

**Black Spruce Regeneration in *Kalmia* Dominated Sites:  
Effects of Mycorrhizal Inoculation and Forest Floor  
Mixing**

**By**

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in partial fulfillment of the requirements for the  
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## Abstract

There can be a shift in vegetation from black spruce (*Picea mariana* Mill.) forests with the ericaceous shrub *Kalmia angustifolia* L. (*Kalmia*) in the understory to ericaceous dominated heath in some areas of Newfoundland. This occurs on low to medium fertility sites after forest fire, insect defoliation, or clear-cut harvesting. *Kalmia* spreads rapidly with the concomitant failure of black spruce regeneration. This is a serious and widespread problem for the forest industry, and for protected area management when human interventions to natural disturbance cause the formation of *Kalmia* heath. The objective of this thesis was to test two alternate methods of enhancing black spruce regeneration in *Kalmia* dominated sites. I examined the growth response and foliar nutrient concentration of black spruce seedlings inoculated with the ectomycorrhiza (EM) *Paxillus involutus* (Batsch. Ex Fr.) Fr, planted in mechanically-mixed forest floors dominated by *Kalmia*. Changes in soil physical and chemical properties following mechanical mixing (tilling) of the forest floor horizon were also studied. The experiment was conducted over two growing seasons in *Kalmia* dominated sites resulting from heavy insect defoliation by spruce budworm and natural fires in Terra Nova National Park, Newfoundland.

I found that inoculated seedlings had higher levels of EM formation, but lower growth than control seedlings, suggesting that the effect of EM on host performance is likely related to the physiological performance of the specific EM isolate under the prevailing soil conditions. Control seedlings that were exposed to nutrient stress in the greenhouse had better growth than the commercial-nursery grown seedlings that were cultured using standard levels of fertilization. This suggests that black spruce growth might be closely related to adaptations to tolerate nutrient stress. Seedlings in insect defoliated sites in general had higher growth and foliar nutrient concentrations than those in burned sites. This was likely due to increased decomposition. Seedlings in all of the mixed forest floors had higher foliar nutrient concentrations than those in non-mixed forest floors, likely due to decreased acidity and competition, and increased decomposition. However, my results did not explain why forest floor mixing resulted in increased growth of black spruce seedlings in insect defoliated sites, but not in burned sites. Most theories of the growth of black spruce in *Kalmia* dominated sites include only below ground effects, but above ground effects may also be important. One possibility is that partial canopy cover is an important factor due to its effects on extremes of soil temperature and moisture, decomposition, litter quality, light levels, and near ground microclimate. My results indicate that it is possible to restore black spruce forest in insect defoliated sites with partial canopy cover that are dominated by *Kalmia* by mixing the forest floor and planting seedlings grown at low soil nutrient levels. However, further research is required to establish a method for restoring black spruce forest in open canopy burned sites.

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# Chapter 1

## General Introduction

Forests dominated by black spruce (*Picea mariana* Mill.) on medium and poor quality sites in eastern Newfoundland commonly become heaths dominated by the shrub *Kalmia angustifolia* L. (*Kalmia*) after disturbance. Many authors have reported this vegetation shift from black spruce forest with *Kalmia* understory to *Kalmia*-dominated heath after burning (forest fire), clear-cut harvest, or insect defoliation (Candy 1951; Damman 1975; Meades 1986; Page 1970; Richardson 1975; Richardson and Hall 1973; Mallik 1995; Yamasaki *et al.* 1998). This vegetation shift occurs primarily on nutrient-poor site types (Mallik 1995). After disturbance, *Kalmia* spreads rapidly and black spruce regeneration is limited (van Nostrand 1971; Richardson 1975; Richardson and Hall 1973; Mallik 1991; English and Hackett 1994). *Kalmia* cover can increase from 30% when forest harvesting occurs, to 85% within 5 years (van Nostrand 1971). Black spruce seedlings occur at low stocking density (Richardson 1975), exhibit slow growth (Richardson 1975; Wall 1977) and mortality after 3-6 years (Mallik 1994). This can result in long-term occupancy of the site by *Kalmia* (Damman 1971; Meades 1986; Mallik 1995). *Kalmia* dominated sites are low in species richness, productivity, and structural diversity (Mallik 2001; Bloom and Mallik 2004). It may take 200 years or more for black spruce forest to re-establish on these sites (Bloom and Mallik 2004).

Research on the causes of shifts from black spruce forest to *Kalmia* heath have focused on the effects of soil conditions and processes on establishment and growth of black spruce. This work suggests that the effects of disturbance on regeneration conditions are partly responsible (Mallik 1994; Bloom and Mallik 2004). For successful germination and establishment, black spruce seeds

require either burned humus (Jeglum 1984) or mixtures of humus and mineral soil (Zasada *et al.* 1983). Rates of germination and establishment of black spruce are low on seedbeds of litter and moss (Richardson 1975; Jeglum 1984). In contrast, *Kalmia* regenerates rapidly by sprouting from rhizomes in the fermentation and humus layers of forest floors (Mallik 1994). Thus, burns with high duff consumption (depth of burn) that create sites with exposed mineral soil and burned humus are generally followed by successful regeneration of black spruce forests. Disturbances that fail to create sites of exposed mineral soil such as insect defoliation, windthrow, and burns with low duff consumption create habitats that are favorable for the establishment of *Kalmia* heath.

Research has suggested several mechanisms to explain the slow growth and eventual mortality of black spruce seedlings that do become established in *Kalmia* dominated sites. Nutrient availability, particularly available N is extremely low in these sites (Damman 1971; Bradley *et al.* 1997a; Inderjit and Mallik 1999). Early research suggested that paludification and low rates of mineralization associated with the accumulation of recalcitrant *Kalmia* humus were responsible (Damman 1971). Some studies have suggested that competition for nutrients must also play a role (Mallik 1994; Wallstedt *et al.* 2002; Yamasaki *et al.* 2002). Bradley *et al.* (1997b) suggested that mineral N is limiting, and black spruce can access it only when mineralization rates are sufficient, whereas *Kalmia* can access organic N through ericoid mycorrhizae. Other studies have reported that secondary compounds from the leachates of *Kalmia* litter have the potential to exacerbate low nutrient availability by interference with mineral N cycling (Inderjit and Mallik 1996b; Bradley *et al.* 2000). Phenols in *Kalmia* leachates can increase soil concentrations of metallic ions such as Al, Fe, and Mn, and decrease N, which may be bound in protein-polyphenol complexes (Inderjit and

Mallik 1996b; Bending and Read 1996a,b; Mallik 2001). Black spruce seedlings in *Kalmia* dominated sites had high foliar concentrations of Al, Fe, and K, but foliar concentrations of N were not significantly low (Mallik 2001). Alternatively, direct allelopathic effects of *Kalmia* leachates may reduce the growth and survival of black spruce (Mallik 1987). *Kalmia* leachates have the potential to inhibit growth and primary root development of black spruce (Mallik 1987; Inderjit and Mallik 1996b). *Kalmia* leachates also have the potential to lower rates of ectomycorrhizal (EM) formation on black spruce seedlings (Mallik *et al.* 1998; Yamasaki *et al.* 1998). Yamasaki *et al.* (1998) hypothesized that *Kalmia* increases the rate of occurrence of root pathogens on black spruce roots. It is not known which of these factors has the greatest impact on black spruce growth in *Kalmia* sites. Yamasaki *et al.* (2002) suggested that the influence of *Kalmia* on black spruce is related to a combination of these and possibly other unknown mechanisms.

This research on mechanisms responsible for regeneration failure of black spruce in *Kalmia* dominated sites is important because there are implications for community ecology. Interspecific competition has been suggested as a key determinant of the structure and dynamics of black spruce-*Kalmia* communities. Early work in community ecology, which assumed that competition is ubiquitous, identified the principle that competition leads to the exclusion of all but the best competitor unless species have different niches (Gause 1934). Subsequent research has largely focused on identifying mechanisms of niche differentiation to explain patterns of species coexistence observed in plant communities. One such theory is that mutualistic associations with mycorrhizal fungi enable species coexistence (Grime *et al.* 1987). This is based on the observation that plants form associations only with a small group of fungi, which have different capacities

and methods of accessing soil resources. Recent research indicates that mycorrhizal associations are important because they result in high levels of variability in plant resource acquisition and growth, not because these relationships are always mutualistic (Klironomos 2003; Reynolds *et al.* 2003). If the growth of black spruce seedlings in *Kalmia* dominated sites depends on EM formation, then perhaps mycorrhizal associations enable niche differentiation and are fundamental to the structure and dynamics of black spruce-*Kalmia* communities. A similar theory is that niche differentiation occurs when species have different strategies for accessing soil nutrients, which vary in supply in space and time (Tilman 1985). Bradley *et al.* (1997b) suggested that niche differentiation occurs in black spruce-*Kalmia* communities because black spruce and *Kalmia* have different strategies for accessing soil N, and this determines the structure and dynamics of these communities.

Some studies have hypothesized that the effects of stress influence the structure and dynamics of black spruce-*Kalmia* communities. Debate continues in the ecological literature about the relative importance of competition and niche differentiation (MacArthur 1972; Tilman 1987; Huston and Smith 1987; Grace and Tilman 1990), as opposed to disturbance and stress (Grime 1973; Diamond and Case 1986; Thompson and Grime 1988; Campbell and Grime 1992), in structuring plant communities. Grime (1979) presented a triangular model of plant strategies arguing that plants have evolved strategies for resource acquisition, growth, and reproduction based on patterns of competition, stress, and disturbance (CSR-model). In this model, the importance of competition decreases as the intensity and frequency of disturbance and stress increases. If *Kalmia* can tolerate highly stressful conditions like extreme microclimatic conditions, very low pH, and nutrient stress, but black spruce is unable to do so,

then perhaps stress plays a role in controlling the structure and dynamics of these plant communities.

Additionally, research on mechanisms responsible for regeneration failure of black spruce in *Kalmia* dominated sites is important because there are significant implications for forest management. Clearcutting has resulted in the conversion of a large amount of black spruce forest to *Kalmia* dominated heath in Newfoundland (Meades 1986; Mallik 1995). This is a serious and widespread problem with respect to timber supply and managing forests for recreational and ecological values, so methods of restoring black spruce forest are needed. Within protected areas, many burned, insect defoliated, or windthrow sites have become *Kalmia* dominated heath. When research indicates that human interventions to disturbance regimes are responsible, it may be desirable to restore black spruce forest on these sites.

Brushing (cutting of stems), burning, and scarification are forestry site preparation methods that do not consistently control *Kalmia* or enhance black spruce regeneration on *Kalmia* dominated sites (Richardson 1979; Mallik 1991; Prevost 1994; Thiffault *et al.* 2004). Two site preparation techniques can improve black spruce growth on *Kalmia* dominated sites originating from clear-cutting, site fertilization (English 1997; Paquin *et al.* 1998; Thiffault *et al.* 2004) and herbicide application (English and Titus 2000; Thiffault *et al.* 2004). However, these methods may not fully release black spruce seedlings from the effects of *Kalmia*. The removal of *Kalmia* using herbicides may release black spruce from competition, but the inhibitory effects related to *Kalmia* humus would likely remain (Bradley *et al.* 1997b). Fertilization may improve nutrient availability for black spruce seedlings in *Kalmia* humus, yet the potential direct effects

associated with *Kalmia* leachates would likely not be relieved (Yamasaki *et al.* 2002).

Forest floor mixing and inoculating seedlings with EM are two possible alternative methods of enhancing black spruce regeneration on *Kalmia* dominated sites, which have not been tested under field conditions. Forest floor mixing is commonly accomplished using a rake, roto-tiller, or plough. The intent is to control competitive vegetation by shredding above and below ground plant parts (Mallik 1991; Frey *et al.* 2003), and to improve nutrient availability by increasing aeration and decomposition (Mallik and Hu 1997; Prescott *et al.* 2000). On *Kalmia* dominated sites, forest floor mixing has potential to also release black spruce from any direct allelopathic effects or nutrient imbalances related to *Kalmia* leachates. Increased microbial activity and decreased inputs of *Kalmia* leaf litter can reduce soil phenols (Inderjit and Mallik 1996b). Forest floor mixing has been used successfully to improve conifer regeneration (Pehl and Bailey 1983; Ross *et al.* 1986; Burger and Pritchett 1988; Messier *et al.* 1995). Inoculating conifer seedlings with site specific EM before outplanting has been successfully employed as a method of improving regeneration on poor quality sites. In general, mycorrhizae increase seedling survival and growth by increasing nutrient uptake, tolerance to low water availability, and resistance to fungal pathogens (Ingleby *et al.* 1994). The EM fungus *Paxillus involutus* (Batsch. Ex Fr.) Fr. may be particularly helpful, because it improved the growth of black spruce seedlings in *Kalmia* soils in a greenhouse experiment (Mallik *et al.* 1998). *P. involutus* may also be capable of degrading allelochemicals in *Kalmia* soils (Zeng and Mallik submitted).

This study was conducted to examine the effectiveness of two methods of improving black spruce regeneration on *Kalmia* dominated sites, each of which

might both increase nutrient availability and ameliorate direct allelopathic effects. The specific objectives of this study were: i) to determine if black spruce growth in *Kalmia* dominated sites can be enhanced by planting seedlings inoculated with EM following mechanical mixing (tilling) of the forest floor and ii) to explain the response of seedlings to these treatments with respect to changes in foliar nutrients and soil physical and chemical properties.

## Chapter 2

# Response of Black Spruce Seedlings to Mycorrhizal Inoculation and Forest Floor Mixing in Sites Dominated by *Kalmia*

### Abstract

In central Newfoundland, regeneration of black spruce (*Picea mariana* Mill.) may fail on sites dominated by *Kalmia angustifolia* L. (*Kalmia*) following forest fire, insect defoliation, or clear-cutting. This is a serious and widespread problem for the forestry industry in nutrient poor sites, and it can be a problem for managers of protected areas. The objective of this study was to experimentally test two alternate methods of restoring black spruce forest cover on *Kalmia* dominated sites. I examined the growth response of black spruce seedlings inoculated with the ectomycorrhizal fungus *Paxillus involutus* (Batsch. Ex Fr.) Fr planted in sites where the forest floor was mechanically mixed (tilled). Uninoculated seedlings and commercial nursery-grown seedlings in non-mixed plots were used as controls. The experiment was conducted in replicated *Kalmia* dominated plots originating from spruce budworm defoliation and forest fire in Terra Nova National Park, Newfoundland. I determined shoot height, root-collar diameter, shoot and root dry mass, shoot/root dry mass ratio, foliar nutrient concentration (N, P, K), and EM formation of the seedlings after one and two growing seasons. The inoculated seedlings had significantly higher EM formation than the control seedlings. However, the inoculated seedlings did not have greater growth (height, root-collar diameter, shoot and root dry mass, and shoot/root dry mass ratio) than the control seedlings in both insect defoliated and burned sites. The control seedlings had significantly higher growth (height, root-collar diameter) than the nursery seedlings in plots with mixed forest floors in insect defoliated sites. In general seedlings in insect defoliated sites had significantly higher increase in height and foliar nutrient concentrations (N, P, K) than that in burned sites. Seedlings in insect defoliated and burned sites had significantly higher foliar nutrient concentrations (N, P, K) in mixed plots than in non-mixed plots. However, forest floor mixing improved seedling growth significantly (height, root-collar diameter, shoot and root dry mass) only in insect defoliated sites. The results suggested that the success of EM inoculation is related to the response of the specific EM isolate to existing soil conditions. They also suggested that black spruce growth might be closely related to adaptations to stress tolerance. Although most hypotheses explaining growth limitation of black spruce in *Kalmia* sites focus on below-ground mechanisms, these results suggested that partial canopy cover also has important indirect effects. It should be possible to restore black spruce forest in insect defoliated sites that are dominated by *Kalmia* by mixing the forest floor and planting seedlings that have been exposed to nutrient stress in the greenhouse.



## Introduction

Forests dominated by black spruce (*Picea mariana* Mill.) on medium to poor quality sites in Newfoundland commonly contain *Kalmia angustifolia* L. (hereafter *Kalmia*) in the understory (Meades and Moores 1989). These forests often become heaths dominated by *Kalmia* after forest fire, clear-cut harvesting, or insect defoliation (Candy 1951; Page 1970; Damman 1975; Richardson 1975; Meades 1986; Mallik 1995). These ericaceous heaths may persist for many decades (Damman 1971; Meades 1986; Bloom and Mallik 2004). Following disturbance, *Kalmia* spreads rapidly and black spruce seedlings exhibit low stocking density, slow growth, and high mortality after 3-6 years (Richardson 1975; Wall 1977; Mallik 1994). The failure of black spruce regeneration has been attributed to competition from *Kalmia* (Mallik 1994), low mineralization rates associated with *Kalmia* humus accumulation (Damman 1971), or allelopathic nutrient interference by phenolic compounds in *Kalmia* leachates (Mallik 1987; Inderjit and Mallik 1996b). In addition, phenols in *Kalmia* leachates may have direct toxic effects on primary root growth and development of black spruce (Mallik 1987; Inderjit and Mallik 1996b) or EM formation in the conifer (Mallik *et al.* 1998). Yamasaki *et al.* (1998) found that seedlings growing near (<1 m) *Kalmia* had lower levels of EM formation and different EM species than those growing far (> 1m) from *Kalmia*.

In Newfoundland, failure of black spruce regeneration in sites dominated by *Kalmia* is a widespread problem for the forestry industry (Mallik 1995). Within National Parks and other protected areas, human interventions have changed historic disturbance regimes and restoration of black spruce forest on some *Kalmia* dominated sites is desirable. For example, Bloom and Mallik (2004) suggested that extinguishing smouldering forest floors after fire in Terra Nova

National Park (TNNP) may cause decreased depth of burn with a concomitant increase in *Kalmia* heath establishment. *Kalmia* grows rapidly from rhizomes and stem-base sprouting in unburned forest floors whereas black spruce requires mineral soil seedbeds for regeneration.

Some forestry site preparation techniques such as brushing (cutting of stems), burning, and scarification likely do not consistently control *Kalmia* or enhance black spruce regeneration on *Kalmia* dominated sites (Richardson 1979; Mallik 1991; Prevost 1994; Thiffault *et al.* 2004). Site fertilization is an effective method of improving black spruce regeneration on *Kalmia* sites (English 1997; Paquin *et al.* 1998; Thiffault *et al.* 2004), as is herbicide application (English and Titus 2000; Thiffault *et al.* 2004). However, these methods may not fully release black spruce seedlings from the effects of *Kalmia*. The removal of *Kalmia* using herbicides may release black spruce from competition, but the inhibitory effects related to *Kalmia* humus would likely remain (Bradley *et al.* 1997b). Fertilization may improve nutrient availability for black spruce seedlings in *Kalmia* humus, yet the potential direct effects associated with *Kalmia* leachates would likely not be relieved (Yamasaki *et al.* 2002).

One possible method of improving black spruce regeneration in *Kalmia* dominated sites involves mechanically mixing the forest floor as a means of controlling *Kalmia* and improving site fertility. Forest floor mixing may promote nutrient availability by enhancing aeration and microbial activity (Mallik and Hu 1997; Prescott *et al.* 2000), and by blending in mineral soil, which increases pH (Messier *et al.* 1995). Forest floor mixing controls the growth of vegetation because roots and rhizomes are shredded (Mallik 1991; Keenan *et al.* 1994; Frey *et al.* 2003). On *Kalmia* dominated sites, forest floor mixing has the potential to also release black spruce from any direct allelopathic effects or nutrient

imbalance related to *Kalmia* leachates. Increased microbial activity and decreased inputs of *Kalmia* leaf litter can reduce soil phenols (Inderjit and Mallik 1996b). Forest floor mixing increased the growth of ponderosa pine (*Pinus ponderosa* Laws.) seedlings in Oregon (Ross *et al.* 1986), and loblolly pine seedlings in plantations in Georgia (Pehl and Bailey 1983) and Florida (Burger and Pritchett 1988). Forest floor mixing reduced competing vegetation and improved the growth of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) in low productivity sites in Vancouver Island (Messier *et al.* 1995). However, the effect of forest floor mixing on black spruce regeneration has not been investigated under field conditions.

Another possible method of improving black spruce regeneration in *Kalmia* dominated sites may be planting spruce seedlings that have been inoculated with ectomycorrhizae (EM). EM have been shown to increase seedling survival and growth by increasing nutrient uptake, tolerance to low water availability, and resistance to fungal pathogens (Ingleby *et al.* 1994), and perhaps by degrading allelochemicals (Perry and Choquette 1987). Therefore inoculation with *P. involutus* has the potential to both improve nutrient availability and ameliorate potential direct allelopathic effects of phenols in *Kalmia* leachates. Inoculating conifer seedlings with EM fungi can be an effective means of enhancing forest regeneration in degraded or nutrient-poor sites. Inoculation of loblolly pine (*Pinus taeda* L.) and Virginia pine (*Pinus virginiana* Mill.) seedlings with *Pisolithus tinctorius* (Pers.) Cok. & Couch was found to increase their survival and growth on degraded, nutrient poor sites in Tennessee (Marx and Bryan 1975; Marx 1980; Marx and Cordell 1988). Inoculation of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) with *Laccaria bicolor* (Maire) P.D. improved seedling growth on well-drained soils in a mixed pine forest in France (Villeneuve *et al.* 1991).

Growth of jack pine (*Pinus banksiana* Lamb.) in the prairies in southern Saskatchewan and Manitoba was improved by seedling inoculation with chopped EM root tips (unidentified) from jack pine forest humus (Whitney *et al.* 1972). Inoculation of jack pine with *Thelephora terrestris* Fr. resulted in larger seedlings on oil sands tailings in northern Alberta (Danielson and Visser 1989). However, inoculation of seedlings with EM may fail to enhance seedling growth, often because the fungi are not adapted to the tree species or site conditions (Trappe 1977; Bledsoe *et al.* 1982; Molina 1980; Molina and Chamard 1983). Mallik *et al.* (1998) identified an EM fungus, *Paxillus involutus* (Batsch. Ex Fr.) Fr. (isolate NF4), which formed abundant EM with black spruce in the presence of *Kalmia* leachates under greenhouse conditions. In addition, inoculation of black spruce with *P. involutus* enhanced the growth of seedlings in soils amended with *Kalmia* leachates, and in the presence of living *Kalmia* plants. Zeng and Mallik (submitted) suggested that *P. involutus* may degrade phenols from *Kalmia* leachates. However, the growth response of inoculated black spruce seedlings in the presence of *Kalmia* has not been tested under field conditions.

