/ha)	Stand height (m)	Latitude	Longitude	Date collected[ii]
CaPc	15	natural	-[a]	3	N53°20'58.9"	W116°52'03.8"	07/10/2010
CaPc	30	natural	34.4	8	N53°12'59.1"	W116°47'09.5"	07/10/2010
CaPc	47	planted	26.4	14	N53°10'48.8"	W117°27'21.2"	14/10/2010
CaPc	19	planted	-[a]	2.5	N53°10'46.3"	W117°28'05.0"	14/10/2010
CaPc	33	natural	16.8	9	N53°11'53.0"	W117°29'09.5"	14/10/2010
CaPc	57	natural	20.8	14	N53°13'43.1"	W117°21'09.8"	14/10/2010
CaPc	33	natural	13.6	9	N53°16'11.0"	W117°20'51.1"	14/10/2010
CaPc	44	planted	24.8	14	N53°16'38.7"	W117°10'11.7"	14/10/2010
SwPc	21	planted	25.6	8	N63° 56'64.0"	E20° 34'55.9"	21/10/2010
SwPc	17	planted	20.4	6	N64° 04'95.7	E19° 48'37.9"	21/10/2010
SwPc	24	planted	25.6	9	N64° 14'80.8"	E19° 48'08.2"	22/10/2010
SwPc	23	planted	20.8	9	N64° 09'26.7"	E19° 35'17.7"	25/10/2010
SwPc	47	planted	27.2	15	N64° 09'44.4"	E19° 34'73.0"	25/10/2010
SwPc	22	planted	22	9	N64° 16'39.0"	E19° 39'33.9"	26/10/2010
SwPc	17	planted	18.8	6	N64° 17'19.5"	E19° 27'43.5"	26/10/2010
SwPc	28	planted	22.4	10	N64° 10'45.7"	E19° 32'99.9"	26/10/2010
SwPs	24	planted	18.8	12	N63° 56'59.5"	E20° 35'76.9"	21/10/2010
SwPs	17	planted	13.2	6.5	N64° 04'97.0"	E19° 48'23.1"	21/10/2010
SwPs	24	planted	26	6	N64° 14'86.5"	E19° 48'13.1"	22/10/2010
SwPs	23	planted	15.2	9	N64° 09'18.4"	E19° 35'68.7"	25/10/2010
SwPs	47	planted	33.2	16	N64° 09'48.7"	E19° 34'75.9"	25/10/2010
SwPs	22	planted	28.4	9	N64° 16'44.5"	E19° 39'50.3"	26/10/2010
SwPs	17	planted	16.4	5	N64° 17'23.2"	E19° 27'49.5"	26/10/2010
SwPs	28	planted	18.4	9	N64° 10'45.7"	E19° 32'99.9"	26/10/2010

[i] Basal area was not recorded for the two stands < 3 m tall.

[ii] Note that bulk density soil cores were collected in spring 2011 immediately after snow melt.

Table 6-2. Means, standard errors (SE), and probability (P)-values for one-way ANOVA and non-parametric Kruskal-Wallis tests to test for significant differences in the response of variables among the three forest types, Canadian *Pinus contorta* (CaPc), Swedish *Pinus contorta* (SwPc), and Swedish *Pinus sylvestris* (SwPs). Significant pairwise contrasts are highlighted with the P-value bolded. Significant differences in means between forest types are indicated by bold letters (a, b).

