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Assessment of post-beetle impacts on natural regeneration of lodgepole pine

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Mountain Pine Beetle Working Paper 2009-07

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MPB Project # 8.03

2009

Library and Archives Canada Cataloguing in Publication

Egger, Keith
Assessment of post-beetle impacts on natural regeneration of lodgepole pine [electronic resource] / Keith Egger ... [et al.].

(Mountain pine beetle working paper; 2009-07)
"MPB Project # 8.03".
Includes bibliographical references.
Type of computer file: Electronic monograph in PDF format.
Issued also in printed form.
Includes abstract in French.
ISBN 978-1-100-13145-0
Cat. no.: Fo143-3/2009-7E-PDF

1. Lodgepole pine--Regeneration--British Columbia--Nechako River Region. 2. Lodgepole pine-- British Columbia--Nechako River Region--Growth. 3. Reforestation--British Columbia--Nechako River Region. 4. Mountain pine beetle--British Columbia--Nechako River Region. 5. Forest fires--British Columbia--Nechako River Region. I. Egger, Keith Norman, 1953 - II. Pacific Forestry Centre III. Series: Mountain Pine Beetle Initiative working paper 2009-07

SB945 M78 A87 2009 634.9'751560971182 C2009-980163-9

Abstract

In 2004, a wildfire near the Kenney Dam in north-central British Columbia burned approximately 10,000 hectares of forests. This ecological disturbance presented a unique opportunity to study natural and artificial regeneration in burned mountain pine beetle (*Dendroctonus ponderosae* Hopkins) infested stands. Mountain pine beetle has been documented as a natural disturbance agent that may precede wildfire in lodgepole pine forests (*Pinus contorta* var. *latifolia*).

In this study, we characterize lodgepole pine regeneration and the related micro-site conditions across a range of disturbance scenarios associated with mountain pine beetle (MPB) infestation and wildfire; identify limitations for the germination, survival, recruitment and growth of natural and artificial regeneration in relation to the effects of site moisture, fire severity, and competition by vegetation; and provide guidance on how to manage beetle-infested lodgepole pine stands subsequently burned by wildfires.

Germination, survival and recruitment of lodgepole pine seedlings over two growing seasons were compared on 18 disturbance plots with three fire severity classes, two moisture regimes, two seed provenances, and two seedbed types. In the growing seasons following the fire (2005 and 2006), seeded plots experienced bursts of spring germination followed by continuous minor waves of new germination that ended by August 2006.

Wet sites experienced a higher level of natural regeneration, and the density of seedlings increased with decreasing fire severity. On dry sites, new germinants were rare due to the impact of high and moderate fire severity, and the highest germination rates existed where fire severity was lowest. Seed provenance did not influence germination and survival rates. In contrast to the germination, survival, and recruitment results, growth rates were highest on the dry sites and increased with increasing fire severity. Thus, although recruitment on dry sites is unlikely to sufficiently restock lodgepole pine stands, the survivors show the highest growth rates. Conversely, recruitment on wet sites was sufficient to restock these stands, but seedling growth rates will likely be impeded by competing vegetation.

We found no evidence that the survival or growth of recruits on dry sites was affected by the adverse impacts of the beetle and burning on soil properties, and lower diversity of ectomycorrhizal communities and nitrogen-cycling communities.

Résumé

La perturbation écologique due aux incendies de forêt (2004) qui ont touché environ 10,000 hectares de forêt près de Kenney Dam était une occasion unique d’étudier la régénération naturelle et artificielle de peuplements infestés par le dendroctone du pin ponderosa (*Dendroctonus ponderosae* Hopkins) brûlés dans le Centre-Nord de la Colombie-Britannique. Le dendroctone du pin a été défini comme un agent perturbateur naturel qui peut précéder les incendies dans les forêts de pins tordus latifoliés (*Pinus contorta* var. *latifolia*).

Dans la présente étude, nous avons tenté de : définir les caractéristiques de la régénération du pin tordu latifolié et les conditions de sa niche écologique, au moyen d’une gamme de scénarios de perturbations associés à l’infestation par le dendroctone du pin ponderosa (DPP) et aux incendies de forêt; déterminer les facteurs qui limitent la germination, la survie, le recrutement et la croissance de la régénération naturelle et artificielle, et qui sont liés aux effets de l’humidité du site, à la gravité de l’incendie et à la végétation concurrente; offrir des conseils concernant les implications sur la gestion opérationnelle des conclusions relatives aux peuplements de pins tordus latifoliés brûlés ensuite par des incendies de forêt.

On a comparé la germination, la survie et le recrutement de semis de pins tordus latifoliés durant deux saisons de croissance, sur 18 parcelles perturbées, parmi lesquelles on comptait trois classes de gravité d’incendie, deux régimes d’humidité, des semis de deux origines différentes et deux types de lits de semences. Durant les saisons de croissance suivant l’incendie (2005 et 2006), les parcelles semées ont connu une forte germination printanière, suivie en continu par des vagues mineures de nouvelle germination, qui se sont achevées en août 2006.

C’est sur les sites humides que la régénération naturelle était la plus forte, et la densité des semences augmentait proportionnellement à la diminution de l’intensité du feu. Sur les sites secs, les nouvelles pousses étaient rares et limitées par les conditions liées à l’intensité moyenne à élevée des incendies, la germination étant plus importante là où les incendies avaient été moins importants. La provenance des semis n’a pas eu d’influence sur les taux de germination et de survie. Contrairement aux résultats de la germination, de la survie et du recrutement, les taux de croissance étaient plus élevés sur les sites secs et augmentaient proportionnellement à l’intensité de l’incendie de forêt. Par conséquent, bien qu’il soit improbable que le recrutement sur les sites secs suffise à restaurer le peuplement de pins tordus latifoliés, ce sont les survivants de ces sites qui montrent les taux de croissance les plus élevés. Inversement, le recrutement sur les sites humides était suffisant pour restaurer le peuplement, mais les taux de croissance des semis seront probablement entravés par la végétation concurrente.

Bien que nous ayons étudié les effets néfastes des dendroctones et du feu sur les propriétés du sol, ainsi que la baisse de la diversité des communautés ectomycorhiziennes et celles fonctionnant selon le cycle de l’azote sur les sites secs, nous n’avons trouvé aucune preuve que ces facteurs éduisent la survie ou la croissance des recrues sur ces sites.

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1 Introduction

The magnitude of mountain pine beetle (*Dendroctonus ponderosae* Hopkins) infestation of lodgepole pine (*Pinus contorta* var. *latifolia*) forests is having immediate social, economic, and ecological impacts in north-central British Columbia. This is most evident among the forests southwest of Vanderhoof (Ebata 2004). The Chief Forester of the B.C. Forest Service estimates that at least 200 million cubic metres of the one billion cubic metres of mountain pine beetle (MPB) infested lodgepole pine in the province will remain unharvested due to operational constraints and protection within parks (Pedersen 2004).

This will have significant consequences for the future regeneration of managed and unmanaged MPB-killed stands because the implications for stand development are unknown given the unprecedented scale of the outbreak. This threat is furthered by the prospect of major losses in lodgepole pine plantations and other species of pine stands also attacked by MPB.

Starting on June 28th 2004, a wildfire consumed 10,000 hectares of MPB-infested forest northeast of the Kenney Dam. The area affected by the fire is in the sub-boreal spruce biogeoclimatic zone, mainly in the dry-cool subzone (SBSdk). In burned areas, MPB-attacked (red) stands experienced higher than expected severities of crown fire (S. Taylor, CFS Forestry Officer, pers. comm.). An outcome documented by Turner et al. (1999) was that pre-fire presence of MPB increased fire severity. Severity is the amount of biomass consumed by the fire event as a function of frontal fire intensity, residence time, and fire cycle (Alexander 1982). Such conditions may inhibit or enhance natural regeneration. The Kenney Dam fire event presented a unique opportunity to study lodgepole pine regeneration in mountain pine beetle-killed stands after wildfire. While our conclusions are based on managed land, they provide an understanding for unmanaged forests as well.