There is an inherent challenge in evaluating the effectiveness of using EM inoculated seedlings on difficult growing sites. To evaluate the specific effect of EM inoculation on growth response of the seedlings, both the inoculated and uninoculated control seedlings must be grown in the greenhouse using low levels of fertilization because high soil nutrient levels can discourage EM formation (Danielson *et al.* 1984). Therefore, the inoculated and uninoculated control seedlings are nutritionally stressed when outplanted. If in the experiment both the inoculated and uninoculated control seedlings grow poorly, it is not clear whether a conventionally grown seedling would perform better than an inoculated seedling. The standard method of growing seedlings for reforestation purposes

is to apply high levels of fertilization to increase foliar nutrient concentrations that sustain nutrient levels after outplanting (Malik and Timmer 1996). Therefore, I used seedlings grown under standard conditions in a commercial nursery as a secondary control.

The objective of this study was to experimentally test the two methods mentioned above in order to improve black spruce regeneration in insect defoliated and burned sites that were dominated by *Kalmia*. I examined the effects of inoculating seedlings with the EM fungus *P. involutus*, and forest floor mixing, on growth and foliar nutrient concentration of black spruce seedlings. I hypothesized that forest floor mixing would improve nutrient availability, and the EM-inoculated black spruce would have improved nutrient uptake, thus both would increase growth and foliar nutrient concentration under field conditions.

## Methods

### *Study area*

The study area was located in Terra Nova National Park (TNNP) (Lat. 48.5° N, Long. 54.0° W, Elev. 100 m asl), approximately 90 km southeast of Gander, Newfoundland. This area belongs to the eastern boreal forest region (Rowe 1972), dominated by black spruce and balsam fir (*Abies balsamea* (L.) Mill.). White spruce (*Picea glauca* (Moench) Voss), tamarack (*Larix laricina* (Du Roi) K. Koch), white birch (*Betula papyrifera* Marsh.), and trembling aspen (*Populus tremuloides* Michx.) occur as sub-dominants (Meades and Moores 1989). The forest understory is dominated by ericaceous shrubs, primarily *Kalmia*, *Vaccinium angustifolium* Ait., *Ledum groenlandicum* Oeder, and *Rhododendron canadense* (L.) Torr., *Kalmia* being the most dominant. The common herbaceous species are *Cornus canadensis* L., *Maianthemum canadense* Desf., *Arctostaphylos uva-ursi* (L.) Spreng., *Clintonia borealis* (Ait.) Raf., *Linnaea borealis* L., and *Gaultheria hispidula* (L.) Muhl. The ground level mosses are *Pleurozium schreberi* (Brid.) Mitt., *Dicranum polysetum* Sw., *Hylocomium splendens* (Hedw.) B.S.G., and *Ptilium crista-castrensis* (Hedw.) De Not. The dominant lichens are *Cladonia* sp. and *Cladina rangiferina* (L.) Nill., *C. mitis* (Sandst.) Hustich., and *C. alpestris* (Opiz) Brodo. Parent materials of the forest soils consist of shale, slate, sandstone, and schists, which are overlain by glacial till (Damman 1964). The forest soils are podzols in well-drained areas, and are gleysols and organic soils in lowland areas (Meades and Moores 1989). This area experiences short cool summers, and moderate winters. The growing season is approximately 150 days, beginning in mid-May and ending in mid-October (Damman 1983). The number of degree-days (> 5 °C) is approximately 1170 (Newton 1992). Mean summer temperature is 12.6 °C, mean summer

rainfall is 448.9 mm, and mean total annual precipitation is 1184.3 mm (Power 1996). The major agents of forest disturbance are fire, windthrow, insect defoliation, and harvesting (Newton 1992).

### *Study sites*

In this study, I considered *Kalmia* dominated sites resulting from two types of common natural disturbances in the area: insect defoliation and forest fire. Three experimental sites were established in each disturbance type. The insect defoliated sites were defoliated by spruce budworm (*Choristoneura fumiferana* Clemens) between 1977 and 1983 (20 to 26 years ago). These sites were located along the Bluehill West Trail in TNNP at 48° 36.2' N, 54° 58.4' W, 75 m elevation (named Bluehill 1), 48° 35.6' N, 53° 59.1' W, 75 m elevation, (Bluehill 2), and 48° 35.6' N, 53° 59.5' W, 75 m elevation (Bluehill 3). Due to tree mortality by insect defoliation these sites had canopy openings ranging from 0.25-0.5 ha (Tracy Harvey, personal communication). The three burned sites, which experienced wildfires and fire suppression activities, were located at 48° 30.6' N, 54° 7.1' W, 165 m elevation (named Terra Nova), 48° 30.0' N, 53° 59.4' W, 140 m elevation (Ochre Hill), and 48° 27.0' N, 54° 8.7' W, 110 m elevation (Dunphy's Pond). The fire at Terra Nova occurred in July 1979 (24 years ago) during extreme fire danger conditions (Canadian Forest Fire Danger Rating System, Van Wagner 1987), burning approximately 314 ha (Power 1996). The Ochre Hill fire occurred in July 1970 (33 years ago) during low fire danger conditions, burning approximately 2 ha. The Dunphy's Pond fire occurred in June 1961 (42 years ago) during extreme fire danger conditions, burning approximately 800 ha. The burned sites were located in the open at least 10 m from the forest edge except Dunphy's Pond, which was located 2-3 m from the forest edge. Structure and composition of tree species were determined in each

site, by measuring stem density, basal diameter, and basal area of each species, and density of black spruce seedlings ( $\leq 3$  cm basal diameter) in five randomly located circular quadrats ( $50 \text{ m}^2$ ) (Table 1). The insect defoliated sites had both remnant canopy trees and advanced regeneration. Therefore, mean tree basal diameter was more variable in those sites than in burned sites, although the means were not significantly different ( $\chi^2 = 8.4$ ,  $p = 0.136$ ). The insect defoliated sites had higher tree species richness than the burned sites, and contained more deciduous species (*Acer rubrum* L., *Populus tremuloides*, and *Betula papyrifera*). The burned sites had only small tree saplings, so variability in basal diameter was lower than in insect defoliated sites. Stem density of trees and density of black spruce seedlings was particularly variable at Dunphy's Pond burn. In all sites, the density of black spruce seedlings was low (mean 160-2400 stems/ha) relative to the standard of 3000 stems/ha considered necessary for black spruce forest regeneration (Newton and Weetman 1993).

Understory species composition was determined by measuring cover (%) of all plants in mid-June in five randomly located quadrats ( $1 \times 1 \text{ m}$ ) in each of the three plots per site (Table 2). All of the insect defoliated sites were dominated by *Kalmia*, and *Kalmia* cover was significantly higher at Bluehill West 3 than in any other site ( $F = 23.0$ ,  $p < 0.001$ ). Abundance and species richness of ground mosses was higher at the insect defoliated sites than in the burned sites. All of the burned sites were dominated by *Kalmia*, and *Kalmia* cover was lower at Dunphy's Pond than at any other site. Abundance and species richness of lichens was higher at the burned sites than at the insect defoliated sites. Height of *Kalmia* (ground level to shoot tip) was also determined in mid-June by measuring fifteen randomly selected stems in each site (Table 2). *Kalmia* was significantly taller in the insect defoliated sites than in the burned sites, with the



Table 1. Stem density, basal diameter, and composition of tree species and black spruce seedling density at three burned, and three insect defoliated experimental sites. Values are mean and (standard deviation) of five circular quadrats (50 m<sup>2</sup>). Black spruce seedlings are  $\leq$  3 cm basal diameter. Dashes (-) represent species absence.

Parameter	Insect defoliated			Burned		
	Bluehill West 1	Bluehill West 2	Bluehill West 3	Terra Nova	Ochre Hill	Dunphy's Pond
Stem density (no./100m <sup>2</sup> )	8.8 (8.6)	18.4 (9.9)	19.2 (6.3)	2.8 (3.0)	33.6 (9.6)	35.6 (27.4)
Basal diameter (cm)	13.2 (10.6)	6.6 (4.9)	4.4 (4.1)	2.3 (2.1)	2.3 (0.3)	3.0 (1.6)
Species composition (% total basal area)						
<i>Picea mariana</i>	47.9 (48.3)	59.8 (50.8)	22.5 (43.6)	80.0 (44.7)	75.6 (31.4)	100.0 (0.0)
<i>Abies balsamea</i>	31.0 (42.5)	1.9 (4.3)	35.7 (48.6)	-	0.3 (0.7)	-
<i>Larix laricina</i>	-	-	-	-	24.1 (31.5)	-
<i>Acer rubrum</i>	4.1 (9.2)	19.3 (43.1)	-	-	-	-
<i>Populus tremuloides</i>	-	-	41.8 (52.3)	-	-	-
<i>Betula papyrifera</i>	16.9 (37.8)	19.0 (42.5)	-	-	-	-
Black spruce seedling density (no./100m <sup>2</sup> )	2.0 (3.5)	8.0 (5.5)	3.2 (3.6)	1.6 (0.9)	24.0 (8.5)	16.8 (27.6)

Table 2. Cover of understory and herbaceous vegetation and *Kalmia* height at three burned, and three insect defoliated experimental sites. Cover values are mean (standard deviation) of five quadrats (1 x 1 m) in each of the three plots per site (n = 15). Dashes (-) represent species absence. *Kalmia* height values are mean (standard deviation) of five stems in each of the three plots per site (n = 15).

Parameter	Insect defoliated sites			Burned sites		
	Bluehill West 1	Bluehill West 2	Bluehill West 3	Terra Nova	Ochre Hill	Dunphy's Pond
Cover (%)						
Vascular plants						
<i>Kalmia angustifolia</i>	57.7 (16.2)	61.0 (19.8)	80.9 (12.1)	54.7 (16.8)	52.7 (11.6)	37.7 (16.9)
<i>Cornus canadensis</i>	7.0 (4.8)	11.1 (6.1)	0.7 (0.6)	0.3 (0.5)	2.4 (1.6)	0.5 (0.5)
<i>Vaccinium angustifolium</i>	6.7 (7.4)	9.7 (12.6)	0.2 (0.6)	1.1 (1.5)	2.5 (1.8)	1.4 (1.2)
<i>Maianthemum canadense</i>	0.7 (0.9)	0.7 (1.8)	0.4 (0.5)	-	0.4 (0.7)	0.3 (0.5)
<i>Ledum groenlandicum</i>	-	-	0.2 (0.8)	0.8 (2.6)	1.0 (1.5)	0.8 (1.5)
<i>Rhododendron canadense</i>	-	-	-	1.7 (2.2)	1.0 (1.3)	6.9 (4.5)
<i>Chamaedaphne calyculata</i>	-	-	-	0.3 (1.3)	0.2 (0.6)	-
<i>Amelanchier bartramiana</i>	1.2 (2.8)	0.1 (0.5)	0.3 (0.9)	0.5 (2.1)	0.6 (1.1)	-
<i>Arctostaphylos uva-ursi</i>	-	-	-	-	0.2 (0.4)	-
<i>Prunus pensylvanica</i>	-	0.4 (1.1)	-	-	0.1 (0.3)	-
<i>Rubus idaeus</i>	0.1 (0.4)	0.4 (0.6)	-	-	-	-
<i>Clintonia borealis</i>	0.7 (0.6)	0.6 (1.1)	-	-	-	-
<i>Linnea borealis</i>	0.7 (0.9)	2.6 (2.5)	-	-	-	-
<i>Fragaria virginiana</i>	0.2 (0.4)	0.1 (0.4)	-	-	-	-
<i>Gaultheria hispidula</i>	0.3 (0.7)	1.1 (1.5)	-	-	-	-
<i>Aralia nudicaulis</i>	0.1 (0.3)	-	-	-	-	-
<i>Coptis trifolia</i>	0.1 (0.3)	0.1 (0.3)	-	-	-	-
<i>Viburnum cassinoides</i>	-	-	-	-	-	0.3 (0.8)
<i>Pteridium aquilinum</i>	-	-	0.1 (0.4)	-	-	-
Mosses						
<i>Dicranum polysetum</i>	0.9 (0.6)	7.5 (8.5)	3.1 (4.7)	5.1 (5.5)	5.2 (6.5)	1.1 (0.7)
<i>Pleurozium schreberi</i>	27.1 (23.5)	27.3 (12.2)	76.3 (20.2)	2.1 (2.5)	12.1 (20.4)	11.1 (21.7)
<i>Hylocomium splendens</i>	16.5 (15.9)	21.9 (11.2)	-	-	-	0.1 (0.3)
<i>Ptilium crista-castrensis</i>	10.6 (13.2)	1.3 (3.0)	0.3 (1.3)	-	-	0.1 (0.3)
<i>Campylium polygamum</i>	2.7 (10.3)	-	-	-	-	-
Lichens						
<i>Cladina rangiferina</i>	-	-	-	5.6 (7.2)	39.0 (14.9)	21.1 (28.0)
<i>Cladina alpestris</i>	-	0.1 (0.3)	-	2.2 (3.1)	25.4 (11.0)	6.0 (10.4)
<i>Cladina mitis</i>	-	-	-	1.3 (2.0)	11.3 (7.9)	6.4 (9.7)
<i>Cladonia sp.</i>	0.4 (1.3)	-	-	0.8 (1.3)	0.2 (0.8)	-
<i>Kalmia</i> height (cm)	65.1 (8.6)	52.5 (7.9)	65.7 (9.0)	36.0 (8.2)	41.9 (6.2)	52.6 (17.7)

exception of Dunphy's Pond ( $F = 20.3$ ,  $p < 0.001$ ). At Dunphy's Pond, mean *Kalmia* height was similar to the insect defoliated sites, and much more variable than any other site. This was likely because two out of three of the plots at Dunphy's Pond were located near the forest edge ( $< 3$  m), whereas all the plots in the other burned sites were located further from the forest edge ( $> 10$  m). Despite these minor differences in plant structure and composition, all of the burned and insect defoliated sites were dominated by *Kalmia* for at least 20 years following disturbance. This is likely the most important factor controlling below ground conditions for black spruce growth (Damman 1971; Inderjit and Mallik 1996b).

#### *Experimental design*

All study sites were located within 3 km of an access road, and distances between the sites ranged from 0.8 to 20 km. Before disturbance, all the study sites were dominated by black spruce, according to forest resource inventory images. There were three randomly selected sites (whole-blocks) nested in each of the two disturbance types, insect defoliated and burned. Within each site, three replicate plots (sub-blocks) (4.5 x 8 m) were randomly located (Fig. 1). Wire-mesh fencing was erected around each plot to protect the seedlings from small mammal herbivory. Each plot was divided in half, and the forest floor in one half (sub-plot) was mixed with the roto-tiller. Each sub-plot was further divided into four 1 x 1 m quadrats. Three of these quadrats were planted with 12 seedlings from each of the control, inoculated, and nursery (commercially grown) seedlings with 0.25 m spacing between them. The fourth quadrat was not planted. The quadrats were separated from one another and from the surrounding vegetation by a 1 m buffer and seedlings in adjacent mixed and non-mixed sub-plots were separated by 2 m. For analyses, quadrats were treated as

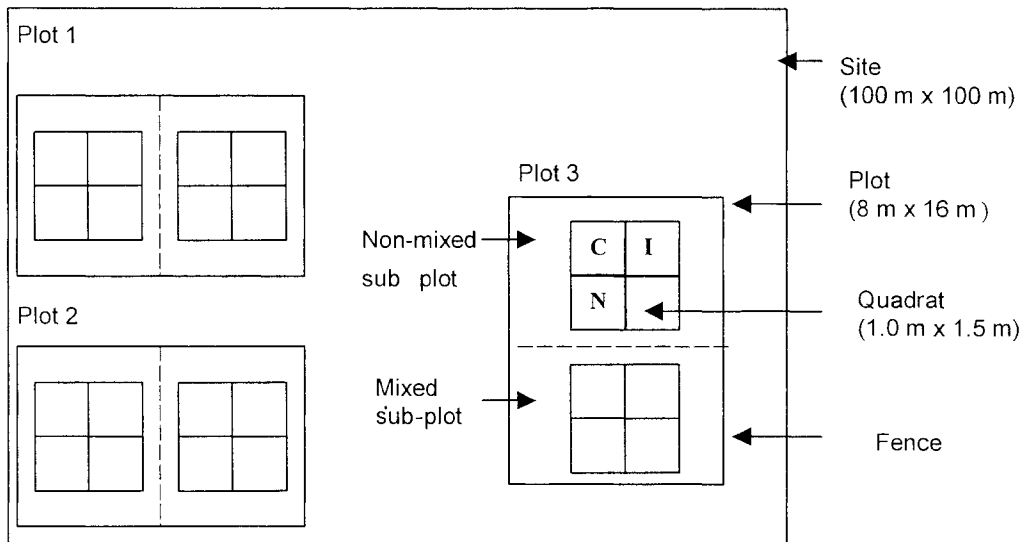


Figure 1. The layout of plots, sub-plots, and quadrats within each experimental site at TNNP, Newfoundland. Quadrats were planted with control (C), inoculated (I), and nursery (N) seedlings.

experimental units (n = 108) and individual seedlings within them were treated as sub-samples.

### *Forest floor mixing*

Mixing was performed during June 27-30<sup>th</sup>, 2002 with a rotary-style mixing machine (Craftsman Roto-tiller, 5.5 horsepower) specially modified to increase its effectiveness in stony soils with tough *Kalmia* rhizomes. Above ground vegetation was first cut with a power brush saw, then the forest floor (L,F,H horizons) and below ground plant parts were mixed to the depth of the mineral soil. Before mixing, the depth of the forest floor was measured and it was significantly greater at the Dunphy's Pond site and Ochre Hill site than at Bluehill site 1 or Bluehill site 2 ( $F = 10.8$ ,  $p < 0.001$ )(Table 3). This was likely a function of time-since disturbance, because the burns at the Ochre Hill and Dunphy's Pond sites are older than the insect defoliation sites or the Terra Nova site burn.

Cover (%) of vascular plants was determined in three randomly located (1 x 1 m) quadrats in each mixed sub-plot at the end of the second growing season (early September 2003). Forest floor mixing successfully reduced cover of competing vegetation in all experimental sites, and this reduction persisted until the end of the second growing season (Table 3). In all sites except Dunphy's Pond site, cover of competing vegetation at mixed plots ranged from 4.1 to 16.4 % at the end of the second growing season. At Dunphy's Pond, cover of competing vegetation (primarily *Kalmia*) was significantly greater than any other site except Bluehill site 3 ( $\chi^2 = 27.3$ ,  $p = 0.001$ ). This was likely because forest floors were deep at Dunphy's Pond, making it difficult to cut all *Kalmia* rhizomes.

Table 3. Forest floor depth before mixing, and cover of competing vegetation at the end of second growing season after forest floors were mixed at three burned, and three insect defoliated experimental sites. Forest floor depth values are mean (standard deviation) of three measurements in each of the three plots per site (n = 9). Cover values are mean and (standard deviation) of three quadrats (1x1 m) in each of the three plots per site (n = 9). Dashes (-) represent species absence. Within rows, values with different letters are significantly different at  $p < 0.05$ .

Parameter	<u>Insect defoliated</u>			<u>Burned</u>		
	Bluehill 1	Bluehill 2	Bluehill 3	Terra Nova	Ochre Hill	Dunphy's Pond
Forest floor depth (cm)	7.6 (2.5) b	8.1 (2.1) b	10.2 (2.6) ab	10.1 (2.4) ab	16.0 (4.5) a	16.4 (3.5) a
Cover (%)						
Total vegetation	12.4 (13.8) b	7.6 (4.3) b	16.4 (15.3) ab	3.6 (3.2) b	4.1 (2.0) b	34.9 (9.3) a
<i>Kalmia angustifolia</i>	9.9 (14.2)	3.7 (3.9)	12.8 (11.3)	3.0 (3.0)	1.9 (1.4)	22.2 (6.2)
<i>Cornus canadensis</i>	1.4 (0.9)	1.4 (2.6)	-	-	0.3 (0.5)	0.3 (1.0)
<i>Vaccinium angustifolium</i>	-	0.8 (0.7)	-	0.6 (0.5)	1.0 (0.5)	2.9 (1.8)
<i>Prunus pennsylvanica</i>	0.2 (0.4)	0.7 (0.7)	0.7 (1.7)	-	-	-
<i>Rubus idaeus</i>	0.1 (0.3)	0.7 (1.4)	-	-	-	-
<i>Viburnum cassinoides</i>	0.4 (1.3)	-	-	-	-	0.3 (0.7)
<i>Rhodora canadense</i>	-	-	-	0.4 (0.5)	0.9 (0.6)	8.3 (5.7)
<i>Pteridium aquilinum</i>	-	-	3.0 (5.0)	0.2 (0.4)	-	0.1 (0.3)
<i>Ledum groenlandicum</i>	-	-	-	-	-	0.7 (1.0)
<i>Betula papyrifera</i>	-	0.3 (0.7)	-	-	-	-

### *Seedling culture and treatments*

I grew black spruce seedlings for two of the three seedling groups (inoculated and control) in the greenhouse at Lakehead University (Thunder Bay, ON). Black spruce seeds were obtained from Woodale Tree Nursery (Grand Falls, NF), sterilized with 2% NaClO<sub>3</sub> solution for 2 min, and then thoroughly rinsed with distilled water. The seeds were placed on filter paper in Petri dishes for two days in a germination chamber at approximately 20 °C. Germinated seeds were sown in growing medium in clean Styroblock multi-pot containers (Beaver Plastics Ltd., Edmonton, AB). Each container had 112 x 128 ml cells. The growing medium was a 1:1 mixture of peat and vermiculite, autoclaved to reduce contamination. Containers with seeds were placed in the greenhouse and irrigated daily with a fine mist of water for two weeks. After emergence, seedlings were irrigated on alternate days. The seedlings were thinned to one per cell 6 weeks after emergence. Beginning at this time, the seedlings received 20:8:20 NPK (nitrogen: phosphorous: potassium) fertilizer (Plant Products, Brampton, ON) in the irrigation water (alternate days) at an N concentration of 100 ppm.