Variable	CaPc		SwPc		SwPs		P
	Mean	SE	Mean	SE	Mean	SE	
pH	4.03**a**	0.10	3.14**b**	0.06	3.20**b**	0.07	**0.0007**[i]
Total N mass (kg/ha)	1.75**a**	0.31	1.01**b**	0.09	1.03**b**	0.09	**0.009**[i]
Total C mass (tons/ha)	14.00	3.33	15.51	1.65	10.68	1.31	0.17 [ii]
C percent	34.34	1.67	36.37	1.57	34.36	1.71	0.68 [ii]
N percent	0.95	0.05	1.08	0.05	0.98	0.05	0.48[i]
Carbon:Nitrogen	36.62	1.21	33.89	0.80	35.46	0.94	0.52 [ii]
SIR-µg CO_2/g/hr	0.11	0.01	0.14	0.01	0.11	0.01	0.15[i]
PLFA biomass (nmol/g)	827	44	823	50	940	46	0.37[i]
Fungi:Bacteria	55.7	4.3	46.6	3.4	45.7	3.0	0.21[i]
PLFA mol%							
Bacteria	47.1	1.1	47.0	0.9	47.1	0.9	1.0[i]
Gram +	11.1	0.4	11.7	0.2	11.5	0.3	0.67[i]
Gram -	34.2	1.0	33.2	0.8	33.8	0.7	0.75[i]
Actinomycetes	1.3	0.1	1.2	0.1	1.1	0.1	0.45[i]
Fungi	24.5	1.3	20.8	1.1	20.6	1.0	0.11[i]
Arbuscular mycorrhizae	3.4	0.2	3.4	0.2	3.7	0.2	0.54[i]

[i] One-way ANOVA, DF=2,21

[ii] Kruskal-Wallis

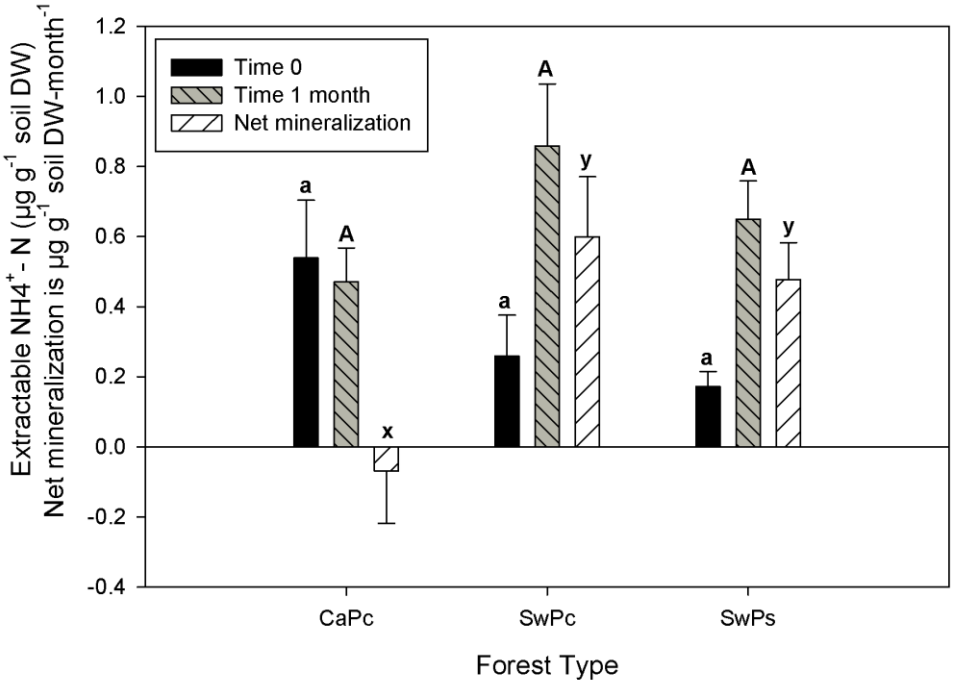

Fig. 6-1. Comparison of ammonia at time zero, after a month of incubation, and net mineralization rates among the three forest types (mean ± SE, n = 8), Canadian *Pinus contorta* (CaPc), Swedish *Pinus contorta* (SwPc), and Swedish *Pinus sylvestris* (SwPs). Within a time (bar pattern), bars with different letters (x, y) indicate significant differences based on pairwise comparisons.