This project characterizes lodgepole pine regeneration and the related micro-site conditions across a range of disturbance scenarios associated with mountain pine beetle infestation and wildfire. It also identifies limitations for the germination, survival, recruitment and growth of natural and artificial regeneration in relation to the effects of site moisture, fire severity, and competition by vegetation; and it offers guidance on how to apply the implications of our findings to managing lodgepole pine stands infected by MPB and subsequently burned by wildfires.

2 Material and Methods

2.1 Selection of Study Area

The 10,000 ha Kenney Dam fire (124º54’27” W, 53º36’34” N) occurred southwest of Vanderhoof, BC. The fire extent was determined by Canadian Forest Products Ltd. - Plateau Division, who provided a global positioning system (GPS) boundary of the wildfire and high-resolution aerial photography (one year before and six weeks after the fire). The post-fire image captured in 2004 was enhanced using proprietary procedures of TDB Forestry Consultants to emphasize MPB-attacked stands (S. Emmons pers. comm.).

To minimize site variation, a geographic information system (GIS) was used to select all potential sites using the criteria in Table 1.

Table 1. Selection criteria used to minimize environmental variation between study sites.

Source	**Criteria**	**Rationale**
DEM	Slopes between 0-3%	minimize variation
DEM	Elevation range between 700-1100m	minimize variation
Buffer	Within 200m of roads but not 30m from road	edge influence
Buffer	Not within 50m of lakes, rivers, swamps (riparian)	minimize variation
VRI	Leading species >90% lodgepole pine, age class >=5	minimize variation
VRI	Age >= 80 years	minimize variation
VRI	Biogeoclimatic subzone	minimize variation

A 25m digital elevation model (DEM) was generated from provincial terrain resource inventory mapping (TRIM) data. From the DEM, slope and aspect were derived and queried based on the criteria in Table 1. Other TRIM layers used included lakes, rivers, and roads with buffers applied to minimize edge effect. Criteria queried in the Vegetation Resources Inventory (VRI) and predictive ecosystem mapping (PEM) databases included age class, species composition, stand density, site index, and ecosystem variant polygons.

Field verification of inventory was completed using three temporary $50m^2$ fixed-radius plots. Height and age were recorded for two site trees and a site index calculated. In severely burned plots where crowns did not exist, diameter at breast height (dbh), rather than crown position, was required to determine dominance. Selection of the study sites was completed in late April and May 2005 using site moisture and fire severity to define disturbance scenarios.

2.1.1 Site Moisture

The soil moisture conditions across the study area were classified into dry and wet sites. A Campbell Scientific time domain reflectometer (TDR) with a 12-cm probe for volumetric water content (%) was used to randomly sample target stands during field reconnaissance. The range in site moisture content across the study area was classified as either dry (<20%) or wet (20-45%).

2.1.2 Fire Severity

The disturbance regime consisted of MPB-killed stands (>80% attack) in combination with high, moderate, and low severity wildfire. Level of MPB attack was determined in unburned stands by a visual estimate and the presence of red crowns. In burned stands, using fixed radius plots in combination with MPB-enhanced aerial photography, visual identification of beetle galleries under the bark and pitch-tubes was necessary to verify MPB attack.

An unsupervised classification of the colour aerial photography (scanned product) was performed to estimate fire severity. Remote sensing software (PCI Geomatics 2005) was used to compare stands identified by the fire severity classes and preliminary site selection data as ground truth (Bertolette and Spotskey 2001). A mask was created using the fire boundary to limit the classification to the fire area and mature forest. The supervised classification was run on the red, green, and blue channels of the imagery using a target of 30 classification classes. The resultant classes were sieved (grouped) to a minimum area of one ha. The classes were then labeled using manual interpretation and the area representing each fire severity class was calculated by a GIS grid summary function. Low fire severity accounted for the greatest proportion of the burned, mature forest (39%) followed by high (32%), and moderate (29%). An accuracy assessment comparing the classification to known fire severity conditions in mature, MPB-attacked stands resulted in 65% accuracy.

A more quantitative methodology is preferable to the remote sensing classification due to the lack of ground truth sites (locations of known conditions) (B. Hawkes, pers. comm.). Ryan and Noste (1985) provided an extensive method to estimate fire severity using the relationship between flame length and depth of char, resulting in a two-dimensional matrix of 20 fire severity classes. However, since their fire matrix does not consider differences between wet and dry sites, severity conditions were visually aggregated into three post-fire severity classes: high, moderate, and low (Figure 1). The distinction was based on the amount of crown and subsequent cone consumption. A relative measure of duff consumption by depth between sites was also used to define the classes, and it was considered acceptable to reduce severity classes in this fashion (B. Hawkes, pers. comm.).

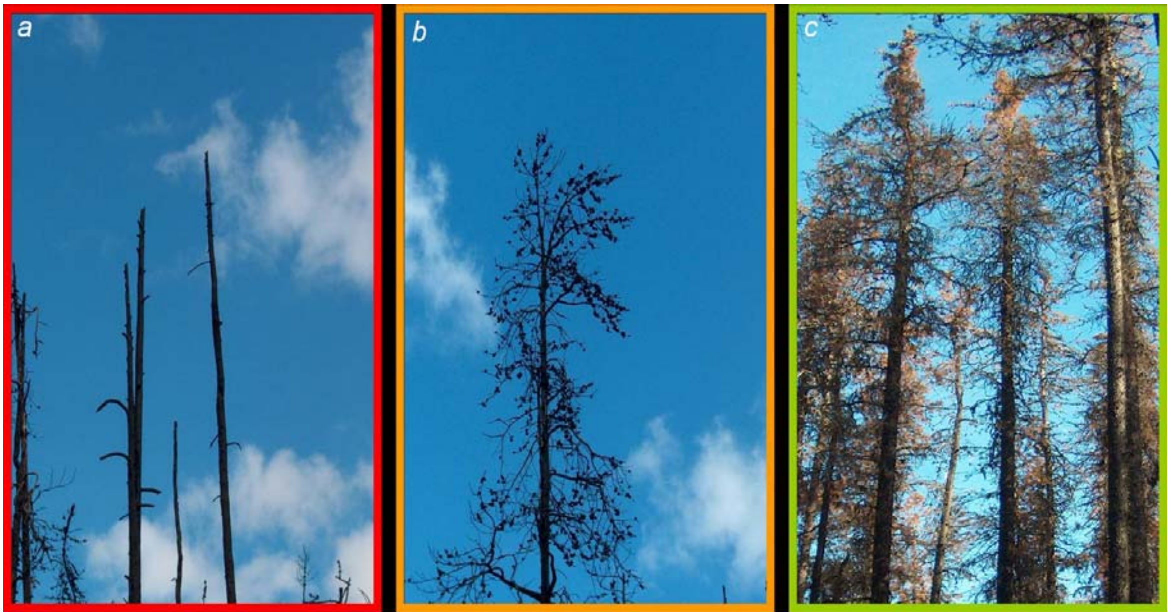


Figure 1. Structural crown differences between high (a), moderate (b), and low (c) fire severity conditions, found in the Kenney Dam fire area.

MPB-killed stands that remained unburned and unsalvaged represented the disturbance regime in unmanaged forests (control) and provided an insight into potential rehabilitation. In this study, three replicate stands were planted with improved class A seedlings on wet and dry sites.