These seedlings were split into two groups five weeks later. One group was inoculated with *P. involutus* obtained from the Canadian Collection of Fungal Culture, Ottawa. Two methods were used to enhance the likelihood of success. First, *P. involutus* was cultured in Petri dishes in the laboratory on sterile Modified Melin-Norkrans (MMN) growth medium (Danielson 1982). Surface-sterilized *Pinus banksiana* seeds (4-5) were added to enhance the growth rate of *P. involutus* (Zeng *et al.* 2003). These cultures were incubated at 25 °C for two weeks. Using sterile technique, *P. involutus* mycelia were carefully separated from the growth medium, and then mixed in a blender (Waring Corp., Hartford,

CT) with sterile water to produce a uniform inoculum. The MMN growth medium was added to vermiculite in glass jars, which were autoclaved. The vermiculite was then inoculated with *P. involutus* and incubated at 25 °C for eight weeks. The growth medium was removed from the vermiculite inoculum by wrapping it in four-ply cheesecloth (Veratec, International Paper, Stamford, CT), and rinsing with water for five minutes. The vermiculite and water inocula were mixed into an autoclaved growth medium of mixed peat and vermiculite (1:1). Approximately 500 randomly selected seedlings were transplanted into this inoculated growth medium. The second group of approximately 500 seedlings, the control, was transplanted into sterile growth medium. Beginning at this time (five weeks), both the inoculated and control seedlings received low levels of fertilizer throughout the remaining greenhouse growth period. They received one application of 20:20:20 NPK fertilizer water at an N concentration of 100 ppm after transplantation, and twice-monthly applications thereafter in the irrigation water for 13 weeks. Greenhouse environmental conditions during the seedling emergence and growth phases (26 weeks) were 18 hours daylight, 60% relative humidity, 24-28 °C daytime temperature, 18-22 °C nighttime temperature, and ambient carbon dioxide concentrations. The pH and electrical conductivity of the growth medium were monitored to ensure that the pH remained at approximately 5.5, and the salinity remained in the 1200 - 2500  $\mu\text{S}/\text{cm}$  range (Landis *et al.* 1990). All seedlings were grown in one area of the greenhouse and the arrangement of seedling containers was changed regularly, to randomize local environmental variability in the greenhouse.

After 26 weeks, a six-week cold hardiness treatment was applied to the inoculated and control seedlings to increase tolerance of environmental stress during and after transportation to the field (D'Aoust and Cameron 1981). Day



length was shortened to eight hours for 14 days to induce bud dormancy. Temperatures were maintained at 15-20 °C (daytime) for three weeks to allow for terminal bud growth and then lowered gradually through the hardening phase. The seedlings were moved outdoors and exposed to low and substantially variable conditions during the daytime (10-15 °C daytime, and 5-10 °C nighttime). During this cold hardiness phase, the inoculated and control seedlings received twice-monthly applications of 20:20:20 fertilizer (Plant Products) in the irrigation water at an N concentration of 50 ppm.

The third group of seedlings (nursery) was grown commercially at Woodale Provincial Tree Nursery in Grand Falls, Newfoundland. Black spruce seeds were from the same source as the inoculated and control groups. The seeds were germinated at approximately 25 °C for two weeks. In early June 2001, the germinated seeds were sown in a growth medium consisting of a 1:1 mixture of peat to vermiculite in plastic containers with 48 cells for seedlings, each approximately 75 ml in volume. Containers with seeds were placed in the greenhouse and irrigated daily with a fine mist of water for two weeks. After emergence the seedlings were irrigated approximately on alternate days. The seedlings were thinned to one per cell six weeks after emergence. Environmental conditions in the greenhouse during the emergence and growth phases (14 weeks) were ambient day-length (approximately 16 hours), relative humidity, carbon dioxide, and air temperature (20-30 °C daytime). The nursery seedlings received high levels of fertilization throughout the emergence and growth phases. They received NPK fertilizer in the irrigation water (alternate days) at an N concentration varying from 35-150 ppm. After 14 weeks, a cold hardening treatment was applied to the nursery seedlings. Using ambient conditions, the seedlings were moved outside and exposed to cold temperatures

(ranging 0-20 °C daytime), low fertilization levels (set to maintain 2.5% foliar N), and reduced irrigation (twice-weekly) for eight weeks. During this period, they were exposed to 275 hours of cold air temperatures (< 5 °C) and 38 hours of freezing soil temperatures. The nursery seedlings were then stored outdoors on the ground for summer outplanting.

To describe the morphology of each seedling group before outplanting, I measured shoot height, root-collar diameter, oven-dry mass (root, shoot, shoot/root ratio), and EM formation of eight randomly selected seedlings.

### *Seedling response*

I used changes in shoot height, root-collar diameter, oven-dry mass (root, shoot, and shoot/root ratio), foliar nutrient concentration, and EM formation as measures of seedling response. I measured initial shoot height and root-collar diameter (immediately after outplanting, July 1-10, 2002), and at the end of the first and second growing seasons (early September 2002, 2003). I determined relative increase in height and root-collar diameter over one and two growing seasons using equations from Evans (1972) (relative height increase (cm/cm) =  $\ln \text{height}_{\text{initial}} - \ln \text{height}_{\text{final}}$ ). To determine oven-dry mass, three randomly selected seedlings were harvested from each quadrat at the end of the first and second growing seasons. To collect the entire root system, seedlings were carefully excavated from the soil. Shoots (stem and needles) and roots were placed in plastic bags and kept cool (4 °C) during transport from Newfoundland to Thunder Bay. Shoots (stem and needles) were separated from root systems, then air-dried and stored in paper bags at room temperature (22 °C). Root systems were stored in a refrigerator (4 °C), gently washed with tap water to remove soil, used to determine EM formation, air-dried (22 °C), and stored on aluminum trays. Roots and shoots were oven-dried at 70 °C for three hours

(Allen 1989) and weighed on an electronic balance. I determined relative increase in shoot and root dry mass that occurred during the second growing season, using the equation above. For change in shoot/root ratio, I calculated the difference between final and initial shoot/root ratio.

To assess foliar nutrient concentration, I harvested the stems of three randomly selected seedlings from each quadrat after the second growing season. They were placed in plastic bags and kept cool (4 °C) during transport, then air-dried and stored in paper bags at room temperature (22 °C). Foliage from the three sub-samples from each quadrat were ground in a mill and mixed together to form one composite sample. Total nitrogen (N) was measured as absorbance (630 nm) using a spectrophotometer (Skalar Auto Analyzer, Skalar Inc., Norcross, GA) after digestion of 0.2 g samples by the Kjeldahl method (Kalra and Maynard 1991). Total phosphorus (P) and potassium (K) were measured by inductively coupled argon plasma spectrometry (Varian Vista Pro ICAP Radial, Varian Inc., Mississauga, ON) after acid digestion (HNO<sub>3</sub>, HCL, H<sub>2</sub>O<sub>2</sub>) of 0.5 g samples (Kalra and Maynard 1991).

For EM formation, I used the root systems of the same seedlings used for dry mass measurements, which were collected at the end of the first and second growing seasons. After soil had been removed by washing, root systems were cut into 2 cm segments to allow separation of roots without damaging EM. Counts of EM and non-EM short roots were made on ten randomly selected root segments from each root system at 10-45x magnification using a dissecting stereomicroscope. From these counts, I determined percentage of total short roots that were EM.

I classified EM short roots into morpho-types and attempted to isolate the EM fungi to distinguish *P. involutus* from volunteer EM species. Classification

was based on EM color, shape, and texture. Using sterile technique, samples of the morpho-types were cut into 0.5 cm segments. Root segments were surface sterilized in 30% hydrogen peroxide for 1 minute, then rinsed for 30 seconds in sterilized distilled water three times. Root segments were placed in Petri dishes on MMN agar (Marx 1969) to which the fungicide benomyl (2  $\mu\text{g}/\text{ml}$ ) and the antibiotic streptomycin sulphate (30  $\mu\text{g}/\text{ml}$ ) were added. To enhance the growth of *P. involutus*, I added three surface sterilized (30% hydrogen peroxide for 30 seconds and rinsed three times) radish seeds (*Raphanus sativus* L.) (McKenzie Seed Co., Brandon, MB) to each Petri-dish (Zeng *et al.* 2003). The dishes were incubated in the dark at 20 °C for three weeks. No EM fungi grew on the dishes, probably because the samples were in storage too long prior to this analysis, consequently I was unable to identify the morpho-types.

#### *Data analysis*

Differences in seedling characteristics at outplanting were tested by analysis of variance (ANOVA) followed by Scheffe's multiple contrasts, or Kruskal-Wallis tests for non-parametric data (unequal variances) followed by Dunnett's T3 multiple comparisons. Relative increase (seedling height, root-collar diameter, and dry mass), EM formation, and foliar nutrient data was analyzed with ANOVA for nested designs followed by Scheffe's multiple contrasts. Relationships between foliar nitrogen and relative increase in shoot dry mass of seedlings were tested using linear regression. Data distributions were examined for normality using the Shapiro-Wilk statistic and variances were tested for homogeneity using Levene's statistic. When variables were not distributed normally or variances were not homogenous, a transformation (square root,  $\text{Log}_{10}$ , reciprocal, or 1/square root) was applied prior to analysis.

Data analyses were performed using Datadesk (Version 6.0, Data Description Inc., Ithica, NY) and SPSS software (Version 9.0, SPSS Inc., Chicago, IL).

## Results

### *Pre-outplanting seedlings*

Before outplanting, the commercial nursery-grown seedlings were 44% larger in shoot height than the control seedlings ( $S = 7.7$ ,  $p < 0.001$ ), and the inoculated seedlings were 33% less in shoot height than the control seedlings ( $S = 5.6$ ,  $p < 0.001$ ) (Table 4). Root-collar diameter of the nursery seedlings was not significantly different from the control seedlings, but the inoculated seedlings were 22% less in root-collar diameter than the control seedlings ( $S = 3.8$ ,  $p < 0.001$ ). The nursery seedlings had about 250% more shoot and root dry mass than the control seedlings ( $S = 10.9$ ,  $p < 0.001$  and  $S = 6.9$ ,  $p < 0.001$  respectively), but the inoculated seedlings were not significantly different from the control seedlings. Shoot/root dry mass ratio of the nursery seedlings was not significantly different from the control seedlings, but the inoculated seedlings were 43% less than that of the control seedlings ( $S = 4.0$ ,  $p = 0.01$ ). The nursery seedlings had significantly higher EM formation (13 short roots/10 cm) than the control seedlings (2 short roots/10 cm) ( $T = 1.9$ ,  $p < 0.001$ ), but the inoculated seedlings had the highest number of mycorrhizal short roots (47 short roots/10 cm) ( $\chi^2 = 20.49$ ,  $p < 0.001$ ) ( $T = 8.1$ ,  $P < 0.001$ ).

### *Shoot height*

Overall, the control and inoculated seedlings had very little relative increase in height after the first growing season (Fig. 2a). Nursery seedlings had significantly more relative height increase than the control seedlings, which did not differ significantly from the inoculated seedlings ( $F = 30.6$ ,  $p < 0.01$ ). In general, relative increase in shoot height of black spruce seedlings was significantly greater in plots with non-mixed forest floors than that of mixed plots for all treatments (control, inoculated, and nursery seedlings) ( $F = 17.9$ ,  $p = 0.01$ ).

Table 4. Shoot height, root-collar diameter, EM formation, and oven-dry mass (shoot, root, shoot/root) of three groups of black spruce seedlings before outplanting. Values are mean and (standard deviation) of eight seedlings. Within columns, values with different letters are significantly different at  $p < 0.05$ .

Seedling group	Shoot height (cm)	Root-collar diameter (mm)	EM formation (short roots/10 cm)	Root dry mass (g)	Shoot dry mass (g)	Shoot/root ratio
Control	15.1 (2.0) b	2.09 (0.13) a	2 (1.2) c	0.114 (0.043) b	0.432 (0.094) b	3.20 (0.907) a
Inoculated	10.1 (0.8) c	1.62 (0.26) b	47 (18.7) a	0.150 (0.045) b	0.336 (0.057) b	1.83 (0.471) b
Nursery	21.8 (2.2) a	2.15 (0.31) a	13 (4.8) b	0.399 (0.131) a	1.512 (0.375) a	3.05 (0.523) a

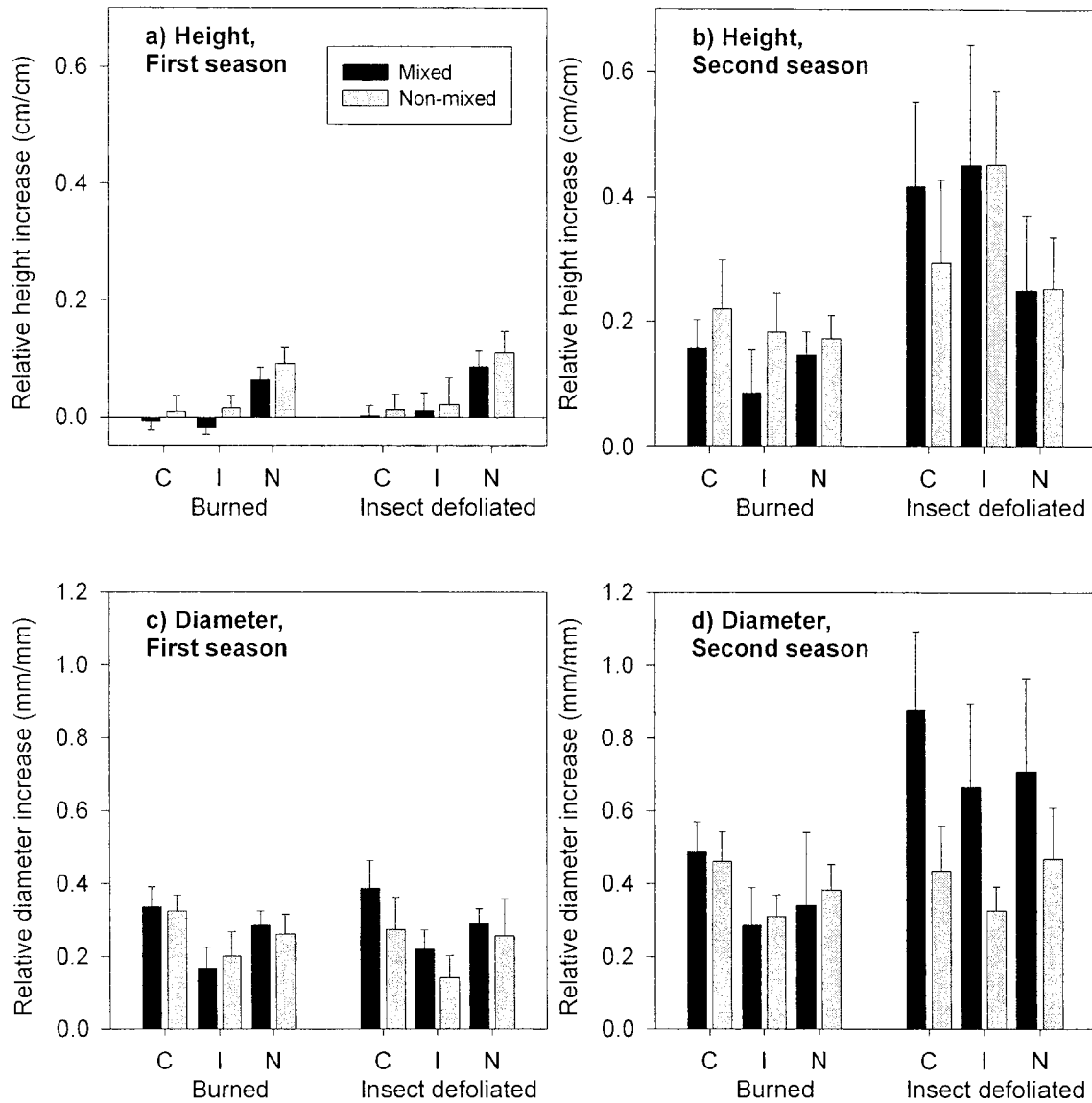


Figure 2. Relative increase in shoot height and root-collar diameter of three groups of black spruce seedlings after the first and second growing seasons. Seedling groups are control (C), inoculated (I), and nursery (N). Values are means of nine seedlings and error bars represent one standard deviation. Negative values of increase over the first season are due to measurement error when there had been little growth.



For relative increase in shoot height after the second growing season, the two-way interaction between disturbance type and seedling type was significant ( $F = 7.5$ ,  $p = 0.01$ ). There were no significant differences in relative height increase between the control, inoculated, or nursery seedlings in the burned sites. In the insect defoliated sites, the control and inoculated seedlings were not significantly different in relative height growth, but the control seedlings were significantly greater than the nursery seedlings (Fig. 2b). Overall, relative increase in shoot height of control, nursery, and inoculated seedlings was significantly greater in the insect defoliated sites than in the burned sites ( $F = 17.1$ ,  $p = 0.01$ ).

#### *Root-collar diameter*

For relative increase in root-collar diameter after the first growing season, the three-way interaction between disturbance type, seedling group, and forest floor mixing was significant ( $F = 8.8$ ,  $p < 0.01$ ). Control seedlings had greater relative increase in root-collar diameter than the inoculated seedlings in all plots (combinations of disturbance and forest floor mixing) (Fig. 2c). Also, control seedlings had higher relative increase in diameter than the nursery seedlings in all plots except in non-mixed plots of insect defoliated sites. Relative diameter increase was highest overall for control seedlings in mixed plots of insect defoliated sites (0.39 mm/mm). For inoculated seedlings, the largest relative increase in diameter was in mixed plots in insect defoliated sites. The nursery seedlings showed no differences in relative diameter increase related to mixing or disturbance. In burned sites, forest floor mixing had no significant effect. However, in insect defoliated sites, relative diameter growth was higher in mixed plots than in non-mixed plots for the control (41%) and inoculated (56%) seedlings (Fig. 2c).

For relative increase in root-collar diameter after the second growing season, the two-way interactions between disturbance type and forest floor mixing ( $F = 12.9$ ,  $p = 0.02$ ), and forest floor mixing and seedling type ( $F = 4.6$ ,  $p = 0.047$ ) were significant. In mixed plots, the control seedlings had significantly greater relative increase in diameter than the inoculated and nursery seedlings (Fig. 2d). In non-mixed plots, the control seedlings had significantly higher relative increase in diameter than the inoculated seedlings, but the control seedlings did not differ from the nursery seedlings. For all the seedling types, relative diameter increase was largest in mixed plots in insect defoliated sites. In burned sites, mixing had no significant effect on relative diameter increase for control, nursery, or inoculated seedlings compared to non-mixed plots. However, in insect defoliated sites, all seedling types had greater relative increase in diameter in mixed plots than in non-mixed plots.

#### *Shoot dry mass*

For relative increase in shoot dry mass after the second growing season, the three-way interaction between disturbance type, forest floor mixing, and seedling type was significant ( $F = 11.81$ ,  $p < 0.01$ ). The control seedlings had significantly greater relative increase in shoot dry mass than the inoculated seedlings in burned sites, but there was no significant difference between these seedling types in the insect defoliated sites (Fig. 3a). The control seedlings had significantly higher relative increase in shoot dry mass than the nursery seedlings in all plots, except in non-mixed plots in the insect defoliated sites. The control and inoculated seedlings in mixed plots in insect defoliated sites had the largest relative increase in shoot mass overall (1.32 and 1.19 g/g). In the insect defoliated sites, relative increase in shoot dry mass was higher in mixed plots

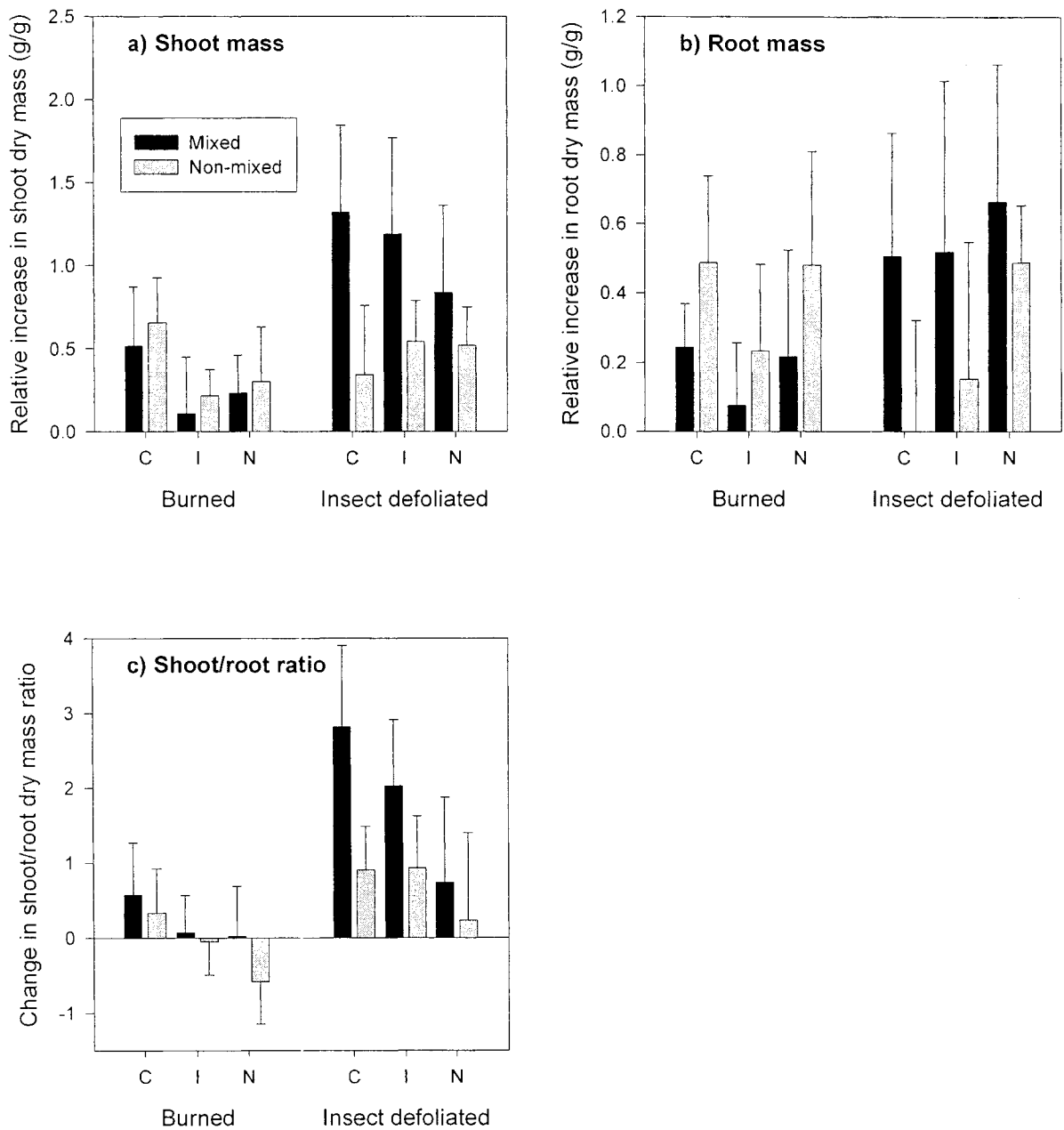


Figure 3. Relative increase in shoot dry mass and root dry mass, and change in shoot/root dry mass ratio of three groups of black spruce seedlings after the second growing season. Seedling groups are control (C), inoculated (I), and nursery (N). Values are means of nine seedlings and error bars represent one standard deviation.

than in non-mixed plots for all of the seedling types. However, in the burned sites, forest floor mixing had no significant effect on relative increase in shoot dry mass for any of the seedling types. Overall, relative increase in shoot dry mass was higher in the insect defoliated sites than in the burned sites.