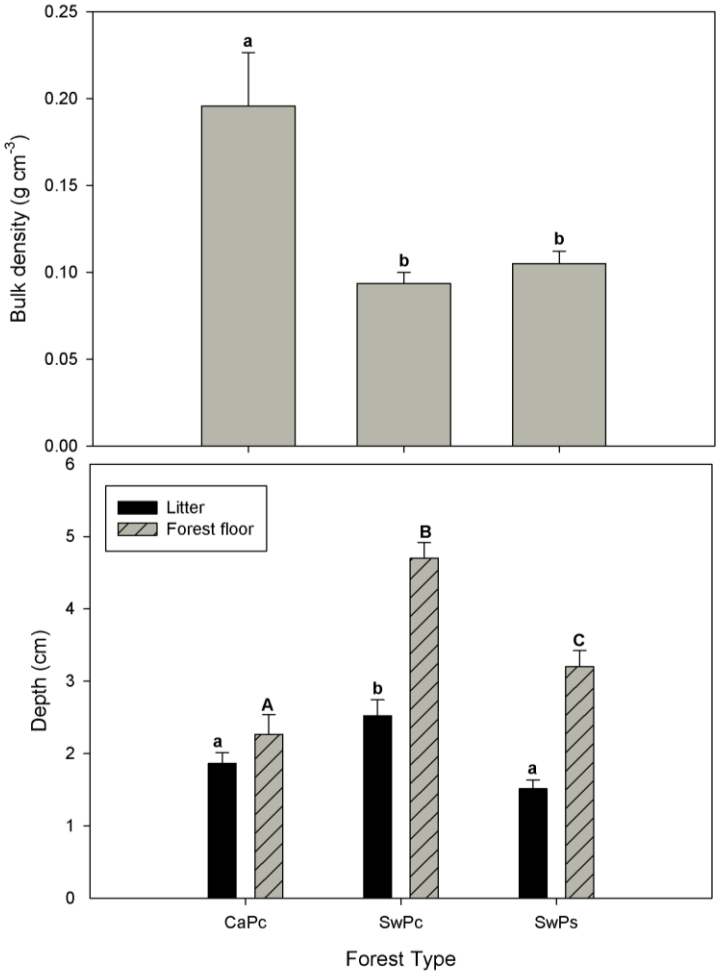

Fig. 6-2. Bulk density and depths of the litter and forest floor (F and H layers) for the three forest types (mean ± SE, n = 8), Canadian *Pinus contorta* (CaPc), Swedish *Pinus contorta* (SwPc), and Swedish *Pinus sylvestris* (SwPs). For each bar pattern, different letters (a, b or A, B, C) indicate significant differences based on pairwise comparisons.