2.2 Experimental Design

2.2.1 Disturbance Plot Establishment

Once the study area was defined, target stands were identified across the two soil moisture and the three fire severity classes. Within identified stands, rectangular disturbance plots were established using a random starting point for the first corner. Point of commencement for plots was recorded using a GPS unit and survey bearing and distances. Boundaries were marked with flagging tape. Site moisture classes (dry and wet), each with fire severity classes (high, moderate, and low) were replicated three times for a total of 18 disturbance plots in burned sites. Similar protocols were used to establish six disturbance plots in unburned sites.

2.2.2 Regeneration Treatments

For each disturbance plot in burned MPB-attacked stands, natural regeneration (control) was complemented by direct seeding (wild and improved seed) and the planting of improved seedlings. Because of limitations of substrate (thick moss layer), interspecific plant competition, and light availability for natural regeneration, the planting of nursery stock was the only regeneration treatment for unburned MPB-killed stands.

2.2.3 Seeded Plot Establishment

Within the 18 disturbance plots (9 dry, 9 wet), three replicate sub-plots measuring 1 x 2 m (1 x 1 m frame times two for a total of 108) were systematically located cardinally (north/south) independently from each other, in an attempt to capture similar micro-site conditions. Where necessary, CWD was removed to reduce natural variability. Metal stakes were driven into the ground at two corners of each 1 x 1 m frame to allow consistent sampling over the course of the experiment. To determine limitations to germination and survival, seeded plots were divided into exposed mineral soil (disturbed) and undisturbed substrate conditions through the careful removal of the organic layer using a small trowel on the north half of the seeded plots to expose mineral soil, leaving the southern half of the subplots undisturbed. A control plot to monitor vegetation and natural ingress was randomly located 7.5 m to either the north or south of the split-plot configuration (Figure 2).

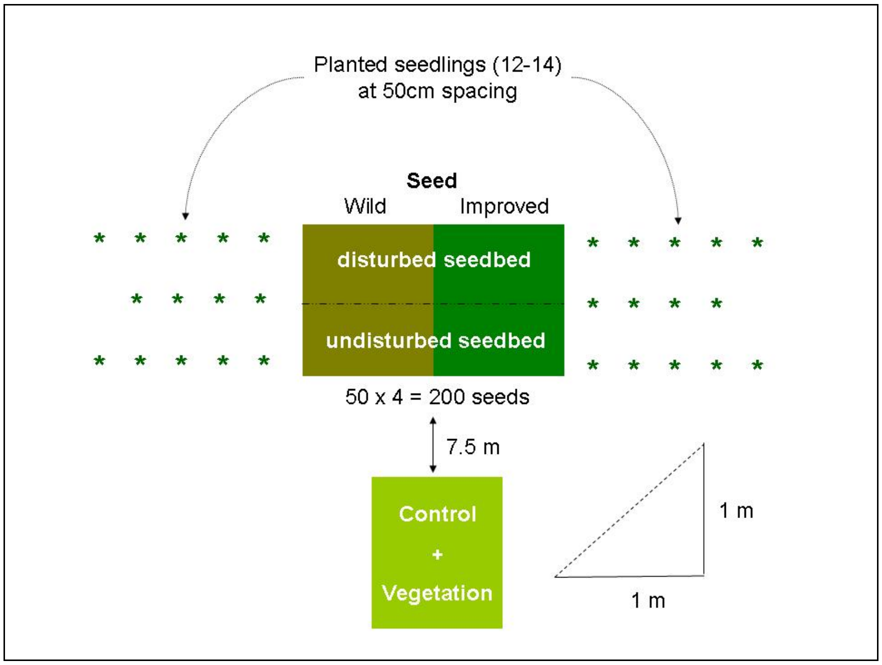


Figure 2. Seeded plot configuration showing the wild seed quadrant, the class-A seed quadrant (improved), both divided to expose the mineral soil. Planted seedlings flank each side.

Plastic seeding/monitoring frames were strung with plastic cord to form a grid of 100 intersections. Seeded plots were grid-planted (50 seeds each), to distinguish experimental from naturally occurring seedlings. The west frame was seeded with wild seed (BC 25110) and the east frame was planted with improved, Class A seed (BC 61041). By weight, improved seed was about 30% heavier than the wild seed. Across all seeded plots, 10,800 planting spots were available, and of these, only 9 had impediments such as rocks and roots that prevented seeding. Seeds were planted in a 1 cm square planting spot in the southeast corner of each intersection. Forceps were used to insert individual seeds 5 mm beneath the mineral seedbed (disturbed) and organic layer (undisturbed). Planting on May 14-15, 2005 was followed by a week of rain that aided germination.

2.2.4 Planted Nursery Seedlings

Class A nursery seedlings (seedlot # 47361) from two Pacific Regeneration Technologies (PRTA) nurseries (Vernon – 3 boxes and Armstrong – 2 boxes) were donated by Canfor - Plateau Division. The stock type was PCT 310B 1+0 and they were lifted in October and November 2004, respectively. Immediately following seeding of burned treatments, seedlings were systematically planted over the following two days (May 16-17, 2005). In burned treatments, half the allotted seedlings for the subplot (12-14) were planted adjacent to the west side of the seeded subplot and the other half to the east side at 50 cm intervals (Fig 2). In unburned treatments, all seedlings were planted in groups of 24-28 with similar spacing (Fig 3). In total, 1828 seedlings were planted in burned and unburned treatments.

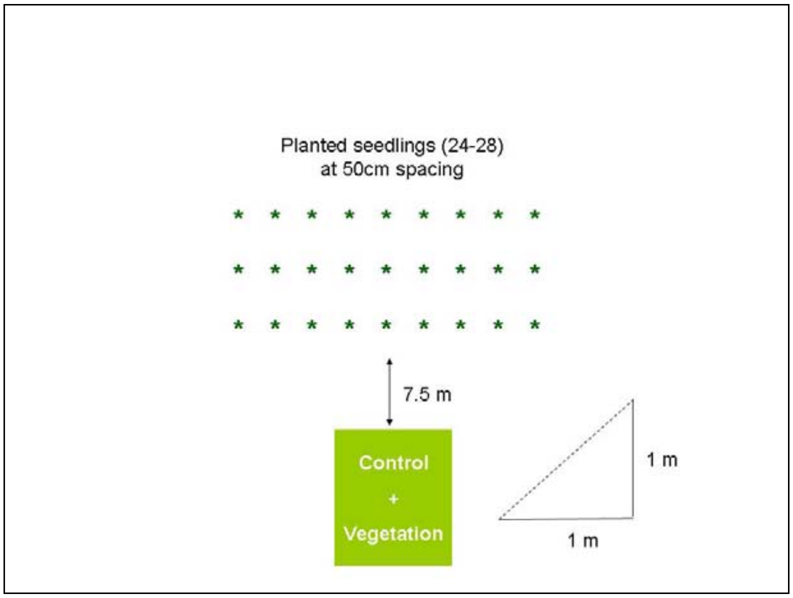


Figure 3. Regeneration subplot for unburned treatment showing the seedling planting spots in relation to the control.

2.3 Data Collection

Measurement in year one for soil moisture, soil temperature, seed germination/survival, and natural regeneration in the controls commenced June 14, 2005 and continued bi-weekly until September 11, 2005. For year two, measurement started on May 26, 2006 and continued monthly (within a day of coinciding year 1 dates) until August 18, 2006. Light data were collected in May 2005 and July 2006. Vegetation data were collected annually in August.

2.3.1 Soil Temperature

Measurements were taken bi-weekly in year one. To capture the range of temperature throughout the growing season, two measurements for each seeded plot were taken from disturbed and undisturbed seedbed conditions using a digital soil probe at the outer edge of each treatment, the rationale being that mineral soil retains heat much better than organic material (Heineman 1998) and could provide valuable information for germination and survival data analysis.

2.3.2 Soil Moisture

Volumetric water content was measured bi-weekly in year one and monthly in year two. Four readings were taken at the seeded plot edge (centre) using a Campbell Scientific time domain reflectometer (TDR) (Fleming et al. 1996). A 12-cm TDR sensor probe was used to capture the range of soil moisture in the newly established, shallow rooting zones.