#### *Root dry mass*

For relative increase in root dry mass after the second growing season, the two-way interactions between disturbance type and seedling type ( $F = 8.7$ ,  $p < 0.01$ ), and disturbance type and forest floor mixing ( $F = 10.2$ ,  $p = 0.03$ ) were significant. The inoculated seedlings had significantly lower relative increase in root dry mass than the control seedlings in burned sites, but there was no significant difference between these seedling types in the insect defoliated sites (Fig. 3b). The nursery seedlings were not significantly different from the control seedlings in relative increase in root dry mass in the burned sites, but the nursery seedlings were significantly greater than the control seedlings in the insect defoliated sites. In the insect defoliated sites, relative increase in root dry mass was higher in the mixed plots than in the non-mixed plots for all of the seedling types. However, in the burned sites, forest floor mixing did not result in significantly different relative increases in root dry mass for any of the seedling groups.

#### *Shoot/root dry mass ratio*

For change in shoot/root ratio, the three-way interaction between disturbance type, seedling type, and forest floor mixing was significant ( $F = 11.9$ ,  $p < 0.001$ ). The inoculated seedlings were not significantly different from the control seedlings in shoot/root ratio change, except in the mixed plots in the insect defoliated sites where the control seedlings had a significantly greater

increase than the inoculated seedlings (Fig. 3c). The nursery seedlings had a significantly lower increase in shoot/root ratio than the control seedlings in all plots (combinations of disturbance type and forest floor mixing). In insect defoliated sites, control and inoculated seedlings had a significantly greater increase in shoot/root ratio in mixed plots compared to that in non-mixed plots. In burned sites, only the nursery seedlings had a significantly greater increase in shoot/root ratio in mixed plots than in non-mixed plots. Overall, shoot/root ratio increase was greater in insect defoliated sites than in burned sites ( $F = 18.7$ ,  $p = 0.012$ ). Of all the plots, the largest increases in shoot/root ratio occurred in mixed plots in insect defoliated sites for all of the seedling types.

#### *Foliar nutrient concentration*

At the end of the second growing season, foliar N, P, and K concentrations were significantly greater for seedlings in insect defoliated sites than those in burned sites (49.8%, 28.5%, 31.9%, respectively) ( $F = 10.4$ ,  $p = 0.03$ ;  $F = 17.4$ ,  $p = 0.01$ ;  $F = 41.8$ ,  $p = 0.003$ ; respectively) (Fig. 4). N concentration was significantly greater (32.2%) for seedlings in plots with mixed forest floors than those in non-mixed plots ( $F = 77.9$ ,  $p = 0.001$ ) (Fig. 4a). P concentration for inoculated seedlings was significantly greater (19.3%) than for control seedlings ( $F = 16.7$ ,  $p = 0.001$ ), which did not differ significantly from the nursery seedlings (Fig. 4b). K concentration of inoculated seedlings was not significantly different from control seedlings, but control seedlings had significantly greater (17.3%) K concentration than nursery seedlings ( $F = 16.1$ ,  $p = 0.002$ ) (Fig. 4c). For K concentration, there was a significant interaction between forest floor mixing and seedling group ( $F = 5.7$ ,  $p = 0.029$ ). K concentration of nursery seedlings was greater in plots with mixed forest floors than those in non-mixed plots. However, for control and inoculated seedlings,

there was no significant difference in K concentration between mixed and non-mixed plots.

Regression analysis of foliar N concentration on relative increase in shoot dry mass showed significant positive relationships for the control, inoculated, and nursery-grown seedlings ( $p \leq 0.001$ ,  $p = 0.001$ ,  $p \leq 0.001$ , respectively) (Fig. 5a-c). However, coefficients of determination indicated that foliar N concentration explained only some of the variability in relative shoot dry mass growth for these three seedling groups ( $r^2 = 0.38$ ,  $r^2 = 0.27$ , and  $r^2 = 0.50$ , respectively).

#### *EM formation*

At the end of the first growing season, EM formation (% of total short roots) was significantly greater (47% and 16% on inoculated and nursery seedlings respectively), compared to the control seedlings ( $F = 143.6$ ,  $p < 0.001$ ) (Fig. 6a). At the end of the second growing season, EM formation was 27% greater on inoculated seedlings compared to the control seedlings, which had increased in EM formation to become equal to the nursery seedlings ( $F = 21.6$ ,  $p = 0.001$ ) (Fig. 6b). At the end of the second growing season, control and inoculated seedlings had significantly greater EM formation in mixed plots than in non-mixed plots (8% and 6% respectively), but nursery seedlings had significantly more EM formation in non-mixed plots than those in the mixed plots (8%) ( $F = 4.6$ ,  $p = 0.047$ ) (Fig. 6b).

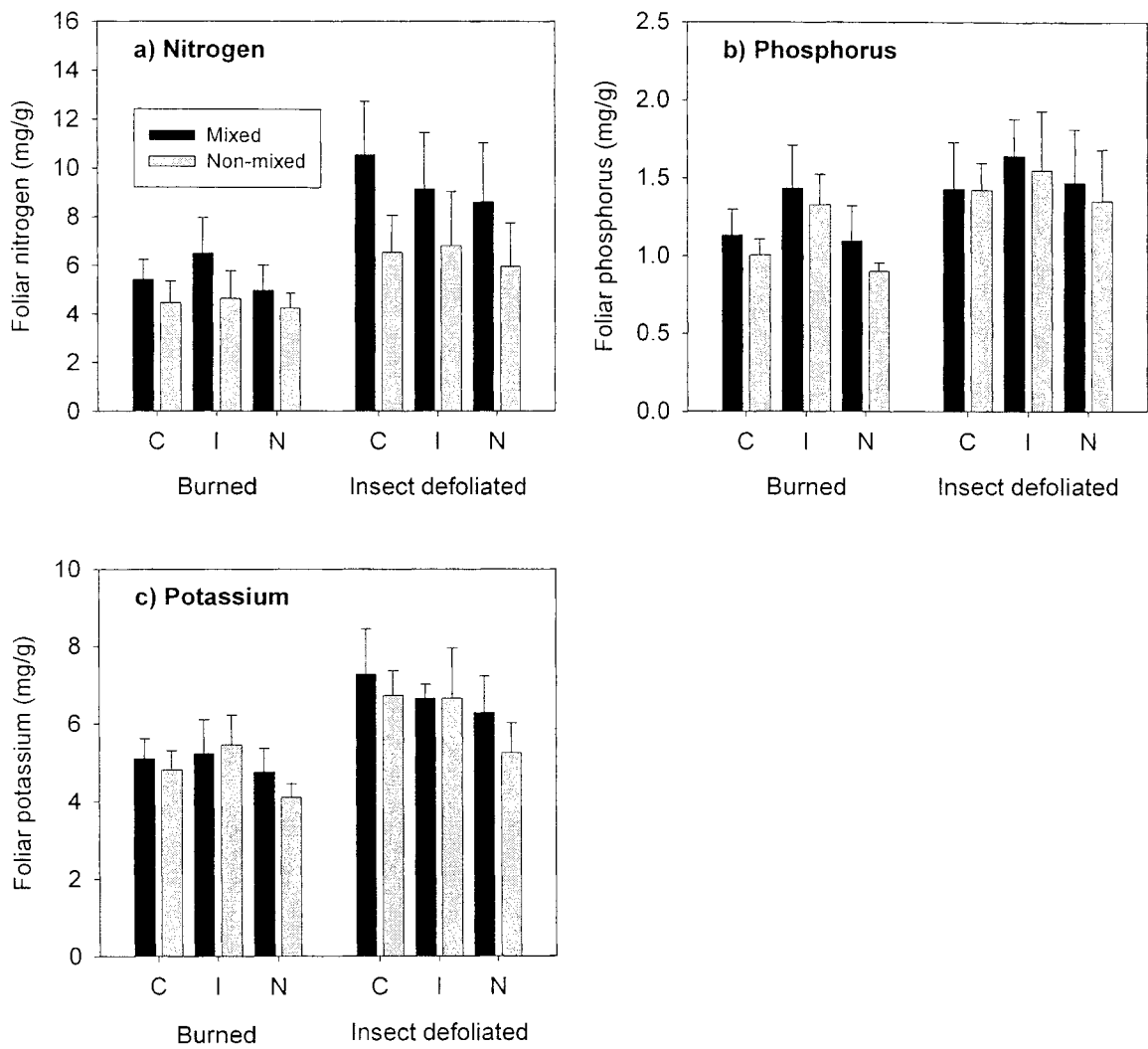


Figure 4. Concentrations of nitrogen, phosphorus, and potassium in foliage of three groups of black spruce seedlings at the end of the second growing season. Seedling groups are control (C), inoculated (I), and nursery (N). Values are means of nine seedlings and error bars represent one standard deviation.

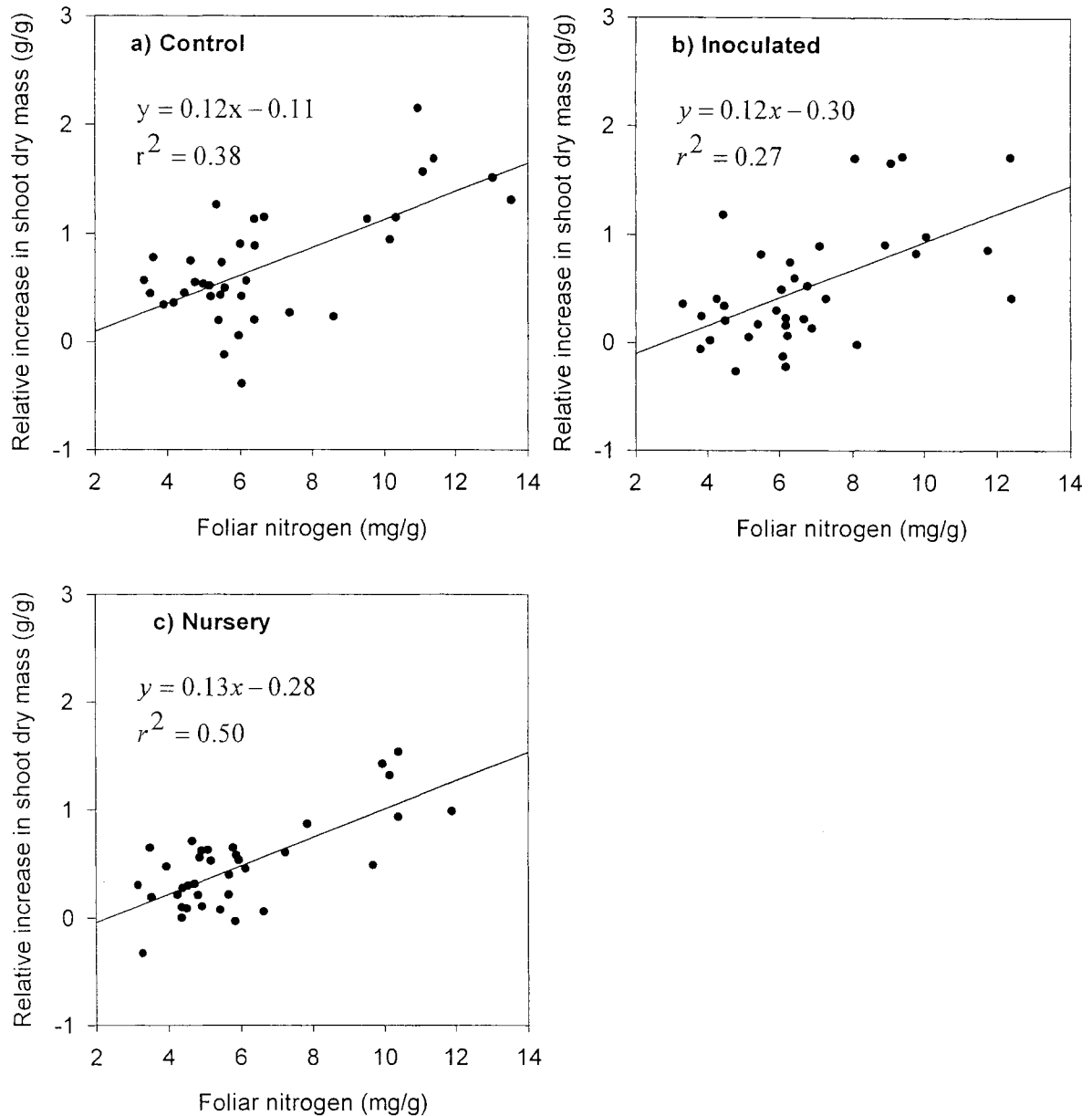


Figure 5. Relative increase in shoot dry mass after the second growing season for 3 groups of black spruce seedlings as a function of foliar nitrogen concentration. Seedling groups were control, inoculated, and nursery. Linear regression equations and line are from analyses of 36 seedlings. Negative values for relative growth are due to measurement error when there was very little seedling growth



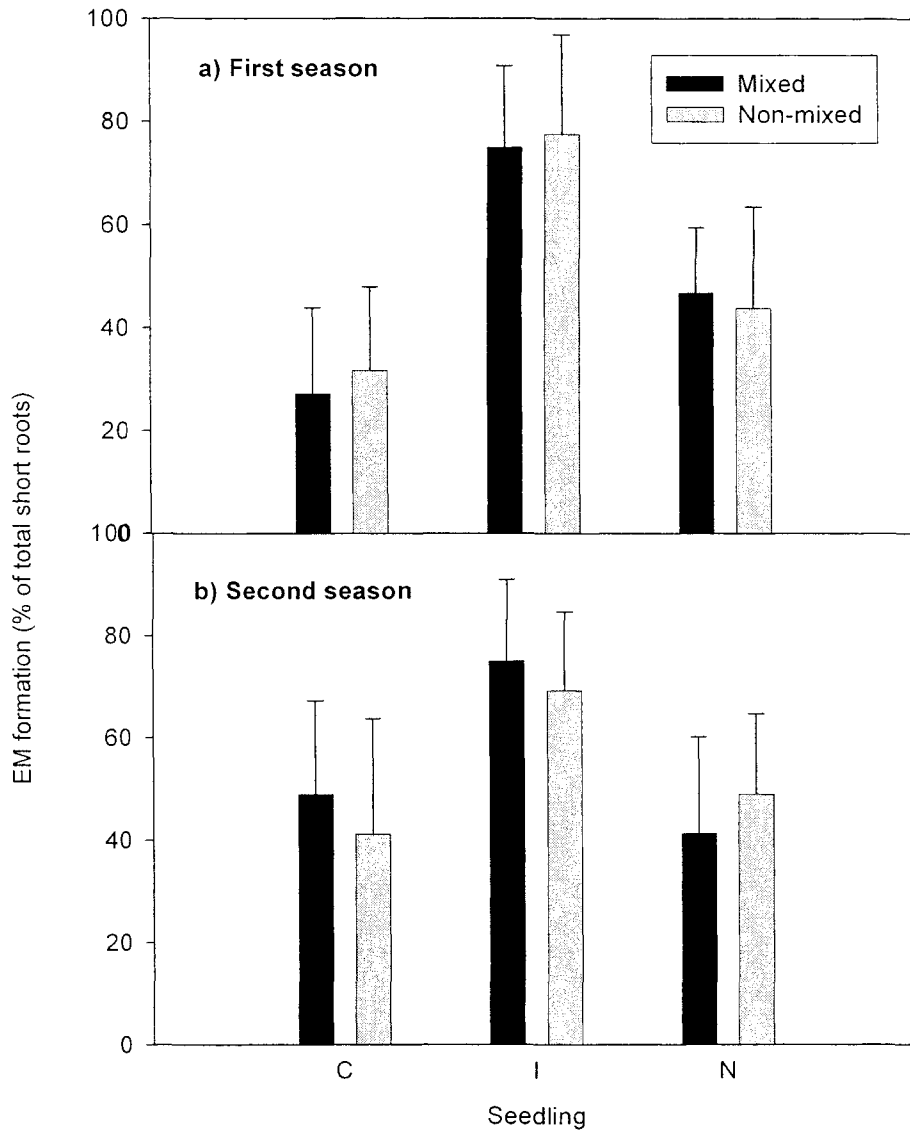


Figure 6. EM formation on three groups of black spruce seedlings at the end of the first and second growing season. Seedling groups are control (C), inoculated (I), and nursery (N). Values represent means of 36 seedlings and error bars represent one standard deviation.

## Discussion

### *Seedling inoculation*

Although the inoculated seedlings had greater EM formation than the control seedlings at the time of outplanting, they did not have greater increases than the control seedlings in shoot height, root-collar diameter, shoot dry mass, root dry mass, and shoot/root dry mass ratio. This was unexpected because, in a greenhouse experiment, black spruce seedlings inoculated with *P. involutus* had improved growth in *Kalmia* soils (Mallik *et al.* 1998). The contrasting result in this study may have occurred because I used a different isolate of *P. involutus* than that used by Mallik *et al.* (1998). I was unable to obtain the isolate (NF4) used in this previous research, which was cultured from central Newfoundland soil. With EM, variability in seedling response occurs at the level of the EM isolate rather than the species (Perry *et al.* 1987).

In a review of inoculation experiments, Perry *et al.* (1987) concluded that, for various reasons, inoculation does not consistently improve seedling growth. In my study, one possible explanation for the inoculated seedlings having less growth is that they were initially smaller than the control seedlings. However, this is unlikely because seedling height at outplanting is not a conclusive indicator of growth. Paterson (1997) found that larger seedlings had greater growth than smaller ones only when they were so much larger that they were comparable in height to competing vegetation. Stenstrom and Ek (1990) found that inoculated seedlings that are initially considerably smaller than control seedlings can grow larger rapidly.

Another possible explanation is that the inoculated seedlings failed to outgrow the control seedlings because the control seedlings had a higher absolute number of EM than the inoculated seedlings. Measuring the quantity of

EM short roots as a proportion of total short roots may not reflect the absolute number of EM if root mass varies between treatment groups. By the end of the second growing season, root mass of the control seedlings had increased substantially (573%), while root mass of the inoculated seedlings had increased only slightly (93%). Therefore, the absolute number of EM on the control seedlings may have become approximately equal to that on inoculated seedlings by the end of the experiment. However, if the control seedlings had a higher absolute number of EM than the inoculated seedlings it was likely a result of growth, not the cause. At the time of outplanting, the inoculated and control seedlings were similar in root mass, but the inoculated seedlings had significantly greater EM formation (%). Therefore, at outplanting, the inoculated seedlings likely had a much higher absolute number of EM than the control seedlings. Additionally, EM synthesis results in increased density of EM short roots. Therefore, the inoculated seedlings likely had a greater absolute number of EM than the control seedlings despite having lower root mass at the end of the second growing season.

The control seedlings had some EM formation and may have been colonized after outplanting with an indigenous EM that was equally or more beneficial than was the isolate of *P. involutus* on my seedlings. One challenge of field trials with inoculated seedlings is that it is impossible to establish proper control seedlings because volunteer EM often colonize them. Some inoculation trials are unsuccessful because the EM inoculum may not be suited to local soil type and environmental conditions (Trappe 1977). The EM that colonized the control seedlings may have been better suited to local conditions and more beneficial for growth than the particular strain of *P. involutus* with which the seedlings were inoculated.

Another possibility is that *P. involutus* EM may have been detrimental to growth of the inoculated seedlings by acquisition of energy produced by the seedlings. Energy and nutrient dynamics in plant-mycorrhizae relationships is not always symbiotic, and may be parasitic depending on environmental conditions (Tinker *et al.* 1994; Johnson *et al.* 1997; Son and Smith 1998; Klironomos 2003). The inoculated seedlings had higher foliar P and similar N and K compared to the control seedlings. This is an indication that reduced growth of inoculated seedlings is related to factors other than nutrient uptake, including perhaps energy dynamics.

Alternatively, it may take longer than two years for the inoculated seedlings to show improved growth. Browning and Whitney (1992) found that black spruce was slow to respond to inoculation compared to jack pine, responding only during the second growing season after outplanting. EM tend to benefit seedlings only during specific stages of seedling development (Perry *et al.* 1987). It is possible that seedling growth was limited because most energy went to establishment of EM and mycelial growth, which will benefit the seedlings in future years.