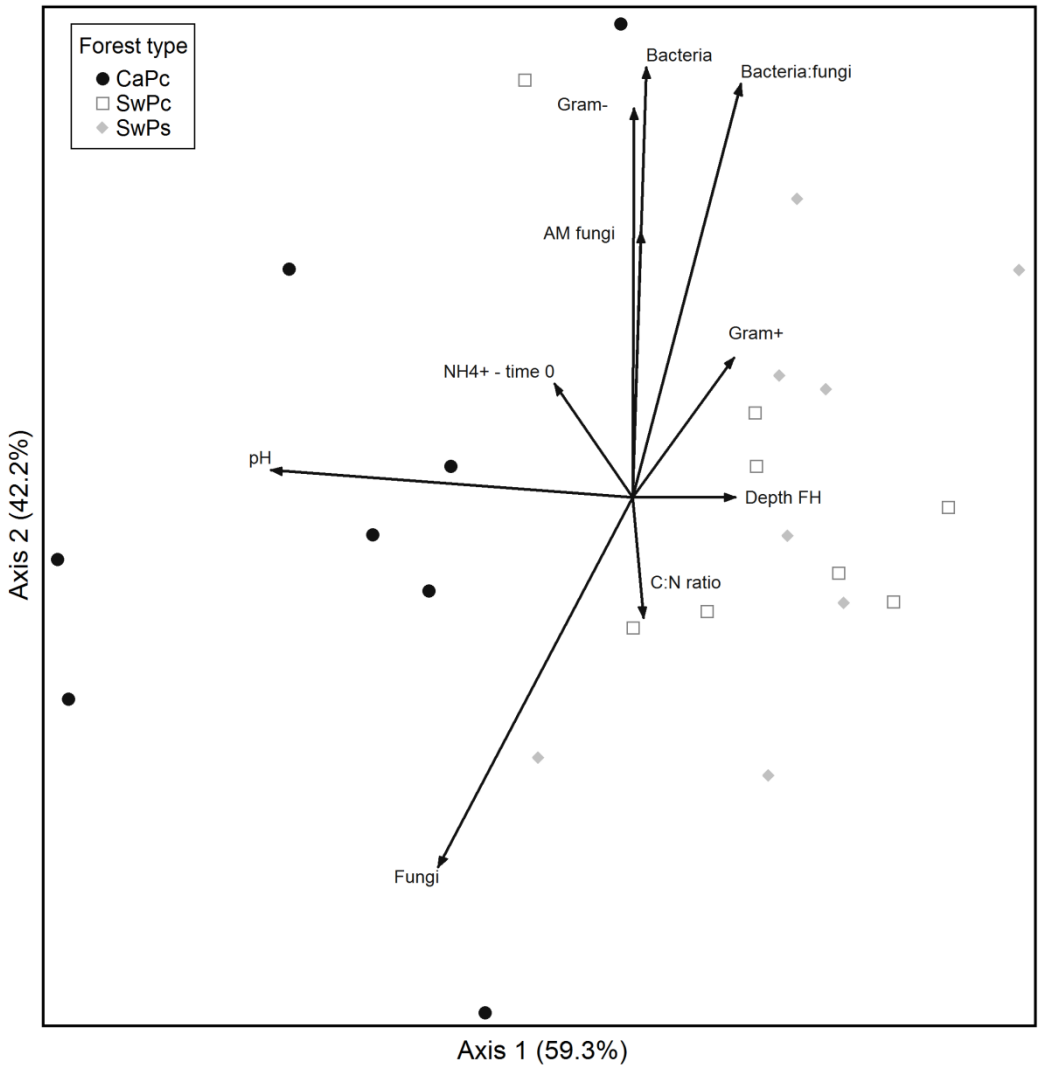

Fig. 6-3. Results of Nonmetric Multidimensional (NMS) scaling ordination for the forest floor phospholipid fatty acid profiles delineated by forest type, Canadian *Pinus contorta* (CaPc), Swedish *Pinus contorta* (SwPc), and Swedish *Pinus sylvestris* (SwPs). The amount of variation explained by each axis is included in parentheses. The angles and lengths of the vectors indicate direction and strength of relationships of the variables with the ordination axes (cutoff for displayed variables was R2 > 0.2).

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Chapter 7. General discussion and conclusions

Overall, my thesis provided novel insights into the relationships between above- and below-ground ecosystem properties and processes in lodgepole pine forests and how they may respond to disturbance, as well as how lodgepole pine itself may act as a disturbance agent when it is introduced outside of its native range.

In the second chapter I investigated the relationships between above-and below-ground resources and how the understory plant community of monoculture pine forests is structured. This chapter increased our understanding of within-stand patterns of variation in understory community composition in mature forest monocultures that are representative of the most common forest type in Alberta's pine forest region. I identified four fine-scale plant community types. As I hypothesized, the majority of environmental variables associated with the differentiation among the plant communities were below-ground, compared with only one significant above-ground variable. My results illustrate the important ecological roles that both above- and below-ground properties and processes play in the functioning of these forested ecosystems, and these findings may be applied to understanding the impacts of partial canopy disturbances on these forests. This study illustrated the significant linkages that exist between below-ground environmental factors, in particular the soil microbial community, and the understory plant communities at the within-stand scale for these homogeneous forests. In the face of shifting disturbance regimes, such as the shift from dramatic forest floor changes after fire to the addition of partial canopy mortality disturbance that leaves the understory community undisturbed in forests attacked by insects such as MPB, these findings have important implications for understanding the effects that partial canopy disturbance events will have in these forests. The type and intensity of disturbance and its relative impacts on the canopy, forest floor, and associated below-ground properties and processes will play an