2.3.3 Available Light

Light availability at the centre of each seeded plot and control was measured using a LiCor LI-1400 photosynthetic active radiation (PAR) sensor and compared with open-sky readings taken in a nearby opening. Measurements were taken at a 24-cm height above the ground on uniformly cloudy days when diffuse light conditions existed underneath the canopy. Using this procedure, measurements were recorded on May 31, 2005.

2.3.4 Vegetation Richness and Diversity

In the three control quadrates within each of the 24 (18 burned and 6 unburned) disturbance plots, vegetation richness, composition, and percent-cover to the nearest five percent were described by an ocular estimation. Following baseline observations (May 2005), vegetation was described annually in August (2005 and 2006) when vegetation growth was at its peak.

2.4 Regeneration Assessments

2.4.1 Seeded Plots

Plots were sampled biweekly for 11 sampling periods, noting the presence of germinated seedlings at each grid intersection. If multiple seedlings were present outside the seeding zone, they were removed as replicates. To ensure field efficiency at each sampling period, seedling presence was recorded independently of previous samples and may have included natural regeneration. This method required data verification in preparation for analysis.

2.5 Germination, Survival and Recruitment

The assumption made for data verification was that, in any given cell, a seed could only germinate once and a seedling should be present in each subsequent sampling period unless it had died. In a Microsoft Excel spreadsheet, a macro-command verified the raw presence/absence data. Germination, survival, and recruitment rates were described for year one, year two, and years one and two combined. Germination rates were calculated as the percentage of planted seed germinating from the total number of seeds by category in year one, in year two, and in years one and two combined (50 seeds per seeded plot). Mean rates at each sampling period were obtained by taking the average germination and survival amongst disturbance plots. Of those seeds that germinated in the first season, the survival rate for year one is the percentage of germinants alive at the end of that season.

An assessment of over-winter mortality captured seedling losses from the last sampling period in year one to the first sampling period in year two. The survival rate in year two was calculated from seed that germinated only in year two. Cumulative germination and survival rates represent total germination and survival across both seasons. Recruitment rates were obtained in year one by multiplying the germination by survival rates for the first season, and in year two by incorporating cumulative germination and survival rates. Mean values for recruitment were determined in each disturbance plot.

To determine the survival rates for planted seedlings, survivors were counted at the final sampling period for year one (September 11, 2005) and year two (August 18, 2006).

Naturally regenerated seedlings were counted during each sampling period over the two growing seasons. Seed rain assessment post-fire was not undertaken.

2.6 Biomass and Resource Allocation

2.6.1 Naturally-regenerating seedlings

A single cohort (germinated 2005) of lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) seedlings were sampled for this study. In 2005, seedlings were collected at two-week intervals through July and August and additionally after the first frost at the end of October (Julian dates 180-306). Where possible, a single seedling was collected close to each of the 54 sub-plots during each sampling period. However, seedlings did not exist for all the sub-plots during all the sampling periods. In 2006, seedlings were sampled after the first frost at the end of October. Where possible, three seedlings that had germinated in 2005 were collected near each of the 54 sub-plots. However, seedlings could not be located for some of the dry sub-plots.

Individual seedlings were sampled based on their proximity to each disturbance sub-plot. Using the disturbance sub-plot center as a reference location, a fixed compass bearing was determined for each sampling period prior to sample collection. For each sampling period, the individual sample for collection was randomly located by walking ten paces on the pre-determined compass bearing. The seedling that met the age requirement and was located closest to the determined sampling location was harvested.

Seedlings were excavated using a trowel, and the entire root systems were placed in individual plastic bags with the associated soil, labelled, and transported to the laboratory in a cooler. Samples were stored for a maximum of two weeks at 4^0C until the samples could be processed.

Seedling samples were processed to determine root and shoot dry mass. Seedling stems were severed at the root collar (i.e. soil interface). Seedling shoots were rinsed using tap water to remove any foreign material. Soil and organic matter were removed from the seedling roots through sequential rinsing. The samples were dried in a drying oven at 70^oC for 72 hours until a constant mass was attained. The dried mass was determined for each root and shoot sample using a digital balance. Root-shoot ratios were then determined for each seedling by dividing the dry root mass (g) by the dry shoot mass (g).

2.6.2 Seedlings derived from germinated seed

A subset of the seedlings derived from germinated seed were removed, stems were severed at the root collar, rinsed to remove soil and organic matter, and dried to constant mass. Dry weight was measured for shoots and roots and root-shoot ratios determined.

2.6.3 Planted seedlings

Planted seedlings were not destructively sampled, and were left on the plots as a legacy for future assessment. Stem diameter was measured for a subset of seedlings on all plots, and stem diameter relative growth (mm/mm initial increment) estimated.

2.7 Ectomycorrhizae (ECM) Morphotyping

Intensive sampling for mycorrhiza assessment occurred on MPB sites on August 25-26, 2006, and consisted of harvesting lodgepole pine seedlings that had been ‘seeded’ (with unimproved wild seed) in the spring of 2005 into previously established grids. These seedlings, depending on date of germination, were approximately two growing seasons old. Disturbance conditions included Dry Burned (low, moderate and high burn) and Wet Burned (low, moderate and high burn), as well as an ‘Unprepared’ (no scarification) versus ‘Prepared’ (organic soil removed down to the mineral layer) treatment.

Reduced seed germination inside and outside the grids was evident while harvesting the dry sites. . To compensate for missing seedlings, we collected similar age seedlings from sites that were ‘off’ grid but in the same plot. ‘Off’ grid seedlings were similar in age, and came from soil determined to be either organic (not prepared) or mineral (‘prepared’ equivalent). All seedlings were randomly selected, harvested with soil surrounding roots, bagged and kept cool. Seedlings were stored at 5˚C until assessed.

A total of 103 seedlings were characterized for ECM diversity. Following EMC assessment, roots were frozen at –20˚C for subsequent molecular (LH-PCR) analysis.

2.8 Fungal and Nitrogen-cycling Bacterial Genotyping

DNA preparation

DNA was extracted from all root samples using the Ultraclean Soil DNA kit (MoBio, Carlsbad, CA, USA) and followed the manufacturer’s recommended alternative protocol for increased yield. Before extraction, samples were frozen at -80°C for 30 min, thawed for 30 min, frozen at -80°C for 30 min, and thawed again for 30 min to weaken cells.

PCR amplification of the fungal community (LH-PCR)

A segment of the fungal intergenic spacer region containing part of the 5.8S rRNA gene and the second internal transcribed spacer (ITS2) was amplified using primer set ITS3 (GCA TCG ATG AAG AAC GCA GC) (White et al. 1990) and NLB4 (GGA TTC TCA CCC TCT ATG AC) (Martin and Rygiewicz 2005). The reverse primer NLB4 was labeled with WellRed fluorescent dye D3 (Integrated DNA Technologies). Each 30 µl PCR reaction contained 0.6 µL undiluted genomic DNA, 1X PCR Buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.04 µM of each primer, and 0.7 U Platinum Taq DNA Polymerase (Invitrogen Life Technologies). Thermocycler conditions were: an initial denaturing, annealing and extension cycle of 94°C for 4 minutes, 48ºC for 1 minute, and 72ºC for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 48°C for 30 seconds, and 72°C for 1 minute 30 seconds. The final extension required 6 minutes 30 seconds at 72°C.