The inoculated seedlings had higher EM formation (% of total short roots) than the control or nursery seedlings throughout two growing seasons. This indicates that inoculating black spruce seedlings with *P. involutus* results in higher EM formation than occurs with uninoculated seedlings that are colonized by indigenous EM. This difference can persist for at least two growing seasons. In contrast, other studies have found that uninoculated seedlings are rapidly colonized by indigenous EM and become equal in EM formation to inoculated seedlings during the first growing season (Amaranthus *et al.* 1987; Berch and Roth 1993; Baum *et al.* 2002).

The difference in EM formation between inoculated and uninoculated seedlings may be due to inhibitory effects of *Kalmia* leachates. Mallik *et al.* (1998) and Yamasaki *et al.* (1998) found that black spruce seedlings in *Kalmia* dominated sites have low rates of EM formation. This is perhaps due to direct toxic effects of *Kalmia* leachates on EM fungi (Mallik *et al.* 1998). However, *P. involutus* may resist the toxic effects of *Kalmia* leachates, which may have helped maintain the high rates of EM formation in *Kalmia* soils (Mallik *et al.* 1998). Zeng and Mallik (submitted) found that *P. involutus* can degrade and detoxify *Kalmia* phenols under laboratory and greenhouse conditions. Alternatively, *P. involutus* may simply maintain higher rates of EM formation than the indigenous EM species that colonized the control seedlings. Browning and Whitney (1992), working with inoculated black spruce seedlings, found differences among EM species in initial rates of EM formation and change after outplanting.

#### *Nursery-grown seedlings*

When comparing the growth of control and commercial nursery-grown seedlings during the first growing season, I found that nursery seedlings had greater height growth than the control seedlings. This was likely because they had greater growth potential due to differences in growing conditions in the greenhouse before outplanting. Much of height growth in a given year, the pre-formed growth, is determined by moisture, light, temperature, and nutrient availability during the previous year when the terminal bud is formed (Grossnickle 2000). The remainder of height growth in a given year, the free-growth, is based on conditions in that year. For the nursery seedlings, greenhouse conditions and fertilization levels were optimal throughout the growth phase. They had well-formed terminal buds, and high potential for pre-formed growth. In the

greenhouse, the control seedlings were exposed to low nutrient levels. The terminal buds of the control seedlings were quite small for black spruce seedlings at the outplanting stage (S. Columbo, personal communication), indicating that they had little potential for pre-formed growth.

In contrast, the control seedlings had greater growth in root-collar diameter than the nursery seedlings during the first growing season. Root-collar diameter growth is closely related to environmental conditions during the current year (Grossnickle 2000). This suggests that the control seedlings tolerated planting stress better and adapted to the site more quickly than the nursery seedlings. However, it is also possible that free-growth during the first growing season was similar for the nursery and control seedlings, except that they were allocating energy to stem diameter and height differently.

The control and nursery seedlings had similar growth over two growing seasons, except in plots with mixed forest floors in insect defoliated sites where control seedlings outgrew nursery seedlings in height and root-collar diameter. There, height growth of control seedlings (mean 8.2 cm) exceeded the estimate of optimal growth for two-year old black spruce (7 cm) reported by Malik and Timmer (1996). As previously discussed, differences in initial size were not likely an important factor. An alternate explanation is that the low nutrient levels that the control seedlings were exposed to in the greenhouse are responsible. These conditions may have given the control seedlings greater capacity to tolerate nutrient stress, resulting in greater growth following outplanting. Mohammed *et al.* (1997) suggested that for black spruce, growth was more closely related to stress tolerance than morphology. Planting sites are normally stressful environments for seedlings, because of extremes of soil moisture and temperature, and low nutrient availability. Planting stress occurs after outplanting

because roots have not yet permeated the soil matrix, thus access to moisture and nutrients are reduced (Grossnickle 2000). To tolerate nutrient and moisture stress, seedlings develop capacity for enhanced root growth. Additional adaptations to moisture stress include physiological adjustments to chloroplasts, osmosis, and stomatal function (Grossnickle 2000). As supporting evidence for greater stress tolerance of control seedlings, I expected increased root dry mass compared to nursery seedlings. The difference was not significant, perhaps because it was small and there was a great deal of measurement error due to the difficulty of extracting the entire root systems. I did not determine any indicators of seedling stress (Mohammed *et al.* 1997), aside from measures of seedling morphology (stem height, root-collar diameter, shoot and root biomass, shoot/root ratio). It would be beneficial to experimentally compare the growth response of seedlings at several levels of nutrient and moisture stress that are planted in burned *Kalmia* dominated sites. Mohammed *et al.* (1997) recommend using a heat stress treatment, then determining chlorophyll fluorescence, net photosynthetic rate, or root growth potential as indicators of stress tolerance in black spruce.

#### *Disturbance type*

All of the seedling groups (control, nursery, and inoculated) had greater height increase in insect defoliated plots than in burned plots over two growing seasons. In addition, seedlings at insect defoliated plots had higher foliar N, P, and K concentrations than those in the plots of burned sites. One explanation for these trends is that burning decreased available nutrients, which were not directly affected by insect defoliation. The overall effects of fire on soil nutrients and the duration of these effects vary depending on fire intensity and severity (Maclean *et al.* 1983, Simard *et al.* 2001), making it difficult to determine the

effects of the fires at the sites in this study. However, given that these burns occurred 24 to 42 years before this experiment, it is likely that the effect of *Kalmia* dominance on nutrient availability is more important than the effects of the historical fires. Brais *et al.* (2000) estimated that the impact of severe fires on total-N content of the forest floor was balanced by inputs of organic matter and site capital. Many authors have shown that *Kalmia* dominance results in low nutrient availability due to low mineralization rates and high acidity (Damman 1971; Inderjit and Mallik 1996b).

A more plausible explanation for the enhanced growth and foliar nutrient content on the insect defoliated sites may be that there were indirect effects of insect defoliation and burning on canopy cover. Burned plots were in large forest openings, but insect defoliated plots had partial canopy cover because they were in smaller gaps containing remnant trees. Also, insect defoliated sites had greater understory cover of vascular plants and structural diversity than burned sites. This occurs because *Kalmia* biomass and vascular plant species richness are greater under partial canopy cover (Bloom and Mallik 2004).

In my study, partial canopy cover in insect defoliated sites may have indirectly enhanced the growth and foliar nutrients of black spruce seedlings due to two belowground effects. First, reductions in extremes of soil temperature due to partial canopy cover may have resulted in greater rates of decomposition and increased nutrient availability (Zhang and Zak 1995; Kurka and Starr 1997). Second, partial canopy cover may affect *Kalmia* litter quality, resulting in increased decomposition rate and nutrient availability. In research on black spruce-*Kalmia* forests, Bloom and Mallik (2004) found a positive correlation between canopy closure and soil respiration. They interpreted this to be an effect of *Kalmia* litter quality, which under partial canopy closure is lower in



phenols and carbon/nitrogen content ratio, resulting in higher rates of decomposition. In leaf litter from a variety of species in Pacific Northwest forests, concentration of phenols was most frequently the most important predictor of decomposition rate, and this relationship was an inverse correlation (Valachovic *et al.* 2004). Also, *Kalmia* litter was found to have lower phenolic content under partial shade than under full sunlight (Bloom and Mallik 2004).

Partial canopy cover in the insect defoliated sites may have also had important effects on above ground factors related to seedling growth. Regression analysis of seedling growth as a function of foliar N concentration suggested that much of the variability in seedling growth was not explained by nutrient availability. As mentioned earlier, partial canopy cover can improve seedling growth by reducing extremes of temperature and relative humidity in the immediate environment of seedlings (Spittlehouse and Stathers 1990). Partial canopy cover reduces light intensity, and Jobidon (1994) reported that maximum shoot growth for black spruce seedlings occurs at 60% full light. Photodamage to plant tissues can occur in full light, but spruce seedlings under partial canopy cover are not affected (Ronco 1975). I did not measure canopy cover and light levels, but doing so would help to further evaluate the role of canopy cover in seedling growth.

#### *Forest floor mixing*

Nursery seedlings had larger height increases in non-mixed plots compared to mixed plots in both burned and insect defoliated sites at the end of the first growing season. One explanation for this is that these seedlings, growing beneath *Kalmia* and other plants, were allocating energy to height growth to compete for light (Grossnickle 2000). However, this did not occur in the second growing season even though the seedlings were still shorter than the

competing vegetation. Also, seedlings in mixed plots did not allocate more energy to root-collar diameter growth than those in non-mixed plots. Alternatively, conditions during the first season may have been better for growth in the non-mixed plots perhaps due to the filter and nurse effects from the shrub cover (Grime 1998). The immediate effect of forest floor mixing may have been extremes of temperature and moisture during the period of planting shock, causing decreased growth in height. A third possibility is that mixing reduced soil compaction, which caused the seedlings to have low root-soil contact compared to the seedlings in the non-mixed forest floors.

In both insect defoliated and burned sites, foliar N concentration was higher for seedlings in the mixed plots than for those in the non-mixed plots over two growing seasons. This is an indication that mixing results in increased nutrient availability, perhaps due to increased soil temperature, pH, increased mineralization, and decreased competitive cover. However, the effects of forest floor mixing on seedling growth differed in burned compared to insect defoliated sites. In insect defoliated sites, the seedlings had better growth in mixed plots than in non-mixed plots. In burned sites on the other hand, mixing had no effect on seedling growth, except for control seedlings which had greater growth in non-mixed rather than in mixed plots. This is another indication that in addition to soil conditions and processes, other factors influence seedling growth, perhaps including partial canopy cover. Although much of the research on growth of black spruce seedlings in *Kalmia* dominated sites has been focused on soil conditions and processes, the effects of canopy cover may also be important.

#### *Management implications*

Inoculating black spruce seedlings with *P. involutus* (isolate 211804) would likely result in high EM formation (%), but would not improve the early

growth of seedlings. In *Kalmia* dominated sites that originate from insect defoliation, planting black spruce seedlings that have been raised in the greenhouse using low fertilization regimes and mixing the forest floor would likely result in enhanced conifer growth and eventual recovery of forest cover. In burned sites that are dominated by *Kalmia*, forest floor mixing would not likely improve black spruce seedling growth or result in forest cover. On burned sites, the use of nurse plants to provide shade could be attempted as a method of improving conifer growth.

Appendix I. F-ratios and p values from analysis of variance testing relative increase in shoot height and root-collar diameter at the end of the first and second growing seasons (results of multiple comparisons not shown).

Source	df	Shoot height		Shoot height		Root-collar		Root-collar	
		<u>First season</u>		<u>Second season</u>		<u>First season</u>		<u>Second season</u>	
		F	p	F	p	F	p	F	p
Disturbance	1	4.92	0.09	17.09	0.01	< 0.01	0.97	6.15	0.07
Site	4	4.72	0.02	2.54	0.09	7.38	< 0.01	4.96	0.01
Plot	12		No test		No test		No test		No test
Mixing	1	17.93	0.01	0.16	0.71	33.05	< 0.01	10.95	0.03
Site*Mixing	4	0.54	0.71	4.48	0.02	0.48	0.75	3.90	0.03
Disturbance*Mixing	1	1.55	0.28	3.12	0.15	31.85	< 0.01	12.89	0.02
Plot*Mixing	12		No test		No test		No test		No test
Seedling	2	30.61	< 0.01	4.74	0.04	40.23	0.01	17.90	< 0.01
Site*Seedling	8	6.51	< 0.01	5.35	< 0.01	2.55	0.04	1.31	0.29
Disturbance*Seedling	2	0.16	0.85	7.48	0.01	0.01	0.99	0.31	0.74
Plot*Seedling	24		No test		No test		No test		No test
Mixing*Seedling	2	2.25	0.17	3.64	0.08	4.85	0.04	4.57	0.05
Site*Mixing*Seedling	8	0.24	0.98	0.67	0.72	0.28	0.97	1.26	0.31
Disturbance*Mixing*Seedling	2	1.81	0.23	3.76	0.07	8.76	0.01	1.12	0.37
Plot*Mixing*Seedling	24		No test		No test		No test		No test
Error	0								
Total	107								

Appendix II. Actual shoot height and root-collar diameter for three groups of black spruce seedlings initially and at the end of the first and second growing seasons. Seedling groups were control, nursery, and inoculated. Values are mean (standard deviation) of nine seedlings.

Disturbance, mixing, seedling group	Shoot height (cm)			Root-collar diameter (mm)		
	Initial	First season	Second season	Initial	First season	Second season
Burned						
Mixed						
Control	16.2 (1.8)	16.1 (1.7)	19.0 (1.7)	2.06 (0.14)	2.88 (0.14)	3.35 (0.19)
Nursery	22.0 (1.9)	23.4 (1.8)	25.4 (1.6)	2.15 (0.08)	2.86 (0.09)	3.06 (0.49)
Inoculated	11.5 (0.7)	11.3 (0.6)	12.5 (0.9)	1.60 (0.03)	1.89 (0.09)	2.14 (0.23)
Non-mixed						
Control	15.9 (1.4)	16.0 (1.4)	19.8 (2.2)	2.02 (0.13)	2.79 (0.14)	3.20 (0.29)
Nursery	22.4 (1.8)	24.5 (1.5)	26.6 (1.5)	2.12 (0.07)	2.76 (0.13)	3.11 (0.17)
Inoculated	11.0 (0.8)	11.2 (0.8)	13.2 (1.2)	1.56 (0.10)	1.91 (0.08)	2.13 (0.08)
Insect defoliated						
Mixed						
Control	16.7 (0.9)	16.7 (0.9)	25.5 (3.6)	2.01 (0.11)	2.96 (0.21)	4.90 (0.90)
Nursery	22.1 (1.1)	24.1 (1.0)	28.6 (3.2)	2.12 (0.08)	2.84 (0.11)	4.42 (1.09)
Inoculated	10.5 (0.7)	10.6 (0.9)	16.5 (2.3)	1.57 (0.08)	1.96 (0.11)	3.09 (0.58)
Non-mixed						
Control	16.3 (0.8)	16.6 (0.6)	22.1 (3.1)	1.93 (0.13)	2.54 (0.13)	3.00 (0.35)
Nursery	22.4 (1.7)	24.9 (1.2)	28.8 (2.7)	2.03 (0.14)	2.62 (0.16)	3.24 (0.34)
Inoculated	10.3 (0.6)	10.5 (0.6)	16.2 (1.9)	1.57 (0.08)	1.81 (0.14)	2.17 (0.08)

Appendix III. F-ratios and p values from analysis of variance testing relative increase in oven-dry mass of shoots, roots, and change in shoot/root ratio at the end of the second growing season (results of multiple comparisons not shown).

Source	df	Shoot dry-mass		Root dry-mass		Shoot/root ratio	
		<u>Second season</u>		<u>Second season</u>		<u>Second season</u>	
		F	p	F	p	F	p
Disturbance	1	8.54	0.04	0.82	0.42	18.67	0.01
Site	4	2.77	0.08	2.53	0.10	1.83	0.19
Plot	12		No test		No test		No test
Mixing	1	3.75	0.13	0.50	0.52	14.90	0.02
Site*Mixing	4	3.74	0.03	1.45	0.28	2.58	0.09
Disturbance*Mixing	1	7.24	0.05	10.23	0.03	4.79	0.09
Plot*Mixing	12		No test		No test		No test
Seedling	2	13.89	< 0.01	12.69	0.00	21.80	< 0.01
Site*Seedling	8	0.68	0.70	0.58	0.78	0.86	0.56
Disturbance*Seedling	2	11.70	< 0.01	8.72	0.01	2.92	0.11
Plot*Seedling	24		No test		No test		No test
Mixing*Seedling	2	7.74	0.01	1.75	0.23	4.97	0.04
Site*Mixing*Seedling	8	0.27	0.97	0.56	0.80	0.27	0.97
Disturbance*Mixing*Seedling	2	11.81	< 0.01	1.27	0.33	11.93	< 0.01
Plot*Mixing*Seedling	24		No test		No test		No test
Error	0						
Total	107						

Appendix IV. Actual shoot dry mass, root dry mass, and shoot/root dry mass ratio for three groups of black spruce seedlings at the end of the first and second growing seasons. Seedling groups were control, nursery, and inoculated. Values are mean (standard deviation) of nine seedlings.

Disturbance, mixing, seedling group	Shoot dry mass (g)		Root dry mass (g)		Shoot/root dry mass ratio	
	First season	Second season	First season	Second season	First season	Second season
Burned						
Mixed						
Control	1.09 (0.18)	1.85 (0.48)	0.62 (0.07)	0.79 (0.06)	1.76 (0.31)	2.33 (0.55)
Nursery	1.63 (0.24)	2.07 (0.35)	0.57 (0.14)	0.70 (0.13)	2.96 (0.56)	2.98 (0.43)
Inoculated	0.53 (0.08)	0.61 (0.16)	0.26 (0.05)	0.28 (0.05)	2.07 (0.42)	2.14 (0.44)
Non-mixed						
Control	0.95 (0.25)	1.82 (0.39)	0.49 (0.11)	0.80 (0.21)	1.97 (0.40)	2.30 (0.32)
Nursery	1.77 (0.38)	2.41 (0.48)	0.46 (0.10)	0.75 (0.20)	3.86 (0.47)	3.28 (0.75)
Inoculated	0.46 (0.08)	0.57 (0.09)	0.21 (0.05)	0.26 (0.06)	2.32 (0.52)	2.28 (0.47)
Insect defoliated						
Mixed						
Control	1.16 (0.24)	4.65 (1.92)	0.52 (0.10)	0.90 (0.28)	2.26 (0.52)	5.07 (1.08)
Nursery	1.69 (0.30)	4.39 (2.24)	0.48 (0.09)	0.98 (0.35)	3.57 (0.65)	4.31 (1.07)
Inoculated	0.46 (0.08)	1.62 (0.62)	0.23 (0.07)	0.38 (0.10)	2.11 (0.36)	4.13 (0.88)
Non-mixed						
Control	1.01 (0.22)	1.46 (0.52)	0.43 (0.07)	0.45 (0.16)	2.42 (0.68)	3.32 (0.57)
Nursery	1.48 (0.25)	2.50 (0.58)	0.35 (0.05)	0.57 (0.10)	4.24 (0.39)	4.47 (1.18)
Inoculated	0.39 (0.05)	0.67 (0.13)	0.21 (0.05)	0.25 (0.07)	1.90 (0.41)	2.83 (0.76)

Appendix V. F-ratios and p values from analysis of variance of foliar N, P, and K concentrations at the end of the second season (results of multiple comparisons not shown). Foliar N values were transformed by  $1/y$  before analysis

Source	df	Foliar N		Foliar P		Foliar K	
		<u>Second season</u>		<u>Second season</u>		<u>Second season</u>	
		F	p	F	p	F	p
Disturbance	1	10.39	0.03	17.36	0.01	41.80	< 0.01
Site	4	4.11	0.03	1.35	0.31	1.84	0.19
Plot	12		No test		No test		No test
Mixing	1	77.90	< 0.01	6.28	0.07	3.71	0.13
Site*Mixing	4	1.12	0.39	0.44	0.78	2.63	0.09
Disturbance*Mixing	1	0.05	0.83	0.67	0.46	0.53	0.51
Plot*Mixing	12		No test		No test		No test
Seedling	2	4.41	0.05	16.75	< 0.01	16.08	< 0.01
Site*Seedling	8	1.87	0.11	1.82	0.12	1.14	0.37
Disturbance*Seedling	2	1.18	0.36	1.88	0.21	2.69	0.13
Plot*Seedling	24		No test		No test		No test
Mixing*Seedling	2	0.33	0.73	0.41	0.68	5.70	0.03
Site*Mixing*Seedling	8	1.96	0.10	1.25	0.31	0.68	0.71
Disturbance*Mixing*Seedling	2	1.40	0.30	0.15	0.87	0.04	0.96
Plot*Mixing*Seedling	24		No test		No test		No test
Error	0						
Total	107						



Appendix VI. F-ratios and p values from analysis of variance testing EM formation at the end of the first and second growing seasons (results of multiple comparisons not shown).

Source	df	EM formation		EM formation	
		<u>First season</u>		<u>Second season</u>	
		F	p	F	p
Disturbance	1	1.52	0.28	2.43	0.19
Site	4	1.91	0.17	2.33	0.11
Plot	12		No test		No test
Mixing	1	8.43	0.04	0.53	0.51
Site*Mixing	4	0.02	1.00	0.64	0.65
Disturbance*Mixing	1	7.43	0.05	2.51	0.19
Plot*Mixing	12		No test		No test
Seedling	2	143.55	< 0.01	21.64	< 0.01
Site*Seedling	8	0.62	0.76	2.29	0.06
Disturbance*Seedling	2	1.42	0.30	0.03	0.97
Plot*Seedling	24		No test		No test
Mixing*Seedling	2	0.26	0.78	4.58	0.05
Site*Mixing*Seedling	8	1.72	0.14	0.58	0.79
Disturbance*Mixing*Seedling	2	0.51	0.62	0.89	0.45
Plot*Mixing*Seedling	24		No test		No test
Error	0				
Total	107				

# Chapter 3

## Changes in Soil Physical and Chemical Properties Following Forest Floor Mixing in *Kalmia* Dominated Sites

### Abstract

Forest floor mixing (tilling) by mechanical means may be an effective method of stimulating black spruce (*Picea mariana* Mill.) regeneration in sites dominated by *Kalmia angustifolia* L. Chapter 2 of this thesis reported on the growth response of black spruce seedlings planted in plots with and without forest floor mixing, in burned and insect defoliated sites in Terra Nova National Park, Newfoundland. The experimental design of this study was the same as that in Chapter 2. I measured soil temperature, soil respiration, and soil moisture near the end of the second growing season. I determined litter mass loss at the end of the first and second growing seasons. Soil samples of forest floor, Ae, and B horizons were collected at the end of first and second growing seasons and analyzed for total-N, total-C, C/N ratio, NH<sub>4</sub>-N, PO<sub>4</sub>-P, K, Fe, Al, Cu, Mg, Na, Ca, Zn, total phenols, and pH. Almost all significant differences were in the forest floor. Insect defoliated sites had significantly higher respiration, litter mass loss, total-N, total phenols, Cu, and Fe than the burned sites. Burned sites had significantly higher total-C, C/N ratio, Mg, and Na than the insect defoliated sites. Mixed plots had significantly higher moisture, litter mass loss, Al, and pH than the non-mixed plots. Non-mixed plots had significantly higher respiration, total-C, C/N, and Ca than in the mixed plots. Decomposition rates were higher in mixed forest floors than in non-mixed forest floors, and higher in insect defoliated sites than in burned sites. In insect defoliated sites, increased decomposition rates were likely due to improved litter quality (C/N), and perhaps higher levels of total phenols, which may have acted as a C source for decomposers. Mixed forest floors may have had increased rates of decomposition due to improved litter quality, and increased soil moisture and pH. However, concentrations of available nutrients were not higher in soils of insect defoliated sites or mixed forest floors compared to burned sites and non-mixed forest floors, respectively. This was likely due to plant uptake and immobilization in microbial biomass, or to leaching and volatilization. These results suggested that higher growth rates of seedlings in insect defoliated sites than in burned sites (Chapter 2) may have been due to improved nutrition. They also indicated that higher levels of foliar nutrients in seedlings in plots with mixed forest floors than those in non-mixed plots (Chapter 2) were likely due to decreased acidity and competition, and increased decomposition. However, these results did not explain why forest floor mixing resulted in increased growth of black spruce seedlings in insect defoliated sites, but not in burned sites (Chapter 2). Although most research has focused on soil ecology, I conclude that other factors must also be taken into account when explaining black spruce growth in *Kalmia* dominated sites, and in plans for restoration of black spruce cover on these sites.