important role in determining the structure and composition of future understory plant communities of these forests.

In the third chapter I examined patterns in the below-ground structural and functional microbial communities of lodgepole pine forests and whether there were linkages of these below-ground communities with other above- and below-ground environmental factors. I identified four structural (using PLFAs as indicators) microbial communities that were primarily influenced by the understory composition. These findings suggest that fine-scale variability in the species composition of the understory plant communities, and their associated litter and rhizosphere resources, contribute to the heterogeneity of below-ground structural microbial communities. I also identified four functional (using carbon substrates as indicators) microbial communities, however there was poor separation among community types and the abiotic and biotic factors we measured had low explanatory power for describing these plant community types. Thus, it appears that other factors that we did not measure in this study have more influence on the functional microbial communities in these forests. Two potential factors that may have an important influence on functional microbial communities at this scale that were not measured in this study are soil moisture and soil temperature. Future research that includes measurements of soil moisture and temperature at this fine scale would help determine their relative influences on the functional microbial community. In addition, repeated measurements across the growing season might also provide additional insights into the temporal dynamics of both the structural and functional below-ground microbial communities. Overall, this study demonstrates the important linkages between the understory and below-ground communities, and that disturbance to one community is likely to influence the others, thus amplifying the potential impacts of disturbances on ecosystem properties and processes.

In the fourth chapter I investigated the potential short-term effects of varying intensities of MPB attack and associated salvage harvest management practices on downed wood, the understory plant community, and below-ground properties and processes. This was the first study that I am aware of that provides an experimental evaluation of the potential impacts of early red-attack MPB attack on these variables, and the first study examining potential responses in newly invaded stands in MPB's expanded range east of the Canadian Rockies. I found short-term resistance to MPB early red-attack in the DWM, understory, and most below-ground properties and processes in these forests. However, there were more immediate changes in the downed wood and understory plant communities in the salvage logged stands. For most ecosystem properties I studied, I found that in the short term post-disturbance they did not differ with undisturbed stands, suggesting that their short-term resistance may give managers time post-attack to manage these forests before ecosystem functions are altered. Research on MPB in other regions that has been carried out over longer periods suggests that as these stands transition from red to grey attack we expect that they will no longer be resistant to MPB attack, and instead will begin to respond to the canopy mortality. However, it remains uncertain what successional trajectory lodgepole pine forests in Alberta will follow in the longer term following introduction of this novel MPB disturbance. Thus, longer-term research that continues to follow MPB-attacked stands temporally post-disturbance is needed to better understand how MPB-attacked stands in Alberta will continue to respond to this novel disturbance agent and shifting disturbance regimes. We have set up our research sites as long-term monitoring plots, and research is ongoing at these sites. Longer-term monitoring of these sites will provide additional insights on the duration of resistance to attack, and at which point the ecological inertia of these systems will be overcome.