PCR amplification of the nitrogen-fixing community (nifH gene)

The half-nested *nif*H protocol used Nh21F (5’-GCIWTITAYGGNAARGGNGG-3’) and WidNhR (5’-GCRTAIABNGCCATCATYTC-3’) for the primary PCR reaction (Widmer et al. 1999) and Nh428R (5’-CCRCCRCANACMACGTC-3’) for the second amplification (et al. 2005). The reverse primer Nh428R was labeled with WellRed fluorescent dye D4 (Integrated DNA Technologies). Each 31.2 µl PCR reaction contained 4.5 µL 1:10 dilutions of genomic DNA, 1X PCR Buffer, 0.2 mM dNTPs, 2.0 mM $MgCl_2$, 0.04 µM of each primer, and 0.75 U Platinum Taq DNA Polymerase (Invitrogen Life Technologies). Thermocycler conditions were the same for both reactions: a 1 minute denaturation step at 94°C, followed by 35 cycles of denaturing, annealing and extension at 94°C for 45 seconds, 53°C for 45 seconds, and 72°C for 1 minute 30 seconds respectively. The final extension required 10 minutes at 72°C.

PCR amplification of the denitrifying community (nosZ gene)

The *nos*Z gene was amplified using the primers nosZ-F (5’-CG(C/T) TGT TC(A/C) TCG ACA GCC AG-3’) (Throbäck et al. 2004) and nos1773R (5’-AAC GA(A/C/G) CAG (T/C)TG ATC GA(T/C) AT-3’) (Throbäck et al. 2004). The reverse primer was labeled with WellRed fluorescent dye D4 (Integrated DNA Technologies). Each 30µl PCR reaction contained 3 µL 1:10

dilutions of genomic DNA, 1X PCR Buffer, 0.2 mM dNTPs, 2.125 mM $MgCl_2$, 0.04 µM of each primer, and 0.75 U Platinum Taq DNA Polymerase (Invitrogen Life Technologies). A touchdown thermocycling program was employed: a 2-minute denaturation step at 94°C was followed by 5 cycles of denaturing, annealing, and extension respectively at 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 1 minute, with the annealing temperature decreasing by 0.5°C each time. This was followed by 29 cycles of denaturing, annealing and extension respectively at 94°C for 30 seconds, 58°C for 1 minute, and 72°C for 1 minute. The final extension required 10 minutes at 72°C.

Assessment and digestion of PCR products

PCR product success and quality was assessed by 1% agarose gel electrophoresis and visualized by staining with ethidium bromide. Bands of expected size were cleaned via ethanol precipitation and then resuspended in ultrapure water (Integrated DNA Technologies).

A restriction digest was performed on *nifH* and *nosZ* amplicons to prepare them for TRFLP analysis. For each reaction, 6 µL of PCR product was digested with 2.5 U MboI (for *nifH*) or HhaI (for *nosZ*) enzyme and 1X of the corresponding REACT buffer (Invitrogen Life Technologies). This enzyme was selected based on the number of restriction sites targeted (and therefore fragments generated); a series of endonucleases were tested on replicate samples and the one that identified the greatest amount of variation was chosen. Digests were incubated at 37°C for at least 3 hours and the reactions were terminated at 65°C for 10 minutes. Digested fragments were desalted by ethanol precipitation and resuspended in 8 µl of ultrapure water (Integrated DNA Technologies).

LH-PCR and TRFLP analysis

Fragments were prepared for analysis as suggested by the manufacturer for the Beckman-Coulter CEQ8000 Fragment Analysis System (Beckman-Coulter Inc.). LH-PCR and *nifH* or *nosZ* TRFLP samples were run together in a multiplexed reaction. 2 µL of LH-PCR product and 1.2 µL of digested *nifH* or *nosZ* PCR product were combined with 35 µL of Sample Loading Solution (SLS, Beckman Coulter Inc.) and 0.5 µL of 600 size standard. Fragment lengths were determined by electrophoresis using a Beckman Coulter (CEQ8000) automated sequencer, version 6.0.2. Analysis of fragment profiles was performed using the Beckman Coulter fragment analysis package 8000, version 8.0.52, fragment analysis algorithm version 2.2.1.

Statistical analysis

Relative abundance of operational taxonomic units (OTUs) was calculated by relativizing the fluorescent signal strength of each fragment peak to the total peak area within each sample (Osborne et al. 2006). Community structure was assessed graphically with Nonmetric Multidimensional Scaling (NMS) using PC-ORD 5.0 software (McCune and Mefford 1999). NMS was calculated on the basis of a Sørensen distance measure with 100 runs with real and randomized data and a maximum of 250 iterations to assess stability (instability criterion was 0.00001).

A stepwise reduction in dimensionality (6D-1D) was used to minimize stress along with a random starting configuration (user-provided seeds). When possible (i.e. for balanced subsets of data), multivariate differences were tested statistically with nested permutational multivariate ANOVA (NPMANOVA) (Anderson 2001); otherwise, univariate differences were tested with Multi-Response Permutation Procedures (MRPP) (McCune and Grace 2002) to examine effects between treatments.

The binary data were used to calculate richness (total number of OTUs per sample). Main effects ANOVA (Statistica 6.0) was used to detect overall significant differences due to treatments.

2.9 Soil Analysis

Soil samples were collected from 75 plots consisting of control (No MPB Unburned), attacked (MPB Unburned) and burned (MPB Burned) sites; an additional 177 samples were collected from rhizosphere and bulk soils to compare physical, chemical, mineralogical and biological properties of soils under attacked and burned pine stand.

3 Results and Discussion

3.1 Seedling Survival and Performance

- Consistent with fire severity classes, available light measured as the percentage of incoming PAR decreased as fire severity decreased and was least available in unburned plots.

- Dry sites had generally one-third the soil moisture of wet sites across all fire severity treatments.

- Growing season soil temperatures were higher on dry sites, especially on high and moderate fire severity sites. Soil temperature declined with decreasing fire severity and was lowest in unburned stands.

- Fire greatly reduced the richness of vegetation on dry and wet sites throughout all the time periods considered. Richness was greatest on wet sites, and although fire reduced richness, the severity of the fire had little impact. On dry sites, species richness increased as fire severity decreased.

- Recruitment (germination and survival) is summarized in Figure 4.

- The overall germination rate for the 10,800 planted seeds after two growing seasons was 33.6%. In both wet and dry sites, germination was highest in the spring and continued until the last monitoring period in the fall.

- Across all fire severities, the rate of germination ($p = 0.00$) and survival ($p = 0.00$) was significantly higher on wet sites versus dry sites.

- Cumulative germination rates on dry and wet sites reflected observations from year one. On dry disturbed ($p = 0.00$) and undisturbed ($p = 0.01$) sites, germination rates were significantly higher under low fire severity. On wet disturbed sites, a significant difference in germination rates only existed between high/moderate ($p = 0.04$) and high/low fire ($p = 0.05$) severities. On wet undisturbed sites, germination rates continued to be variable and were significantly higher ($p = 0.00$) in moderate severity as compared to high and low severities.

- Survival rates were significantly higher on dry sites in year 1 under low fire severity as compared to high (0.05) and moderate ($p = 0.03$) severities. On wet sites, survival declined with declining fire severity however, survival was only significant between high and low fire severity ($p = 0.02$).

- There were no significant differences in recruitment rates between seed provenance

- On dry disturbed sites, there was significantly higher recruitment on low severity fires as compared to high (p = 0.00) and moderate (p = 0.02) fire severities, which were not significantly different from each other (p= 0.51). On dry undisturbed sites, there was also significantly higher recruitment on low severity fires as compared to high (p = 0.02) and moderate (p = 0.02) fire severities, which were not significantly different from each other (p = 0.93). This trend continued for cumulative recruitment rates on dry sites that were significantly higher than high and moderate severity on disturbed (p = 0.00, p=0.01, respectively) and undisturbed seedbeds (p= 0.00, p=0.01, respectively).