## Introduction

Following canopy disturbance in black spruce (*Picea mariana* Mill.)–*Kalmia angustifolia* L. (hereafter referred to as *Kalmia*) forests, *Kalmia* may gain dominance, forming ericaceous heath (Richardson 1975; Mallik 1995). Such disturbances include clearcut harvesting, forest fire, or severe insect defoliation. Heaths may persist for decades or longer (Mallik and Bloom 2004). Several mechanisms have been reported as being responsible for the failure of black spruce to regenerate and the dominance of *Kalmia* on these sites. These include competition (Mallik 1994), paludification and low mineralization rates (Damman 1971), nutrient imbalance caused by phenols in *Kalmia* leaf litter (Inderjit and Mallik 1996b), and direct toxic effects of these phenols on primary root development (Inderjit and Mallik 1996b) and formation of ectomycorrhizae by black spruce (Mallik *et al.* 1998; Yamasaki *et al.* 1998).

The formation of heath dominated by *Kalmia* on sites that formerly supported black spruce forests is a serious problem for both the forest industry and managers of protected areas. Therefore, efforts to develop methods of improving black spruce productivity on *Kalmia* sites have been underway for many years. Site fertilization (English 1997; Paquin *et al.* 1998; Thiffault *et al.* 2004) and herbicide application (English and Titus 2000; Thiffault *et al.* 2004) can improve black spruce growth on *Kalmia* dominated sites. However, these methods may not fully release black spruce seedlings from the effects of *Kalmia*. The removal of *Kalmia* using herbicides may release black spruce from competition, but the inhibitory effects related to *Kalmia* humus would likely remain (Bradley *et al.* 1997b). Fertilization may improve nutrient availability for black spruce seedlings in *Kalmia* humus, yet the potential direct effects

associated with *Kalmia* leachates would likely not be relieved (Yamasaki *et al.* 2002).

Mechanical mixing (tilling) of the forest floor horizon (L,F,H) may provide an alternative means of improving regeneration of conifer seedlings on *Kalmia* dominated sites. Forest floor mixing has the potential to control competing vegetation, improve nutrient availability, and reduce any direct allelopathic effects or nutrient imbalances related to *Kalmia* leachates. Mallik (1991) simulated soil mixing by mulching *Kalmia* plants and the associated forest floor, and found that *Kalmia* growth was effectively controlled. As well, other studies have found that mixing effectively controls vegetation (Keenan *et al.* 1994; Frey *et al.* 2003). Forest floor mixing results in fragmentation and redistribution of humus through the organic horizon, exposing it to decomposers (Ross and Malcolm 1982). Soil mixing may result in increased rates of microbial activity and nutrient mineralization (Burger and Pritchett 1988; Mallik and Hu 1997; Prescott *et al.* 2000). Forest floor mixing often results in increased soil pH (Keenan *et al.* 1994; Messier *et al.* 1995). Furthermore, because rhizosphere microbes can degrade phenols, increased microbial activity and decreased inputs of *Kalmia* leaf litter may correct soil nutrient imbalances caused by phenols (Inderjit and Mallik 1996b). Forest floor mixing has been shown to enhance conifer regeneration (Ross and Malcolm 1982; Ross *et al.* 1986; Burger and Pritchett 1988; Messier *et al.* 1995). However, the effects of forest floor mixing on physical and chemical properties of *Kalmia* dominated sites are not known.

The objective of this study was to examine the effects of forest floor mixing in insect defoliated and burned sites dominated by *Kalmia*, on physical and chemical properties of soil that influence black spruce growth. I hypothesized that forest floor mixing would result in increased nutrient availability because of

decreased competitive cover and acidity, and enhanced decomposition due to changes in soil temperature, moisture, litter quality (C/N), and pH.

## Methods

### *Study sites and experimental design*

The study area and experimental design described in the previous section (Chapter 2) were used in this study.

### *Soil physical properties*

I measured volumetric soil moisture and soil temperature at five random locations in each sub-plot during the second growing season (August 23, 2003). Measurements were made at 6-8 cm depth in the forest floor, within an 8-10 hour period, beginning 48 hours after a sustained rainfall. An electronic probe and meter (Thetaprobe ML2x and Thetameter HH2, Delta-T Devices Ltd., Cambridge, UK) were used for soil moisture, and a thermocouple and meter (Tri-sense KX-37000-00, Cole-Parmer Instrument Co., Vernon Hills, IL) were used for soil temperature determination. I measured soil bulk density at four random locations in each sub-plot near the end of the second growing season (August 13-18, 2003). I collected samples of undisturbed forest floor soil (5-10 cm deep) using a cylindrical steel corer (54.3 cm<sup>3</sup>) after removing the litter layer. These bulk density samples were placed in plastic bags and kept cool (4 °C) during transport, air-dried in paper bags at room temperature (22 °C), oven-dried at 105 °C to constant mass (5 hours), and weighed on an electronic balance.

### *Litter mass loss and respiration*

I installed litterbags at the beginning of the first growing season (July 11<sup>th</sup>, 2002) to estimate decomposition rate. Litter consisting of leaves, twigs, and mosses from a *Kalmia* dominated site was collected and air-dried for one week. Litterbags were constructed using two sheets (20 x 20 cm) of 1 mm fiberglass mesh fastened together using inert alloy staples, then filled with 10 g of air-dry

litter. At this time, 10 g samples of air-dry litter were retained to estimate the initial oven-dry mass of the litterbags. Litterbags were placed on the fermentation layer of the forest floor after the litter layer had been brushed aside, and inert alloy pins were used to fix them in position. Some litterbags were transported to the field sites, but were not installed to estimate mass loss during transportation. Two groups of three replicate litterbags were installed in each sub-plot at the beginning of the first growing season. The first group of bags was collected at the end of the first growing season (60 days later), and the second was collected at the end of the second growing season (410 days later). Litterbags were gently cleaned of external debris, placed in plastic bags and kept cool (4 °C) during transport, air-dried and stored in paper bags at room temperature (22 °C), oven-dried at 105 °C to constant mass (five hours), and weighed on an electronic balance.

To estimate decomposition rate, I measured soil respiration at five random locations in each sub-plot at the end of the second growing season. I used an infrared gas analyzer (IRGA, Nortech, Ottawa, ON) (Parkinson 1981), which measured CO<sub>2</sub> evolution in a sampling chamber covering 78.5 cm<sup>2</sup> of soil surface. The dry litter layer was brushed aside and the sampling chamber was inserted 1 cm deep into the forest floor.

#### *Soil chemical properties*

I sampled soil from four random locations in each sub-plot at the end of the first and second growing seasons (September 2002, 2003). I excavated soil columns of approximately 10 x 10 cm, measured the depths of each soil horizon [forest floor (L,F,H), Ae (eluviation), and B (mineral)], then collected samples of approximately 200 g. After the first growing season only the forest floor was sampled, but after the second growing season the forest floor, Ae, and B

horizons were sampled. The soil samples were placed in plastic bags and kept cool (4 °C) during transport, then air-dried and stored in paper bags at room temperature (22 °C) in an air-tight container. Air-dry soil samples were sieved (2 mm), and equal volumes of the four samples from each sub-plot were mixed into one composite sample (n = 36). Air-dry moisture content was determined by weighing 1 g samples on an electronic balance before and after oven-drying them at 105 °C to constant mass (five hours). Air-dry soils were used for all analyses, then the results were converted to oven-dry mass.

All soil samples were analyzed for pH, total water-soluble phenols, exchangeable inorganic ions (Ca, K, Mg, Na, Al, Cu, Fe, Mn, Zn), total nitrogen total-N (organic + ammonium), ammonium nitrogen (NH<sub>4</sub>-N), and inorganic phosphorus (PO<sub>4</sub>-P). Soil pH was measured with a potentiometer (Accumet pH probe, Fischer Scientific, Nepean, ON) after preparing suspensions of air-dry soil in distilled water and shaking them for 30 minutes. For forest floor soils, the soil-to-distilled water ratio was 1:4, and for Ae and B-horizon soils it was 1:2 (Kalra and Maynard 1991). Total phenolic concentration of soils was determined by comparing extractions (distilled water) against a standard curve developed using phenol crystals (Swain and Hillis 1959). I used Folin Ciocalteu Reagent (Yu and Dahlgren 2000) and measured absorbance (725 nm) of the filtrate using a spectrophotometer (Skalar Auto Analyzer, Skalar Inc., Norcross, GA).

Cations were extracted with NH<sub>4</sub>OAc (pH 7.0), and 0.1 N HCL was used for metals (Allen 1989). After extraction, cations and metals were measured by inductively coupled argon plasma spectrometry (Varian Vista Pro ICAP Radial, Varian Inc., Mississauga, ON). Total-N was measured as absorbance (630 nm) using a spectrophotometer after digestion of 0.2 g samples by the Kjeldahl method (Kalra and Maynard 1991). NH<sub>4</sub>-N was extracted with 1.0 M KCL and



measured as absorbance (535 nm) using a spectrophotometer (Kalra and Maynard 1991). PO<sub>4</sub>-P was extracted using the Bray P1 method and measured as absorbance (882 nm) with a spectrophotometer (Kalra and Maynard 1991). For all extractions I used 1.5 g soil samples and Whatman # 5 filter paper. The forest floor soil samples collected after the second growing season were analyzed for total carbon (total-C) using finely ground and sieved (1 mm) samples in a dry combustion carbon analyzer (LECO Analyzer, Laboratory Equipment Corporation, St. Joseph, MI).

#### *Data analysis*

Data on soil physical and chemical properties were analyzed by analysis of variance (ANOVA) for nested designs followed by Scheffe's multiple contrasts. Data distributions were examined for normality using the Shapiro-Wilk statistic and variances were tested for homogeneity using Levene's statistic. When variables were not distributed normally or variances were not homogenous, a transformation (square root, Log<sub>10</sub>, reciprocal, or 1/square root) was applied prior to analysis. The data for bulk density and Mn had unequal variances that could not be homogenized using transformations, so I excluded these variables from the analysis. Data analyses were performed using Datadesk (Version 6.0, Data Description Inc., Ithica, NY) and SPSS software (Version 9.0, SPSS Inc., Chicago, IL).

## Results

### *Disturbance type*

Burned and insect defoliated sites had different soil physical properties but the differences were not significant. Plots in burned sites had 64% deeper forest floors ( $F = 6.4$ ,  $p = 0.06$ ) with 33% higher soil moisture than those in the insect defoliated sites ( $F = 6.1$ ,  $p = 0.07$ ) (Table 1). Soil temperature did not vary significantly between the burned and insect defoliated sites (Table 1, Appendix I).

In both sampling years, litter mass loss did not vary significantly between plots in burned and insect defoliated sites (Fig. 1a, Appendix I). However, soil respiration was 51% higher in plots in the insect defoliated sites than in those of the burned sites ( $F = 10.8$ ,  $p = 0.03$ ) (Fig. 1c).

After one growing season, forest floor soils in plots in burned sites had significantly higher Mg concentration than plots in insect defoliated sites ( $F = 19.6$ ,  $p = 0.011$ ) (Table 2, Appendix II). After the second growing season, forest floors in plots in burned sites had significantly higher total-C ( $F = 14.9$ ,  $p = 0.018$ ), C/N ratio ( $F = 92.5$ ,  $p = 0.001$ ), Mg ( $F = 49.6$ ,  $p = 0.002$ ), and Na ( $F = 11.7$ ,  $p = 0.027$ ) than plots in insect defoliated sites (Fig. 2c, g, k, l). Whereas plots in insect defoliated sites had significantly higher total-N ( $F = 8.7$ ,  $p = 0.042$ ), Fe ( $F = 8.3$ ,  $p = 0.045$ ), total phenols ( $F = 8.2$ ,  $p = 0.046$ ), and Cu ( $F = 23.1$ ,  $p = 0.009$ ) (Fig. 2a, f, i, n) than the plots in burned sites.

Soils in the Ae horizon in plots of insect defoliated sites had significantly higher total-N ( $F = 6.9$ ,  $p = 0.058$ ), Al ( $F = 9.2$ ,  $p = 0.039$ ), and K ( $F = 9.5$ ,  $p = 0.037$ ) than those in the burned sites (Table 3). Soils in the B horizon in plots of insect defoliated sites had significantly higher K ( $F = 76.1$ ,  $p = 0.001$ ), Cu ( $F = 8.0$ ,  $p = 0.047$ ), total-N ( $F = 8.7$ ,  $p = 0.042$ ), and pH ( $F = 25.9$ ,  $p = 0.007$ ) than those in the burned sites (Table 3).

Table 1. Depth, temperature, and moisture of forest floors at the end of the second growing season in plots with mixed and non-mixed forest floors, in burned and insect defoliated sites. Values are mean (standard deviation) of nine plots. In a column, values with different letters are significantly different at  $p < 0.05$ .

Disturbance, mixing	Depth (cm)	Temperature (° C)	Moisture (%)
Burned			
Mixed	12.8 (3.6) a	9.8 (1.7) a	46.5 (9.7) a
Non-mixed	15.6 (5.0) a	9.9 (1.5) a	34.0 (5.3) b
Insect defoliated			
Mixed	7.9 (2.4) b	12.2 (1.6) a	36.7 (6.6) a
Non-mixed	9.4 (2.5) b	11.3 (1.9) a	22.4 (6.7) b

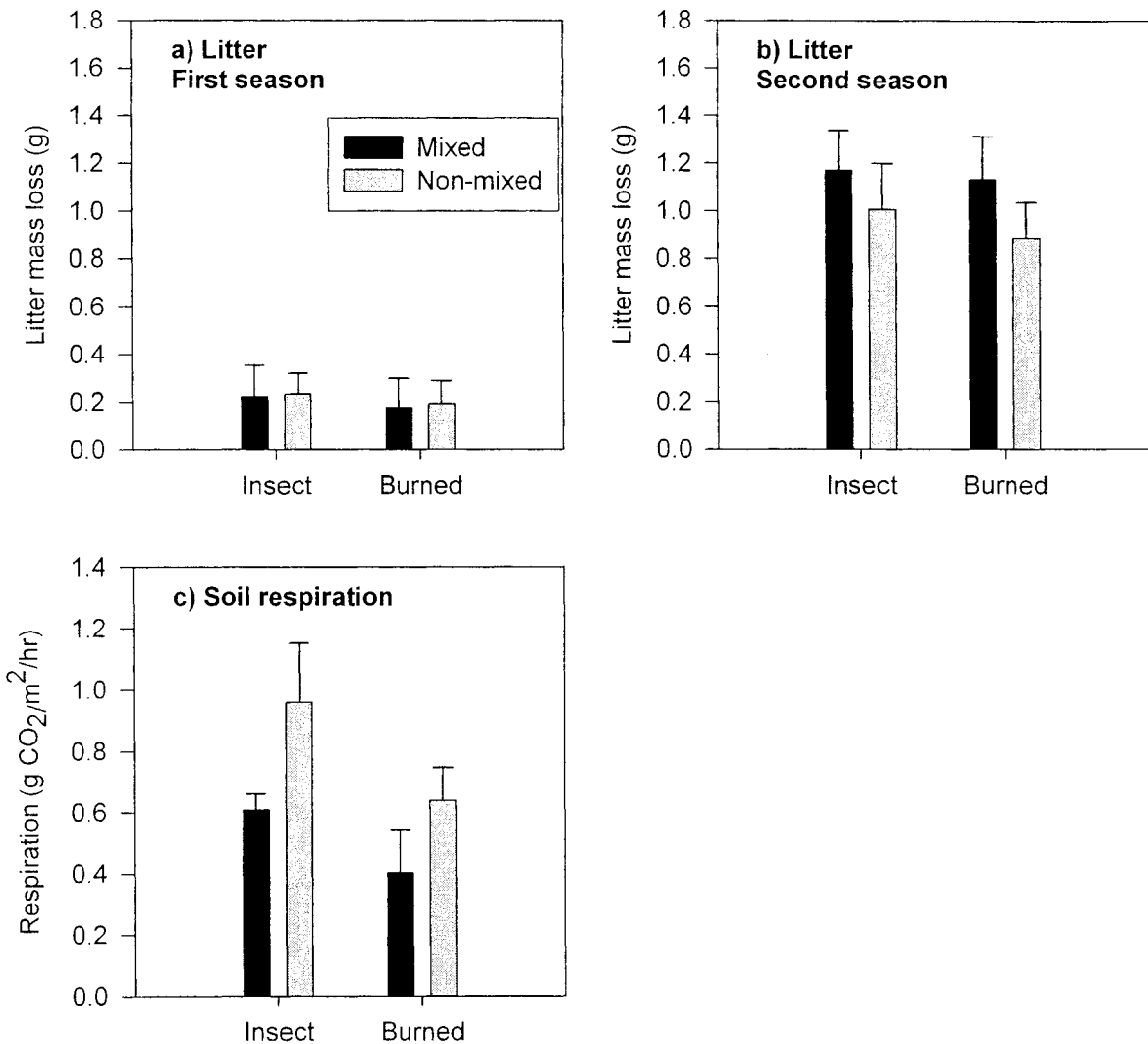


Figure 1. Litter mass loss at the end of the first growing season (a), litter mass loss at the end of the second growing season (b), and soil respiration at the end of the second growing season (c), for the forest floor horizon. Values are means of nine plots and error bars represent one standard deviation.

Table 2. Chemical properties of forest floors at the end of the first growing season in plots with mixed and non-mixed forest floors, in burned and insect defoliated sites. Values are mean (standard deviation) of nine plots. In rows, values with different letters are significantly different at  $p < 0.05$ .

Parameter	Insect defoliated		Burned	
	Mixed	Non-mixed	Mixed	Non-mixed
Ca ( $\mu\text{g/g}$ )	796.7 (342.7) a	1013.8 (439.4) a	546.3 (212.3) a	609.3 (143.6) a
K ( $\mu\text{g/g}$ )	272.9 (76.2) a	284.8 (92.1) a	243.4 (58.3) a	229.4 (50.0) a
Mg ( $\mu\text{g/g}$ )	227.4 (63.7) b	285.2 (63.1) b	396.1 (81.5) a	440.1 (91.7) a
Na ( $\mu\text{g/g}$ )	116.1 (31.1) b	179.0 (67.2) a	131.8 (56.2) b	147.8 (69.0) ab
Al ( $\mu\text{g/g}$ )	319.9 (199.6) a	157.7 (149.4) ab	127.3 (101.3) b	155.9 (154.4) ab
Cu ( $\mu\text{g/g}$ )	0.20 (0.11) a	0.16 (0.13) a	0.05 (0.06) a	0.16 (0.28) a
Fe ( $\mu\text{g/g}$ )	50.2 (23.6) a	20.0 (14.3) b	14.2 (9.9) b	17.4 (12.7) b
Zn ( $\mu\text{g/g}$ )	11.8 (8.9) a	13.4 (8.4) a	6.3 (2.0) a	13.5 (17.5) a
P <sub>04</sub> ( $\mu\text{g/g}$ )	30.0 (11.7) a	33.2 (7.1) a	26.4 (6.6) a	29.9 (7.8) a
NH <sub>4</sub> -N ( $\mu\text{g/g}$ )	36.8 (13.6) a	41.6 (13.2) a	35.4 (18.4) a	41.3 (10.7) a
Total-N (mg/g)	9.2 (1.3) b	12.6 (2.4) a	9.3 (1.1) b	10.0 (1.2) b
pH	3.39 (0.13) a	3.28 (0.16) b	3.21 (0.11) c	3.19 (0.10) c

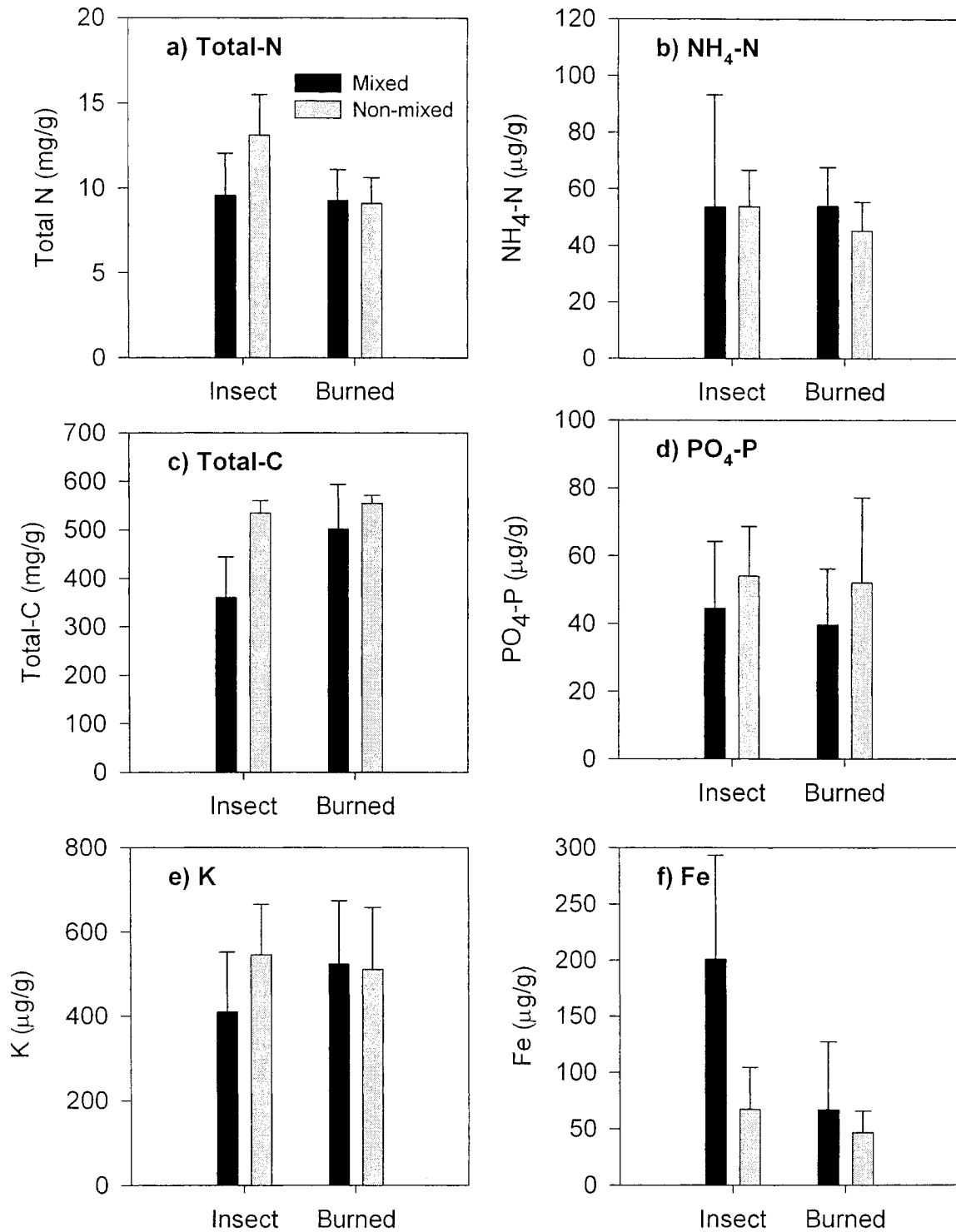


Figure 2. Total-N (a), NH<sub>4</sub>-N (b), total-C (c), PO<sub>4</sub>-P (d), K (e), and Fe (f) in forest floors at the end of the second growing season. Values are means of nine plots and error bars represent one standard deviation.