In the fifth chapter I investigated the potential for pine regeneration in MPB attacked stands by quantifying advanced regeneration and new recruitment of individual pine seedlings after MPB attack. I found minimal advance regeneration, no natural recruitment post-MPB attack, and the highest recruitment rates on mineral soil and decayed wood seedbed types, which were scarce across all four stand treatment types. My findings suggest that significant silvicultural intervention will be required to supplement regeneration if the goal is a stocked future pine forest. For attacked forests similar to those in our study that are left unmanaged, which may comprise a large portion of the landscape depending on the magnitude of MPB outbreak in Alberta, it appears that regeneration towards a replacement forest will be slow, owing to the lack of advance regeneration and suitable seedbed types as well as unsuitable environmental conditions. Another potential concern may be that by the time favorable seedbed types become available for germination (as dead trees begin to fall), seed may have already been released from the cones. Future research is needed that monitors recruitment and longer-term survival of seedlings in attacked forests as they transition to grey attack, and eventually fall to the ground, in order to better predict the future successional pathway of MPB-disturbed stands that lack additional anthropogenic or natural wildfire disturbances. Our research sites provide an area where we can monitor recruitment over the longer term and evaluate what will happen in the absence of silvicultural intervention in both MPB-attacked and salvage logged stands. Long-term monitoring of the release of seeds from cones, the fall rates of dead and dying trees, and the resultant changes in seedbed availability and light transmission to the forest floor are needed to better understand the potential for recruitment in the longer term post MPB attack.

In the sixth chapter I examined the potential for lodgepole pine to be a disturbance agent in a region where it was introduced outside its' native range. Understanding the ecosystem-level consequences of introduced and invasive organisms

has great societal importance. Our study provided a rare evaluation of the effect of non-native species on ecosystem-level properties by comparing a species' effects on ecosystem properties and processes in both its native and introduced range, and by making comparisons to the comparable native species in the region of introduction. The differences among the Canadian and Swedish forest types we observed suggested that regional factors that differed between the native and introduced ranges appeared to exert a stronger influence on most forest floor properties and processes than did species differences; whereas some forest floor properties, notably litter and forest floor depth, appeared to be driven by a positive linkage between lodgepole pine and its introduced location. Our results suggest that the impact of species introductions on ecosystem processes will be a function of both regional influences and ecological differences between the introduced and comparable native species. An introduced species that is functionally similar to a native species may have minor ecosystem-level effects, but we currently lack an understanding of how functionally different an introduced species must be to cause ecosystem-level change. These concepts could be explored in the future by studying the impacts following introduction of a species into a range of ecosystem types and regions. Further, interactions between regional influence and species could be explored through studies involving reciprocal introductions of comparable species between regions.

Overall, the results of my thesis have provided novel insights into the ecology of lodgepole pine forests in the face of shifting disturbance regimes and forest management practices, including the important ecological roles that both above- and below-ground properties and processes play in these forest ecosystems and the important linkages among the below-ground and above-ground communities of them. My results can be applied to forest management and silvicultural practices for pine forests, providing an increased understanding of the linkages between the above- and below-ground

components of these systems and how they will respond to evolving disturbance regimes. Forest management should consider that partial canopy disturbances that kill trees but leave them standing will have different impacts on ecosystem properties and processes than will the salvage of dead trees after MPB attack. Evidence of ecological inertia in ecosystem responses in the short term (1 year) after MPB attack suggests that forest managers will have time post attack to evaluate potential management scenarios for these disturbed pine forests, including whether they salvage harvest these stands or instead leave them standing without active management to undergo natural successional processes. However, if the goal of forest management is a replacement fully stocked lodgepole pine forest, my findings of low natural regeneration levels and lack of available microsites in the short term after MPB attack suggest that significant silvicultural intervention will be required. This will likely require salvage harvest that removes the MPB-killed overstory to create better microsite conditions for this shade-intolerant pioneer species, and may also require tree planting if germination of seed released from serotinous cones is insufficient to attain desired levels of regeneration. Further research that builds on this study is needed over longer time periods post disturbance to better understand the longer-term patterns in ecosystem properties and processes following MPB attack. This future research will help us better understand the future successional trajectories of these attacked stands under both active management through salvage harvest, and through passive hands off management that leaves attacked stands to undergo natural successional processes after MPB attack. Overall, my dissertation provides an important contribution to understanding the structure and ecological function of lodgepole pine forests in western Alberta.