- On dry sites, the control quadrats failed to capture natural regeneration under the high and moderate fire severity treatments. On dry high and moderate severity sites, the random location of quadrats did not capture any natural regeneration, but preliminary fixed plots had densities of 100-200 stems/hectare (sph). On a per hectare basis, the mean density of natural seedlings in the dry, low fire severity sites for year 1 and year 2 was 45,185 sph and 64,074 sph, respectively. Overall, the occurrence of natural regeneration was more prevalent on wet sites. In fact, the average amount of regeneration on wet, high fire severity treatments (46,667 and 76,296 sph) was similar to the dry, low severity fires. Regeneration density and the variability in density increase as fire severity decreases (year 1: moderate 281,481 sph, low 481,852 sph; year 2: moderate 441,111 sph, low 732,222 sph).

- Overall, 77.5% survival of the planted seedlings was observed. On dry sites, wildfire reduced survival of under planted seedlings to 67.8% as compared to the unburned control (90.9%).

- On wet sites, the effect of fire was not as pronounced but wet survival rates (86.9%) were higher than those on dry sites (66.9%). Mortality on wet sites was highest under low fire severity and in the unburned control in year two.

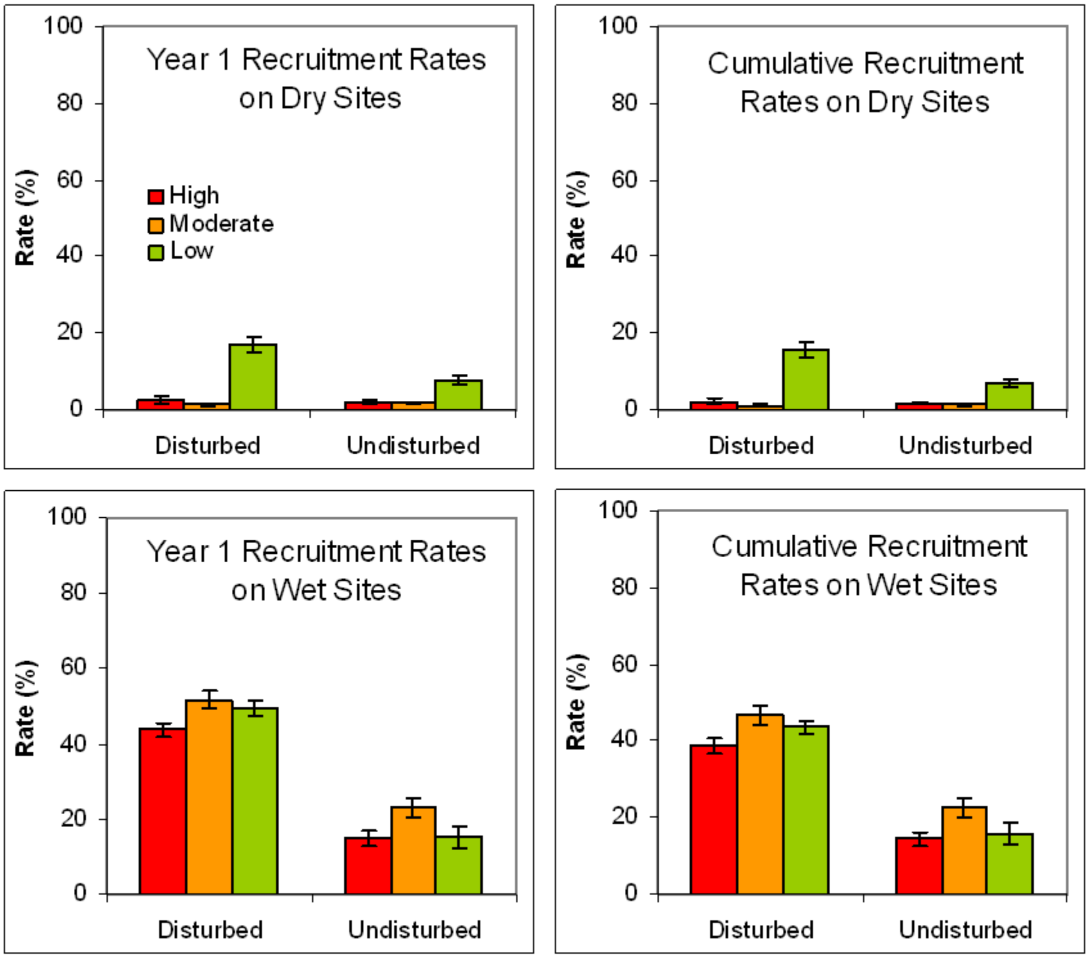


Figure 4. Mean (± S.E.) recruitment rates using pooled data illustrate higher rates in wet sites compared to dry sites both for year one and cumulatively.

3.2 Biomass and Resource Allocation

3.2.1 Naturally-regenerating seedlings

- In naturally-regenerating seedlings, there was no consistent relationship between disturbance type and the expressed average root-shoot ratios during the first growing season.

- While not significantly different, mean root-shoot ratios decreased in all measured treatments in 2006 relative to 2005, with the exception of the wet-low fire severity sites.

- No significant differences in total biomass were found between disturbance treatments in 2005. However, in 2006, the wet-moderate (wm) fire severity sites had significantly greater biomass than the wet-low fire (wl) (*p*=0.0003), wet-high fire (wh) (*p*=0.004) and the dry-low fire (dl) (*p*=0.00006) severity sites. In addition, a significant difference in total biomass was found between the dry-low fire severity (dl) and the wet-high fire severity (wh) sites (p=0.003).

- The biomass in 2006 was always higher in the wet sites compared to the dry sites.

3.2.2 Seedlings derived from germinated seed

- Seedling dry weight increased with increasing fire severity on both wet and dry sites.
- Seedling dry weight was higher on dry sites than on wet sites.
- Seedling root/shoot ratio decreased with increasing fire severity on dry sites but increased on wet sites.

3.2.3 Planted seedlings

- Relative growth (RG) results for height and diameter are given in Figure 5.
- In 2005, there were no clear differences between stem height relative growth either between sites or between treatments, although RG was higher on the wet site at moderate and high fire severities. For stem diameter, relative growth appeared to increase somewhat with fire severity on both wet and dry sites.
- In 2006, stem relative growth (for both height and diameter) responded strongly and positively to fire severity on the dry site, but not on the wet site.
- The less apparent growth trends in 2005 suggest that acclimation to the field conditions may have muted any growth responses to treatments. In addition, similar seedling growth likely reflected conditioning from greenhouse culture as seedlings likely possessed comparable carbon reserves for growth.
- The divergent responses on wet and dry sites in 2006 may reflect strong competitive effects limiting growth on the wet site and on the dry site under low fire conditions. These results suggest that planting may be utilized most effectively on dry sites (particularly given the exceeding low incidence of natural regeneration) and potentially on wet site under highest fire severity conditions. Planted seedlings will be monitored in future years to confirm the continuing patterns or to identify any changing patterns.