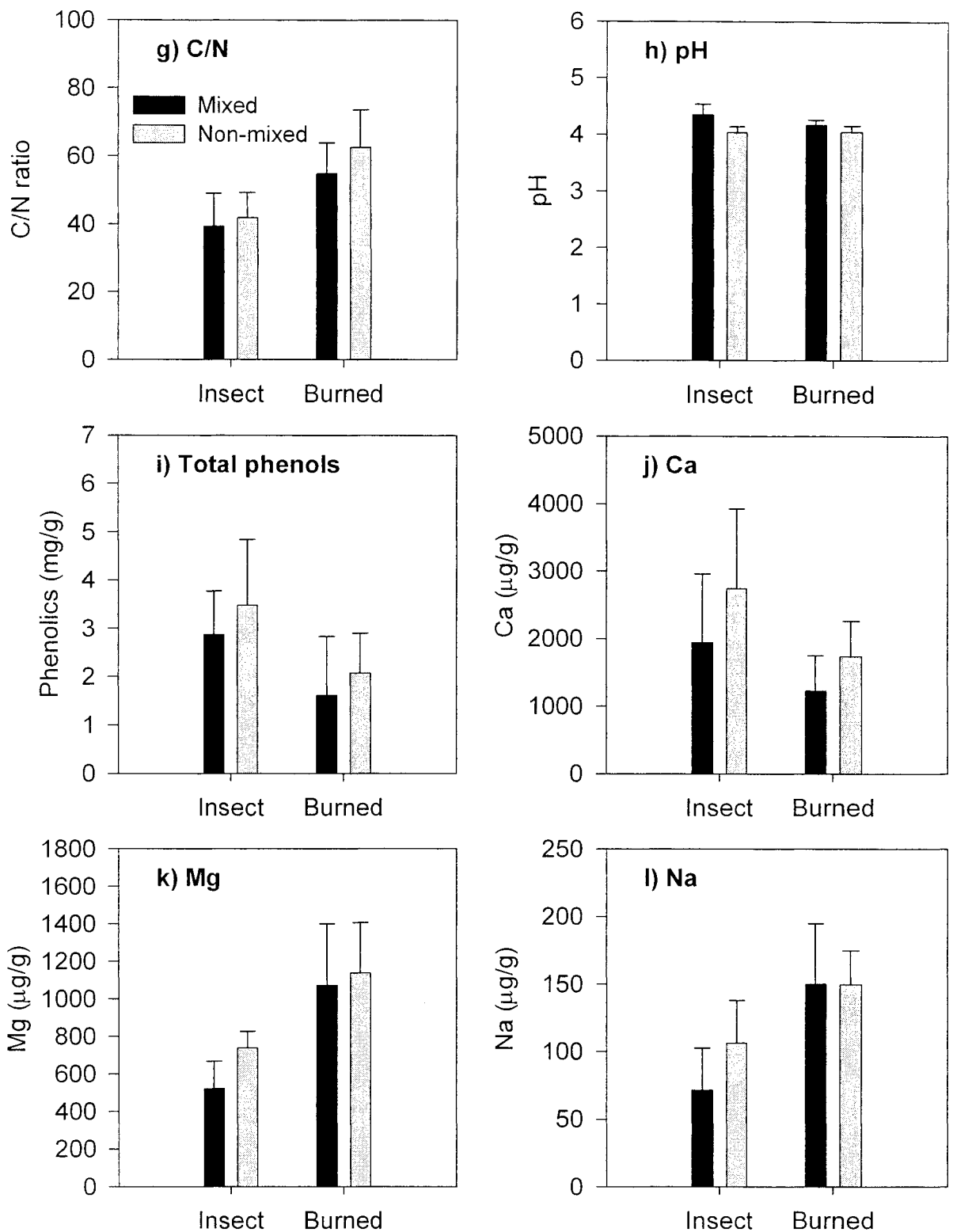


Figure 2. Continued. C/N ratio (g), pH (h), total phenols (i), Ca (j), Mg (k), and Na (l) in forest floors at the end of the second growing season. Values are means of nine plots and error bars represent one standard deviation.

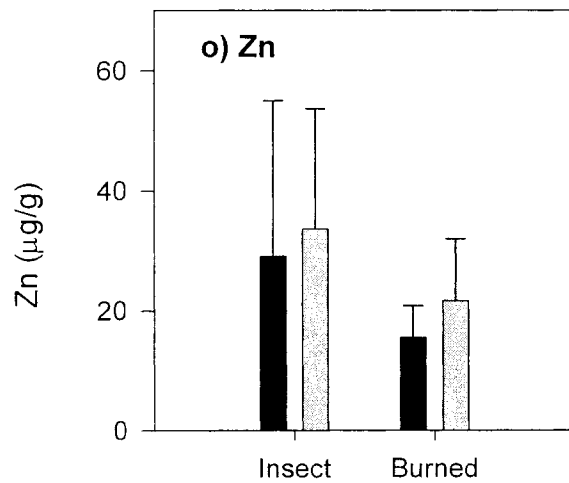
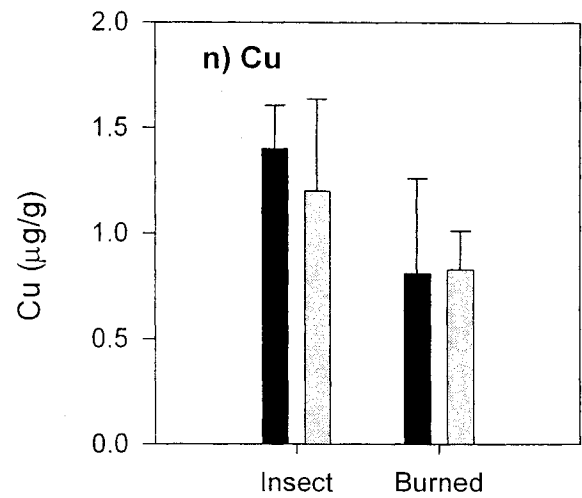
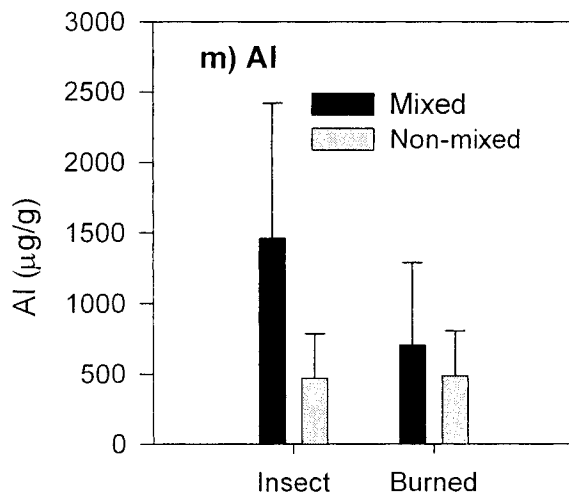


Figure 2. Continued. Al (m), Cu (n), and Zn (o) in forest floors at the end of the second growing season. Values are means of nine plots and error bars represent one standard deviation.



Table 3. Soil chemical properties of Ae and B horizons at the end of the second growing season in plots with mixed and non-mixed forest floors, in burned and insect defoliated sites. Values are mean (standard deviation) of nine plots. In rows, values with different letters are significantly different at  $p < 0.05$ .

Parameter	Horizon	Insect defoliated		Burned	
		Mixed	Non-mixed	Mixed	Non-mixed
Ca ( $\mu\text{g/g}$ )	Ae	93.5 (144.3) a	70.8 (67.9) a	13.2 (7.0) b	12.8 (5.4) b
	B	32.3 (27.6) a	17.3 (20.4) a	13.9 (7.4) a	15.6 (9.6) a
K ( $\mu\text{g/g}$ )	Ae	39.9 (16.4) a	35.6 (25.6) a	16.4 (2.5) b	14.9 (6.4) b
	B	51.0 (11.0) a	41.4 (20.9) ab	25.4 (11.7) b	22.9 (9.9) b
Mg ( $\mu\text{g/g}$ )	Ae	25.8 (21.1) a	23.5 (15.8) a	9.7 (2.7) b	9.9 (3.5) b
	B	16.0 (10.1) a	11.5 (5.8) a	11.4 (3.0) a	10.7 (3.5) a
Na ( $\mu\text{g/g}$ )	Ae	11.2 (4.8) a	11.7 (5.4) a	7.9 (3.5) a	8.9 (2.6) a
	B	15.4 (4.6) a	12.8 (3.0) a	16.5 (9.1) a	14.4 (8.0) a
Al ( $\text{mg/g}$ )	Ae	0.7 (0.3) a	0.5 (0.2) b	0.4 (0.1) c	0.3 (0.1) c
	B	7.5 (1.8) ab	9.7 (1.9) a	5.6 (5.3) b	5.1 (3.8) b
Cu ( $\mu\text{g/g}$ )	Ae	0.76 (0.27) a	0.70 (0.39) a	0.43 (0.09) a	0.76 (0.74) a
	B	1.53 (0.47) a	1.44 (0.57) a	0.72 (0.22) b	0.73 (0.46) b
Fe ( $\mu\text{g/g}$ )	Ae	194.9 (287.3) a	83.4 (107.4) a	135.8 (190.8) a	73.9 (84.5) a
	B	502.6 (263.7) a	590.4 (235.3) a	944.2 (670.4) a	750.4 (430.5) a
Zn ( $\mu\text{g/g}$ )	Ae	1.3 (0.9) ab	1.7 (1.6) a	0.6 (0.3) c	0.8 (0.4) bc
	B	1.5 (1.4) a	1.6 (0.8) a	1.2 (0.4) a	1.1 (0.3) a
Phenols ( $\text{mg/g}$ )	Ae	0.81 (0.81) a	1.09 (0.98) a	0.86 (0.51) a	0.90 (0.58) a
	B	3.33 (1.15) a	3.86 (0.98) a	2.87 (0.61) a	3.39 (1.71) a
pH	Ae	4.18 (0.12) b	4.13 (0.12) b	4.33 (0.18) a	4.38 (0.13) a
	B	4.83 (0.25) a	4.66 (0.24) b	4.50 (0.25) bc	4.46 (0.22) c
P <sub>04</sub> ( $\mu\text{g/g}$ )	Ae	8.4 (3.6) a	8.4 (4.8) a	8.8 (4.6) a	6.7 (2.8) a
	B	17.1 (4.7) a	17.0 (5.7) a	14.6 (8.0) a	17.7 (11.5) a
NH <sub>4</sub> ( $\mu\text{g/g}$ )	Ae	11.5 (7.8) a	12.9 (6.8) a	11.7 (4.9) a	11.9 (2.8) a
	B	16.9 (6.1) a	16.0 (6.8) a	15.6 (6.0) a	17.9 (7.4) a
Total N ( $\text{mg/g}$ )	Ae	0.61 (0.30) a	0.61 (0.37) a	0.28 (0.12) b	0.25 (0.14) b
	B	1.63 (0.48) ab	1.92 (0.60) a	1.02 (0.58) b	1.02 (0.61) b

### *Forest floor mixing*

Soil moisture was significantly greater (48%) in mixed plots than in the non-mixed plots ( $F = 16.3$ ,  $p = 0.016$ ) (Table 1). After two growing seasons, litter mass loss was significantly (21.5%) greater in mixed plots compared to the non-mixed plots ( $F = 9.8$ ,  $p = 0.035$ ) (Fig. 1b). However, soil respiration was significantly (58%) higher in non-mixed plots than in mixed plots ( $F = 29.7$ ,  $p = 0.006$ ) (Fig. 1c).

After one growing season, forest floor soils in non-mixed plots had significantly higher total-N ( $F = 17.8$ ,  $p = 0.014$ ) and Na ( $F = 11.8$ ,  $p = 0.026$ ) concentrations than those of the mixed plots (Table 2). After the second growing season, forest floor soils in non-mixed plots had significantly higher total-N ( $F = 15.8$ ,  $p = 0.016$ ), total-C ( $F = 105.9$ ,  $p = 0.001$ ), K ( $F = 7.1$ ,  $p = 0.057$ ), and Ca ( $F = 8.8$ ,  $p = 0.041$ ) concentrations, as well as higher C/N ratio ( $F = 12.2$ ,  $p = 0.025$ ), than the mixed plots (Fig. 2a, c, e, g, j). Mixed plots had significantly higher Fe ( $F = 37.6$ ,  $p = 0.004$ ) and Al ( $F = 9.9$ ,  $p = 0.035$ ) concentrations, as well as higher pH ( $F = 27.6$ ,  $p = 0.006$ ), than the non-mixed plots (Fig. 2f, h, m). At the end of the second growing season, soils in the Ae layer in the mixed plots had significantly higher Al ( $F = 28.4$ ,  $p = 0.006$ ) concentrations than those in the non-mixed plots (Table 3).

### *Interactions between disturbance type and forest floor mixing*

Significant interaction effects between disturbance type and forest floor mixing occurred only with chemical properties of forest floors. At the end of the first growing season, mixed plots in insect defoliated sites had significantly more Fe ( $F = 17.2$ ,  $p = 0.014$ ) and higher pH ( $F = 16.2$ ,  $p = 0.016$ ) than non-mixed plots, but not in burned sites (Table 2). At the end of the second growing season, the two-way interaction between disturbance type and forest floor mixing

was significant for Fe ( $F = 20.4$ ,  $p = 0.011$ ), K ( $F = 10.2$ ,  $p = 0.033$ ), total-C ( $F = 30.0$ ,  $p = 0.005$ ), and total-N ( $F = 19.2$ ,  $p = 0.012$ ). In insect defoliated sites, non-mixed plots had significantly higher total-N and K than the mixed plots (Fig. 2a and e), and mixed plots had significantly higher Fe than non-mixed plots (Fig. 2f). In burned sites there was no significant difference in total-N, K, or Fe between the mixed and non-mixed plots (Fig. 2a, e, f, respectively). Total-C was significantly higher in non-mixed plots than in mixed plots in both insect defoliated and burned sites, but the difference was much greater in insect defoliated sites than in the burned sites (Fig. 2c).

## Discussion

### *Disturbance type*

I did not find significant differences in soil temperature and moisture between plots in the insect defoliated and burned sites. I used instantaneous measures of temperature and moisture on one day in late summer, which may not reflect the differences of temperature and moisture extremes experienced during the growing season. In addition, it was difficult to measure soil temperature and moisture at all plots in a period of time short enough to control error due to diurnal changes. Burned sites may have been subject to greater extremes of temperature and moisture than insect defoliated sites. The fires at the burned sites occurred 24 to 42 years before this experiment. The long-term impacts of burning on soil temperature and moisture would be indirect effects associated with vegetation cover and soil properties. Rouse and Mills (1976) found that during summer, surface soils in burns without canopy cover are 3-5 °C warmer and 12-20% drier than adjacent closed canopy forests.

Soil respiration and litter mass loss were higher in insect defoliated plots than in burned plots, suggesting that decomposition rate was greater in the insect defoliated plots. Increased decomposition rate in the insect defoliated sites may have been due to better litter quality (C/N ratio). The ratio of C/N of forest floors was lower in the insect defoliated sites than in the burned sites. As C/N ratio increases, N availability limits microbial activity and decomposition rate (Pritchett 1979). The ratio of C/N may have been lower in the insect defoliated sites due to the effects of partial canopy closure. Bloom and Mallik (2004) found that degree of canopy closure in *Kalmia*-black spruce forest was positively correlated with soil respiration. They interpreted this to be an effect of *Kalmia* litter quality, which under partial canopy closure has a lower C/N ratio than the open canopy

heath condition, resulting in higher rates of decomposition. Also, enhanced decomposition rate in insect defoliated plots was accompanied by higher concentrations of total phenols, which could have been a C source that increased microbial activity (Brady 1990).

Forest floors in insect defoliated sites had higher concentrations of total phenols than those in the burned sites. This result was unexpected and it is not clear why total phenols would be higher in insect defoliated plots. Concentrations of total phenols in plants commonly increase with ultraviolet light exposure (Hattenschwiler and Vitousek 2000), and there was greater light exposure in burned sites. Also, with higher decomposition rates in insect defoliated sites, levels of total phenols should decrease due to microbial degradation (Inderjit and Mallik 1996b). However, some phenols may be resistant to microbial degradation (Hattenschwiler and Vitousek 2000). One possible explanation is that total phenols were higher because of increased biomass of the partially shaded *Kalmia* in insect defoliated sites. *Kalmia* attains its maximum above-ground biomass under partial shade (Mallik 1994). Both cover and height of *Kalmia* was higher in insect defoliated sites than the burned sites. Another possibility is that phenols in the burned sites were adsorbed onto organic matter (Rice 1984), because organic matter (total-C) was higher in burned sites than in insect defoliated sites.

Plant phenols have been implicated in inhibiting the growth of black spruce seedlings in sites dominated by Labrador tea (*Ledum groenlandicum*) (Inderjit and Mallik 1996a) and in soils amended with *Kalmia* leachates (Inderjit and Mallik 1996b). These authors suggested that phenols cause nutrient interference or have direct toxic effects. Phenols, even in low concentrations, have been shown to alter the accumulation and availability of plant nutrients in

soils (Inderjit and Dakshini 1994). In mineral soils from *Kalmia* sites, total phenols decreased concentrations of total-N, and increased concentrations of Fe, Mn, Al, and PO<sub>4</sub>-P (Inderjit and Mallik 1996b). Black spruce seedlings grown in *Kalmia* soils had high foliar concentrations of Ca, Al, Fe, and K (Mallik 2001), and lower concentrations of N (Yamasaki *et al.* 1998). In contrast, I found higher levels of total phenols occurring concurrently with enhanced growth of black spruce seedlings (Chapter 2) in forest floors of the insect defoliated plots, compared to the burned plots. Higher levels of total phenols in insect defoliated sites were not accompanied by lower concentrations of total-N, but concentrations of Fe were higher. This suggests that *Kalmia* soils do not always cause reduced total-N concentrations, increased concentrations of PO<sub>4</sub>-P and metallic ions, or inhibition of black spruce seedling growth.

#### *Forest floor mixing*

I found no difference in temperature between mixed and non-mixed forest floors. As previously discussed, I used instantaneous measures that do not reveal daily differences in temperature mean or range, which may have been greater in mixed forest floors (see Thiffault *et al.* 2004). For example, mixing increased the mean temperature by 0.5 °C and the diurnal range of temperatures by 2-3 times in an upland heath soil in Scotland (Ross and Malcolm 1982). Also, clearing of vegetation and harrowing soils resulted in increased soil temperatures in a pine plantation in Florida (Burger and Pritchett 1988). Higher soil surface temperatures occurred after removal of plant cover by prescribed burning in *Calluna* heath (Mallik 1986).

Soil moisture was greater in mixed than in non-mixed forest floors. Similarly, clearing of vegetation and harrowing soils resulted in increased soil moisture in a pine plantation in Florida (Burger and Pritchett 1988). In contrast,

Keenan *et al.* (1994) found that forest floor mixing decreased soil moisture in coastal forests of British Columbia. These authors suggested that this was due to increased mineral soil content, which increased thermal conductivity and lowered moisture-holding capacity. However, in my study mixing blended only small amounts of mineral soil into the forest floor layers because forest floors were deep and Ae layers were typically hardened by iron pan. An indication of this was that there was no difference in organic matter content (total-C) between mixed and non-mixed forest floors. Increased moisture in the mixed forest floors could have been due to vegetation removal. Most studies (McColl 1977; Mallik 1986; Adams *et al.* 1991) show that soil moisture increases after vegetation removal due to decreased evapotranspiration and rainfall interception by vegetation. In addition, increased infiltration due to mixing may have played a role in increasing soil moisture in mixed forest floors (Ross and Malcolm 1982).