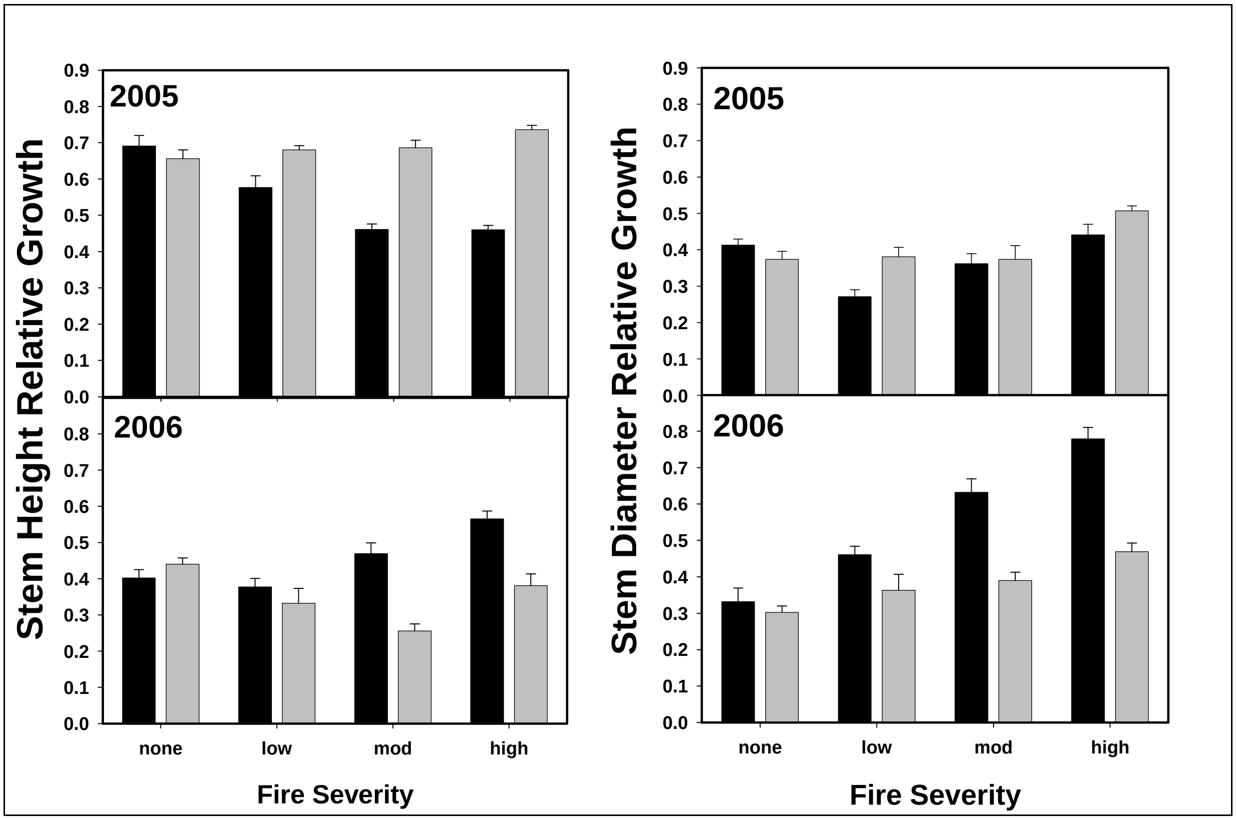
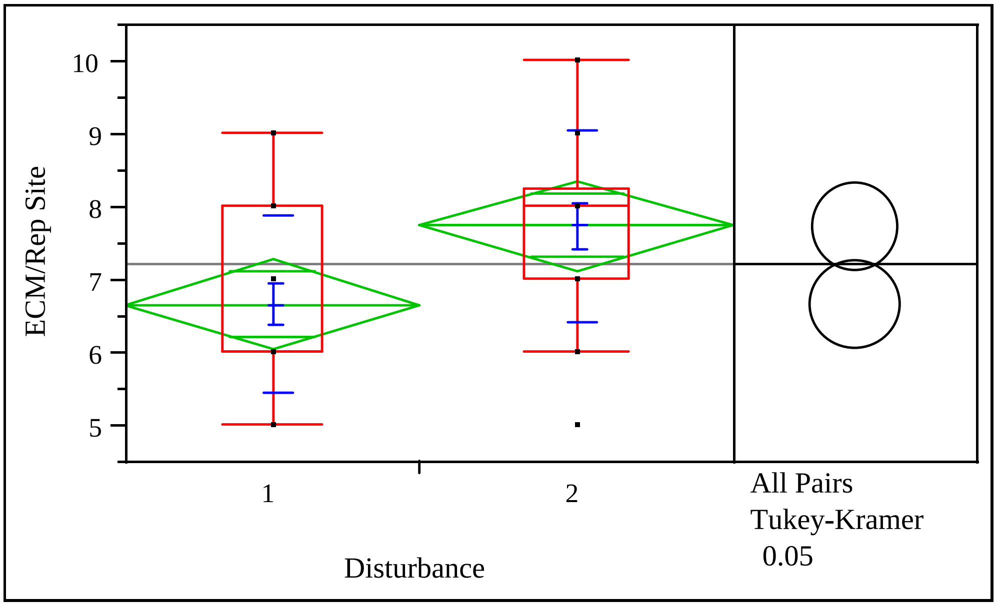


Figure 5. Planted seedling relative growth (±se) for stem height and stem diameter between dry sites (black bars) and wet sites (gray bars) for 2005 and 2006.

Ectomycorrhiza (ECM) Morphotyping

- ECM assessment found 29 morphotypes for young seeded seedlings on post MPB/fire sites (25 from the Dry disturbance and 27 from the Wet).
- Seedlings had from two to six ECM types each; replicate plots had from five to 10 morphotypes.
- No significant difference was found between the ‘on’ or ‘off’ grid seedlings, although those ‘off’ grid from wet sites (total seven seedlings) appeared to have higher ECM counts, but did not have bigger root systems or more root tips.
- ‘No Preparation’ compared to ‘Prepared’ sites, and for the Burn level (low vs moderate vs high burn), showed no significant differences within either the Dry or Wet disturbance. When data for the two disturbances was combined, results were the same.
- Disturbance effect (dry compared to wet sites), examined at both the seedling response as well as the plot response level for ECM morphotypes, showed that the ECM morphotype

diversity was significantly greater on wet compared to dry sites (Prob > F = 0.0177, replicate plot ECM response). (Fig. 6).



1 = Dry 2 = Wet

Figure 6. One-way ANOVA of ECM diversity by plot for wet and dry sites.

- Two-way ANOVA was used to determine possible interactions occurring between disturbance (Dry/Wet) and the 'No preparation/Prepared' as well as the Burn level (low/moderate/high burn) variables. All outcomes supported one-way ANOVA results: the ECM morphotype diversity was significantly greater on wet compared to dry sites, and no significant differences were seen between No preparation and Prepared sites, or among Burn levels. No interactions were identified between variables.

- To test the possibility that the number of root tips scored may have differed between comparisons and thus affected ECM outcomes, we repeated all analysis using the number of ECM 'root tips' per seedling as well as the number of ECM root tips per replicate plot. Both response levels indicated that significantly more ECM root tips were assessed for the Dry disturbance compared to the Wet disturbance (Prob > F = 0.0002 for seedling, and 0.0066 for plot responses). In addition, significantly fewer ECM tips were scored from low burn compared to moderate and high burn sites (Prob > F = 0.0046 for seedling and 0.0189 for plot responses). Despite fewer tips being assessed in the Wet disturbance, these sites still had greater ECM diversity compared to dry sites, so richness estimates from wet sites may have been underestimated. One reason for differences in the number of tips may be that wet sites had fine textured clay-like soils. This may have caused more tips to be lost during excavation and root processing compared to seedlings from the dry, coarser sandy soils. We are uncertain why seedlings from low burn sites would have fewer root tips, but soil properties might account for some of these differences. It does not appear that fewer tips in low burn sites affected the ECM morphotype diversity outcomes.

- By far the most frequently described ECM morphotypes in both the Dry and Wet disturbance were ascomycetes: *Cenococcum*, *MRA* and E-strain-like fungi. These were abundant and found on most seedlings/plots. Some Russulaceae and *Lactarius*-like species were also common to both Dry and Wet disturbances, as well as several *Tomentella*-like species and *Thelephora*. *Suillus/Rhizopogon*-like species, *Piloderma* and *Amphinema* mostly occurred on wet sites.

3.3 Fungal and Nitrogen-cycling Bacterial Genotyping

- The only treatment factor significantly affecting fungal community structure was moisture. PERMANOVA of a balanced subset of the data revealed that moisture had a significant impact, while burn and soil were non-significant. This was confirmed by MRPP and NMS analysis of the full data set, with MRPP analysis resulting in p=0.0006 for moisture, and NMS analysis revealing groupings according to moisture (Fig. 7).