Mixed forest floors had higher pH compared to non-mixed forest floors. Similarly, Keenan *et al.* (1994) found decreased acidity after forest floor mixing in the coastal forests of British Columbia, which they attributed to mixing mineral soil with low acidity into organic matter with high acidity. In my study little mineral soil was mixed into the forest floor, but it may have been enough to raise the pH. Decreased acidity in soils generally results in enhanced nutrient availability (Pritchett 1979).

Decomposition rate appears to have been higher in mixed forest floors than in non-mixed forest floors. Non-mixed forest floors had higher rates of respiration than mixed forest floors. However, soil respiration is not always a clear measure of decomposition because it includes both microbial and root respiration. The mixed forest floors had little vegetation cover and thus would have had little root respiration, but an unknown portion of the respiration of non-

mixed forest floors would have been root respiration. Under these circumstances, litter mass loss is a better measure of decomposition rate, and it was greater in mixed than in non-mixed forest floors. Similarly, clearing vegetation and harrowing soils resulted in increased decomposition rate in a pine plantation in Florida (Burger and Pritchett 1988).

Increased decomposition in mixed forest floors was likely due to a combination of improved litter quality (C/N), and increased soil moisture, and pH. C/N ratio was greater in the non-mixed forest floors than in mixed forest floors, indicating that mixing improved litter quality. Ugolini *et al.* (1990) found that mixing improved litter quality and decomposition rate in forests in coastal Alaska because mineral soils blended into the forest floor horizon were high in nutrients. In contrast, Keenan *et al.* (1994) found that mixing decreased litter quality and decomposition rate in the coastal forests of British Columbia because mineral soils and humus mixed into the forest floor layer were low in nutrients, particularly total-N. In my study, litter quality and decomposition rate may have been improved by mixing because humus high in nutrient content was blended into the surface litter layer. In forest floors from *Kalmia* sites, organic matter in the humus and fermentation layers was higher in total-N than organic matter in the litter layer (Damman 1971).

Mixed forest floors had much higher Fe concentrations than non-mixed forest floors in insect defoliated sites but not burned sites. This may have occurred because the forest floor was thinner in insect defoliated than in burned sites, causing more mineral soil rich in Fe to be mixed into forest floors. Mixing increased soil concentrations of Ca and Mg due to blending of mineral soil (Schmidt *et al.* 1996; Frey *et al.* 2003). Also in insect defoliated sites, mixing forest floors resulted in decreases in total-N, total C, and K compared to non-



mixed forest floors. Lower concentrations of total N and total C in mixed plots in insect defoliated sites were likely the result of higher rates of mineralization (decomposition) in the mixed plots than in the non-mixed plots. It is not clear why concentrations of K would have been lowered by forest floor mixing in insect defoliated sites, but not in burned sites. In *Kalmia* soils, K concentration generally decreases with depth of the forest floor (Damman 1971), so mixing may have brought humus to the surface that was low in K concentration. However, if this is the mechanism that resulted in lower K in mixed forest floors, it should have occurred in both insect defoliated and burned sites.

#### *Implications for black spruce growth*

In chapter 2, I found that black spruce seedlings in insect defoliated sites generally had higher growth (height and shoot mass) and foliar nutrient concentrations (N, P, K) than those in burned sites. In that study, I speculated that those responses were likely due to the higher decomposition rates and nutrient cycling in insect defoliated sites compared to burned sites. I suggested that seedlings in insect defoliated sites were not exposed to the same extremes of soil temperature and moisture that the seedlings in burned sites were. I found that seedlings in plots with mixed forest floors generally had higher foliar nutrient concentrations (N, P, K) than those in plots with non-mixed forest floors. This was likely due to decreased acidity and competitive cover, and higher decomposition in mixed forest floors compared to non-mixed forest floors. Concentrations of available N and P ( $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$ ) were not higher in the soils of insect defoliated sites than in the burned sites, or in mixed forest floors compared to non-mixed forest floors. This was likely because increased mineralization of these nutrients was balanced by plant uptake and immobilization in microbial biomass, or leaching and volatilization. Immobilization

of nutrients is commonly associated with increases in microbial activity (Pritchett 1979; Vitousek and Matson 1985). Frey *et al.* (2003) also found that soil mixing did not result in an increase in available-N, and suggested that this occurred because mixing introduced litter containing labile-C, which in turn caused immobilization by microbes. I did not directly measure nutrient mineralization or immobilization, which would help to further substantiate this conclusion. This could be accomplished using isotope dilution techniques (Kirkham and Bartholomew 1954; Hart *et al.* 1994) to estimate N mineralization and immobilization rates in these soils. The soil variables I measured did not explain why forest floor mixing results in increased black spruce seedling growth in insect defoliated sites, but not in burned sites (Chapter 2). This suggests that growth of black spruce in *Kalmia* dominated sites is only partly explained by soil properties.

#### *Management implications*

*Kalmia* dominated soils in sites originating from insect defoliation have better physical and chemical conditions for black spruce growth than those originating from burning. Mixing of the forest floor of *Kalmia* dominated sites results in improved physical and chemical properties relative to soils that are not mixed. Factors in addition to soil properties must be taken into account when explaining black spruce growth in *Kalmia* dominated sites, and in designing methods for restoring of black spruce cover to such sites. Forest floor mixing is likely to result in satisfactory regeneration and re-establishment of black spruce forest cover on insect defoliated sites, but not on burned sites.

Appendix I. F-ratios and p values from analysis of variance of soil temperature, moisture (volumetric) and soil respiration at the end of the second growing season; and litter mass loss after the first and second growing seasons (results of multiple comparisons not shown).

Source	df	<u>Temperature</u>		<u>Moisture</u>		<u>Respiration</u>		<u>Litter mass loss</u>			
		F	p	F	p	F	p	<u>First season</u>		<u>Second season</u>	
Disturbance	1	2.02	0.23	6.15	0.07	10.79	0.03	0.84	0.41	5.53	0.08
Site	4	12.48	< 0.01	5.61	0.01	5.15	0.01	1.43	0.28	0.28	0.88
Plot	12	No test									
Mixing	1	3.10	0.15	16.29	0.02	29.66	0.01	0.14	0.73	9.83	0.04
Disturbance*Mixing	1	5.88	0.07	0.08	0.79	1.16	0.34	< 0.01	0.96	0.38	0.57
Site*Mixing	4	0.42	0.79	4.45	0.02	2.96	0.06	1.28	0.33	1.34	0.31
Plot*Mixing	12	No test									
Error	0										
Total	35										

Appendix II. F-ratios and p values from analysis of variance for chemical properties of forest floors at the end of the first growing season (results of multiple comparisons not shown).

Source	df	<u>Ca</u>		<u>K</u>		<u>Mg</u>		<u>Na</u>		<u>Al</u>		<u>Cu</u>	
		F	p	F	p	F	p	F	p	F	p	F	p
Disturbance	1	1.64	0.27	1.61	0.27	19.60	0.01	0.04	0.86	3.02	0.16	1.63	0.27
Site	4	24.44	< 0.01	2.15	0.14	2.35	0.11	10.09	< 0.01	3.40	0.04	1.15	0.38
Plot	12	No test											
Mixing	1	6.23	0.07	< 0.01	0.96	4.96	0.09	11.82	0.03	2.10	0.22	0.26	0.63
Disturbance*Mixing	1	1.89	0.24	0.33	0.60	0.09	0.78	4.16	0.11	4.29	0.11	1.27	0.32
Site*Mixing	4	1.33	0.32	1.19	0.36	1.00	0.45	0.62	0.66	0.48	0.75	1.71	0.21
Plot*Mixing	12	No test											
Error	0												
Total	35												

Source	df	<u>Fe</u>		<u>Zn</u>		<u>PO4-P</u>		<u>NH3-N</u>		<u>Total-N</u>		<u>pH</u>	
		F	p	F	p	F	p	F	p	F	p	F	p
Disturbance	1	9.30	0.04	0.20	0.67	0.57	0.49	0.01	0.92	1.42	0.30	3.05	0.16
Site	4	2.91	0.07	3.64	0.04	2.16	0.14	7.53	< 0.01	6.40	0.01	2.94	0.07
Plot	12	No test											
Mixing	1	11.21	0.03	2.09	0.22	4.12	0.11	1.04	0.37	17.77	0.01	11.03	0.03
Disturbance*Mixing	1	17.16	0.01	0.84	0.41	0.01	0.94	0.01	0.93	7.12	0.06	16.22	0.02
Site*Mixing	4	0.38	0.82	0.92	0.48	0.69	0.61	1.72	0.21	2.15	0.14	0.23	0.91
Plot*Mixing	12	No test											
Error	0												
Total	35												

Appendix III. F-ratios and p values from analysis of variance for chemical properties of forest floors at the end of the second growing season (results of multiple comparisons not shown). Al values were transformed by square root ( $x$ ) before analysis.

Source	df	<u>Ca</u>		<u>K</u>		<u>Mg</u>		<u>Na</u>		<u>Al*</u>		<u>Cu</u>		<u>Fe</u>		<u>Zn</u>	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Disturbance	1	1.86	0.24	0.25	0.64	49.56	< 0.01	11.72	0.03	3.47	0.14	23.11	0.01	8.26	0.05	1.16	0.34
Site	4	8.80	< 0.01	3.48	0.04	0.45	0.77	2.90	0.07	1.08	0.41	0.64	0.64	2.07	0.15	5.19	0.01
Plot	12	No test															
Mixing	1	8.80	0.04	7.06	0.06	3.89	0.12	2.45	0.19	9.89	0.03	0.46	0.53	37.64	< 0.01	4.61	0.10
Disturbance*Mixing	1	0.42	0.55	10.17	0.03	1.10	0.35	2.60	0.18	3.61	0.13	0.65	0.47	20.44	0.01	0.10	0.77
Site*Mixing	4	1.90	0.18	0.29	0.88	2.36	0.11	1.43	0.28	0.85	0.52	1.86	0.18	0.42	0.79	0.46	0.77
Plot*Mixing	12	No test															
Error	0																
Total	35																
Source	df	<u>PO<sub>4</sub></u>		<u>NH<sub>3</sub></u>		<u>Total-N</u>		<u>pH</u>		<u>Phenols</u>		<u>Total-C</u>		<u>C/N</u>			
		F	p	F	p	F	p	F	p	F	p	F	p	F	p		
Disturbance	1	1.64	0.27	1.87	0.24	1.77	0.25	1.77	0.25	8.19	0.05	14.86	0.02	92.48	< 0.01		
Site	4	0.10	0.98	0.13	0.97	2.68	0.08	2.68	0.08	2.27	0.12	0.97	0.46	0.24	0.91		
Plot	12	No test															
Mixing	1	2.57	0.18	0.36	0.58	27.61	0.01	27.61	0.01	3.25	0.15	105.9	< 0.01	12.24	0.02		
Disturbance*Mixing	1	0.05	0.84	0.39	0.57	5.58	0.08	5.58	0.08	0.06	0.82	30.00	0.01	3.13	0.15		
Site*Mixing	4	2.22	0.13	0.86	0.52	1.19	0.37	1.19	0.37	0.55	0.71	0.21	0.93	0.24	0.91		
Plot*Mixing	12	No test															
Error	0																
Total	35																

Appendix IV. F-ratios and p values from analysis of variance for chemical properties of Ae horizon soils at the end of the second growing season (results of multiple comparisons not shown). Ca, K, Mg, Cu, Zn values were transformed by square root ( $x$ ), and Fe values were transformed by  $\text{Log}_{10}(x)$ .

Source	df	<u>Ca*</u>		<u>K*</u>		<u>Mg*</u>		<u>Na</u>		<u>Al</u>		<u>Cu*</u>		<u>Fe*</u>		<u>Zn*</u>	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Disturbance	1	3.48	0.14	9.47	0.04	3.71	0.13	3.59	0.13	9.18	0.04	1.02	0.37	0.17	0.70	0.73	0.44
Site	4	4.19	0.02	2.69	0.08	4.17	0.02	1.64	0.23	1.00	0.44	2.61	0.09	2.23	0.13	7.77	< 0.01
Plot	12	No test															
Mixing	1	1.80	0.25	1.24	0.33	0.53	0.51	0.22	0.67	28.40	0.01	0.37	0.58	1.23	0.33	6.34	0.07
Disturbance*Mixing	1	1.74	0.26	0.11	0.75	0.64	0.47	0.04	0.86	5.06	0.09	1.05	0.36	0.05	0.83	0.22	0.66
Site*Mixing	4	0.13	0.97	2.09	0.15	0.80	0.55	1.07	0.41	0.58	0.68	2.68	0.08	0.91	0.49	0.57	0.69
Plot*Mixing	12	No test															
Error	0																
Total	35																
Source	df	<u>PO<sub>4</sub></u>		<u>NH<sub>3</sub></u>		<u>Total-N</u>		<u>pH</u>		<u>Phenols</u>							
		F	p	F	p	F	p	F	p	F	p						
Disturbance	1	0.10	0.77	0.01	0.91	6.93	0.06	4.81	0.09	0.25	0.64						
Site	4	3.12	0.06	3.83	0.03	1.48	0.27	4.80	0.02	0.38	0.82						
Plot	12	No test															
Mixing	1	2.44	0.19	0.66	0.46	0.09	0.78	0.01	0.94	0.61	0.48						
Disturbance*Mixing	1	2.28	0.21	0.33	0.59	0.20	0.68	1.43	0.30	0.37	0.58						
Site*Mixing	4	0.34	0.85	0.72	0.59	0.98	0.45	3.11	0.06	0.49	0.74						
Plot*Mixing	12	No test															
Error	0																
Total	35																

Appendix V. F-ratios and p values from analysis of variance for chemical properties of B horizon soils at the end of the second growing season (results of multiple comparisons not shown). Zn values were transformed by square root ( $\sqrt{x}$ ), Ca values were transformed by  $\text{Log}_{10}(x)$ , and Mg and Na values were transformed by  $1/(x)$ .

Source	df	<u>Ca*</u>		<u>K</u>		<u>Mg*</u>		<u>Na*</u>		<u>Al</u>		<u>Cu</u>		<u>Fe</u>		<u>Zn*</u>	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Disturbance	1	0.01	0.92	76.06	< 0.01	0.08	0.79	0.22	0.67	15.65	0.02	8.02	0.05	1.85	0.24	0.36	0.58
Site	4	13.84	0.00	0.23	0.91	0.80	0.55	0.66	0.63	0.24	0.91	8.79	< 0.01	3.54	0.04	4.45	0.02
Plot	12	No test															
Mixing	1	0.32	0.60	1.19	0.34	0.31	0.61	7.11	0.06	0.77	0.43	0.15	0.71	0.07	0.81	0.08	0.79
Disturbance*Mixing	1	0.64	0.47	0.41	0.55	< 0.01	0.95	0.04	0.84	4.05	0.11	0.26	0.64	0.48	0.53	0.55	0.50
Site*Mixing	4	2.22	0.13	1.63	0.23	3.27	0.05	0.33	0.85	0.83	0.53	0.41	0.79	3.38	0.05	2.62	0.09
Plot*Mixing	12	No test															
Error	0																
Total	35																
Source	df	<u>PO<sub>4</sub></u>		<u>NH<sub>3</sub></u>		<u>Total-N</u>		<u>pH</u>		<u>Phenols</u>							
		F	p	F	p	F	p	F	p	F	p						
Disturbance	1	0.09	0.78	0.02	0.91	8.69	0.04	25.92	0.01	1.54	0.28						
Site	4	1.13	0.39	1.03	0.43	1.88	0.18	0.44	0.78	1.31	0.32						
Plot	12	No test															
Mixing	1	0.51	0.51	0.06	0.82	0.54	0.50	7.29	0.05	3.85	0.12						
Disturbance*Mixing	1	0.62	0.48	0.35	0.59	0.49	0.52	2.42	0.19	< 0.01	0.99						
Site*Mixing	4	0.77	0.57	1.95	0.17	1.71	0.21	0.19	0.94	0.30	0.87						
Plot*Mixing	12	No test															
Error	0																
Total	35																

## Chapter 4

### General Discussion

The results of my study have several implications for understanding regeneration failure of black spruce in *Kalmia* dominated sites. First, as in previous studies, I found that black spruce growth is likely limited due to low nutrient availability (Damman 1971; Bradley *et al.* 1997b). Increases in seedling growth and foliar nutrient concentrations occurred on insect defoliated sites where decomposition was increased. However, I also found that factors other than nutrient availability are likely important in determining the growth of black spruce on these sites. In sites originating from burning, forest floor mixing increased decomposition and foliar nutrient concentrations, but not seedling growth. Also, regression analysis of seedling growth as a function of foliar N concentration suggested that much of the variability in seedling growth was not explained by nutrient availability. One possible explanation is that partial canopy cover is an important factor. Partial canopy may ameliorate extremes of soil temperature, soil moisture, and near ground microclimate; increase decomposition rate; and reduce light levels to a range that is optimal for black spruce growth. If so, the presence of canopy cover is an under-appreciated effect on regeneration of black spruce in *Kalmia* dominated sites, which should be considered in plans for restoring black spruce to these sites. Secondly, in contrast to previous studies (Inderjit and Mallik 1996b), I found both high total phenols and high seedling growth rates in the forest floor in insect defoliated sites. This suggests that, in this case, phenols did not result in nutrient imbalance or growth inhibition of black spruce seedlings. The potential allelopathic effects of phenols from *Kalmia* leachates on soil nutrients and growth of black spruce are likely complicated by other soil properties and processes under field conditions. Lastly, I found that seedlings inoculated with *P. involutus*



had high EM formation (%), but did not have greater growth than uninoculated control seedlings. If growth inhibition of black spruce seedlings is related to mycorrhization, it is likely not only due to an allelopathic effect of *Kalmia* leachates on levels of EM formation as proposed by Yamasaki *et al.* (1998). Rather, it may be related to physiological responses of specific EM isolates to conditions in *Kalmia* soils.

My results suggested that tolerance of nutrient stress may be an important determinant of the structure and dynamics of black spruce-*Kalmia* communities (Grime 1979; Campbell and Grime 1992). I found that forest floor mixing can improve below ground conditions (increased decomposition, nutrient availability, pH) and reduce competition in burned sites, and yet not improve seedling growth. This suggests that black spruce may be limited on some sites by above-ground conditions, whereas *Kalmia* may be able to tolerate these conditions. This supports the proposal that *Kalmia* dominated heath that establishes after fires of low duff consumption can initiate retrogressive succession (Bloom and Mallik, 2004). *Kalmia* may maintain dominance by tolerating stressful above-ground conditions, making it unlikely that other plant species will become established and change the prevailing conditions.

In contrast, Bradley *et al.* (1997b) suggested that the ability of *Kalmia* and black spruce to acquire soil N influences the structure and dynamics of black spruce-*Kalmia* communities. These authors reported that *Kalmia* and black spruce have different strategies for competing for N. They proposed that black spruce may rely on active nutrient cycling to obtain mineral N, and stimulate nutrient cycling with root exudates containing C. In contrast, *Kalmia* may not require active nutrient cycling because it can access organic N through ericoid mycorrhizae (Leake and Read 1989). Therefore, in *Kalmia* soils with low rates of

nutrient cycling and available N, *Kalmia* is a better competitor than black spruce. My study suggested that while competition for soil N is important, it does not fully explain the structure and dynamics of black spruce-*Kalmia* communities. Increases in nutrient cycling and uptake by black spruce can improve their growth, as occurred in insect defoliated sites. However, in burned sites with open canopies, stimulating nutrient cycling and uptake by forest floor mixing did not result in improved growth of black spruce seedlings even in the absence of competition from *Kalmia*. Growth of black spruce may also have been limited by stressful above ground conditions and tolerance of these conditions may play a role in determining structure and function of black spruce-*Kalmia* communities.

#### *Management implications*

In areas where forest harvesting occurs, it may be possible to address the problem of regeneration failure of black spruce by changing from clear-cutting to retention or shelterbelt harvesting systems on sites that are of poor quality. Van Nostrand (1971) reported that strip-cutting in black spruce forests resulted in adequate control of *Kalmia* and improved natural regeneration of black spruce. My study suggests that, when partial canopy cover is retained, sites with poor regeneration could be reforested with black spruce by mixing the forest floor and planting seedlings grown at low nutrient levels.

Within protected areas, in places where high-severity prescribed burning is not possible, improving black spruce regeneration by mixing the forest floor and planting seedlings tolerant of nutrient stress would be successful in sites with partial shade such as insect defoliated sites. In burned sites, near ground microclimate and light levels would likely limit seedling growth in the absence of cover. Further research is needed to examine if it is possible to promote reforestation of burned sites with black spruce by mixing the forest floor, planting

seedlings that are stress tolerant, and planting species such as alder or red maple for cover and nitrogen fixation.

#### *Research directions*

In *Kalmia* dominated sites, relationships between canopy cover, near ground microclimate and light levels, and growth of black spruce are understudied. The effects of canopy cover (in different disturbance types) and forest floor mixing on soil temperature, moisture, and mineralization rates remain unclear. This could be addressed by studying extremes and means of soil temperature and moisture on a daily, weekly, or growing season basis. Further research in this area might clarify the mechanisms responsible for improved growth of black spruce seedlings due to partial cover and forest floor mixing, and the role of above ground effects in failure of black spruce regeneration.

The range of growth responses of black spruce to inoculation with different EM species and isolates under field conditions on *Kalmia* dominated sites is unknown. This research would help to identify the range of variability of physiological responses of EM to *Kalmia* dominated sites, and whether some EM enhance black spruce growth. Future research should include identification of EM on seedlings to species before outplanting and when growth response measurements are made. Also, uninoculated control seedlings should be used to identify positive (mutualistic) and negative (parasitic) growth responses, although for this to work a method of discouraging volunteer EM is needed. Subsequently, soil analyses should be carried out to identify which soil variables are related to variability in growth response of black spruce with different EM.

Lastly, the effect of growing black spruce seedlings to develop tolerance to nutrient stress for outplanting on burned *Kalmia* dominated sites is unknown. It would be beneficial to conduct this experiment using seedlings raised in the

greenhouse under several different levels of nutrient stress, using measures of physiological stress tolerance (Mohammed *et al.* 1997).

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