- Fungal richness varied widely between samples. In general, mineral soils had higher fungal richness than organic soils, but these differences were not significant. Despite the impact of moisture on community structure, similar numbers of fungal OTUs were found in wet and dry samples.

- Fungal richness decreased with increasing fire severity, but again these differences were not significant.

- N-cycling bacterial communities (N-fixers and denitrifiers) were not significantly affected by any of the treatment factors.

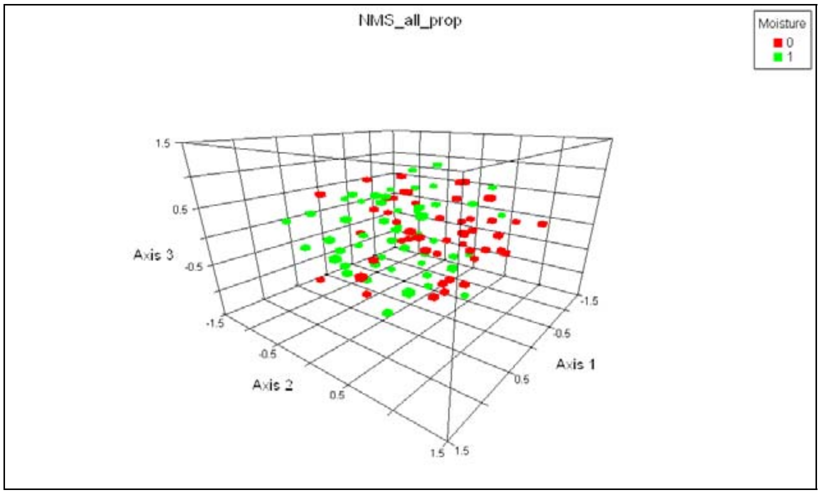


Figure 7. Non metric multidimensional scaling (NMS) plot of all samples, coded by moisture class. Samples from dry areas are red (0), while those from wet areas are green (1).

3.4 Soil Analysis

- Soils under MPB Unburned stands have mean pH $CaCl_2$ 5.8 compared to pH $CaCl_2$ 6.0 in No MPB Unburned stands.
- Soils under MPB Unburned stands have a significantly higher number of ectomycorrhizal associations than in MPB Unburned sites (20 *vs* 16).
- The effects of forest fires in soils under MPB-killed stands are different between dry and wet sites. In wet sites, only pH water varies in burned and unburned sites. In dry sites, total C, N, pH in water, conductivity, exchangeable Al, Fe, Ca, Mg, K, Mn, and Na, and CEC, Bray P1, and mineralizable NH_4-N in MPB Burned sites are significantly lower than MPB Unburned sites. We attributed the differential responses to the lower volumetric heat capacity of soil in dry sites compared to wet sites.
- Available N had mean (and standard error, in mg NH_4^+ per kg soil) values of 6.13 and 6.57 for rhizosphere and non-rhizosphere soils, respectively.
- Mean (standard error) available PO_4^{3-} (mg PO_4^{3-} per kg soil) was significantly higher in rhizosphere soils at 0.53 (0.028) than non-rhizosphere soils with 0.40 (.026).

4 Conclusions

4.1 Seedling Survival and Performance

- Site conditions following MPB and wildfire limited germination, survival, and recruitment rates.
- The extent of vegetation establishment depended on fire severity: wetter sites provided better conditions for plant re-establishment than dry sites.
- The density of natural regeneration was highest in stands that experienced less severe fire severity effects.
- There was no difference in early survival rates of wild and improved Class-A seed.
- Using ordered logistic regression to estimate the probability of survival, the model found that the odds of wild seeds surviving were slightly lower in burned stands than in unburned stands.
- Survival rates of planted seedlings indicated that growth limitations were higher in burned stands than in unburned stands.
- Germination and survival rates from our study support findings from other trials, which found that low germination, survival, and recruitment rates of seed may limit reforestation by way of direct seeding.

4.2 Resource Allocation

4.2.1 Naturally-regenerating seedlings

- In this study, naturally-regenerating lodgepole pine seedlings did not express any clear acclimation response until after the first growing season.

- The divergence in root-shoot ratios among disturbance treatments in year two suggest that by the end of the second growing year, the seedlings are acclimating to their localized environmental conditions.

- Among all sampled site conditions, the root-shoot ratios at the end of the first growing year (October) had a similar value of 0.35.

- Although there were significant differences within the same cohort on different disturbance sites in the second growing season, these differences could not be attributed to soil moisture and fire severity alone. Additional environmental factors such as nutrients, vegetation competition, light, and mycorrhizal associations likely contributed to the observed results.

4.2.2 Seedlings derived from planted seed, and planted seedlings

- The growth patterns and resource allocation for the seedlings derived from planted seed and from the planted seedlings showed a clearer relationship between site and treatment and growth.

- Although germination and survival were highest on wet sites, these seedlings had much slower growth rates, presumably due to greater competition.

- Although stocking of dry sites was limited, especially at higher fire severity, the seedlings grew much faster than on wet sites.

- We revisited the sites in the fall of 2008 to measure the planted seedlings again to determine if this is a consistent growth trend.

4.3 Ectomycorrhiza Morphotyping

- Lower ectomycorrhizal diversity on the dry sites may have contributed to the lower survival rates of seedlings derived from planted seed.

- Although ectomycorrhizal diversity was lower on the dry sites, seedling growth was not impaired. In fact, the seedlings on the dry site grew the fastest, suggesting that colonized trees can grow rapidly on the dry burned sites.

4.4 Fungal and Nitrogen-cycling Bacterial Genotyping

- We found no evidence that seedling growth is impaired by reduced fungal diversity or reduced diversity of nitrogen-cycling bacteria.

4.5 Soil Analysis

- Although fire damaged soil structure and may have created hydrophobic layers, seedling growth was unaffected.

- It is unclear whether salvage-logging of standing dead trees on the burned sites would sufficiently damage the soil to impair regeneration.

5 Recommendations

We recognize that managers will invest in sites to maximize their return on investment over the entire rotation, and as such, the following recommendations are based on burned MPB stands where seed supply is not limited (i.e. pine leading stands), and only relate to the early stages of regeneration.

- Suitable natural regeneration is more likely wet sites than dry ones.
- Where wet sites are not salvaged, managers should consider naturally restocking, as the seed bank, survival and recruitment should be sufficient on these sites in most years, particularly at low to moderate fire severity.
- Managers should monitor wet sites exposed to high fire severity to determine if natural recruitment is sufficient; and if found insufficient, should consider planting these sites.
- Our findings suggest that planting would be utilized most effectively on dry sites (particularly given the low incidence of natural regeneration in most years).
- Plantings on dry sites, particularly those experiencing moderate to high fire severity, may exhibit higher growth rates and a better investment ratio.
- Change to soil properties increased, and mycorrhizal fungal diversity decreased with increasing fire severity, but neither factor significantly affected seedling growth. Although we did not assess cumulative effects in our study, managers should try to assess whether additional perturbation, such as salvage logging of burned stands, would affect soil function sufficiently to impair seedling survival and growth.
- While our preliminary results show the best early gains on dry sites, we are continuing to monitor the planted seedlings to see if these relative growth advantages are maintained as yearly weather fluctuations and other factors affecting regeneration occur.

6 Acknowledgements

This project was funded by the Government of Canada through the Mountain Pine Beetle Initiative, a three-year, $100 million program administered by Natural Resources Canada, Canadian Forest Service. Publication does not necessarily signify that the contents of this report reflect the views or policies of Natural Resources Canada, Canadian Forest Service. Some of these results were the basis of a Master of Science thesis by Scott R. Scholefield (2007) and an undergraduate report by Patrick McMechan (2007).

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