Black spruce (*Picea mariana*) regeneration in post-fire cryptogamic mats

By

Imrul Kayes

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Abstract

Post-fire black spruce (*Picea mariana* Mill.) regeneration has received considerable attention in management of the boreal forest of eastern Canada. Seedbed quality is a key factor for seed germination and early seedling establishment. The objective of this research was to investigate the potential role of cryptogamic seedbeds in black spruce germination and seedling establishment. To compare lichen-dominated seedbeds with thin and thick organic matter, *in situ* seeding experiments were conducted at three sites in Terra Nova National Park, Newfoundland burned 11 (Rocky Pond, RP), 17 (Spracklin Road, SR) and 37 (Terra Nova Road, TNR) years ago. At each site, three manipulation treatments (mat-intact, mat-mixed and mat-removed) were applied to the seedbed with four replications in plots of 30 x 30 cm where 200 black spruce seeds were broadcasted. To compare lichen vs. moss seedbed, the same experimental design was extended to moss dominated seedbeds at the SR site. A laboratory germination bioassay was conducted to test the possible chemical effects of four cryptogamic species on black spruce germination and primary growth. Seed germination was highest in mat-intact and mat-removal plots with thick OM at RP, mat-mixed plots with thin OM at SR and mat-mixed plots with both thin and thick OM at TNR. There was significantly higher germination and seedling establishment on moss seedbeds than lichen seedbeds. In moss seedbeds, mat-mixed plots with thick OM had the highest germination but mat-intact plots of both thin and thick OM had higher seedling establishment. Moss seedbeds had higher soil moisture and lower surface temperature than lichen seedbeds. Laboratory bioassay with lichen *Cladonia cristatella* Tuck. had significantly lower germination and seedling growth than control but no difference between the control and moss treatments. HPLC analysis of *C. cristatella* indicated the presence of a germination inhibiting allelochemical, usnic acid. Lichen mat provides physical barrier to seed germination and seedling growth in late post-fire site TNR but not in early post-fire sites. It appears that moss dominated seedbeds facilitate black spruce regeneration by increasing seedbed moisture retention and maintaining low surface temperature, while lichen seedbeds inhibit seedling regeneration by their adverse physical and chemical effects. Cryptogamic seedbed type, OM thickness, soil moisture and temperature are limiting factors in poor black spruce seedling regeneration after fire.

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1. Introduction

The role of disturbance-driven modified seedbeds on seedling regeneration has been a fundamental focus in ecology of disturbed ecosystems. In the boreal forests of Canada and northern Europe, wildfire is a predominant disturbance agent that modifies abiotic and biotic conditions of post-fire seedbeds (Steijlen et al. 1995, Mallik 2003). Wildfires leave an ash or charcoal layer and an unburned or partially burned residual organic matter layer (hereafter referred to as OM) depending on fire severity. The thickness of unburned or partially burned residual OM depends on fire severity (Siegwart Collier and Mallik 2010).

Fire severity had a strong positive effect on conifer seedling recruitment after fire and growth of transplanted seedlings in severely burned sites was higher than that of low severity burn sites (Siegwart Collier and Mallik 2010). Seedling mortality of small seeded plants like black spruce was higher in lightly burned soil in the boreal forest of Alaska (Johnstone and Chapin III 2006). However, Siegwart Collier and Mallik (2010) reported that naturally regenerated black spruce seedling density was higher in thin OM than thick OM in post-fire sites and they found that $\leq$ 2cm OM thickness in post-fire seedbed is suitable spruce regeneration. Kravchenko (2014) found OM thickness $\leq$ 5cm was suitable for natural regeneration of black spruce in post-fire sites in the boreal forest of Newfoundland.

In the absence of high severity natural fires, dominant understory plants such as ericaceous shrubs, mosses and lichens become dominant drivers affecting conifer regeneration by influencing physical and chemical characteristics of seedbed and belowground processes such as OM decomposition and nutrient cycling (Sedia and

Ehrenfeld 2005, 2006, Lavoie et al. 2007, Thiffault et al. 2013). They eventually steer forest succession, even to the point that succession turns into a retrogressive mode (Mallik 1995, Nilsson and Wardle 2005, Hyppönen et al. 2013). Usually, lichens recover very slowly after fire in a severe burn compared to a low severity burn (Lutz 1953). Slow lichen recovery rates were reported from the Seward Peninsula of Alaska, while rapid recovery rates have been reported from the open lichen woodlands of Newfoundland (Racine 1981), where the climate is warmer than in Alaska. Cryptogamic species form a carpet or mat-like structure on top of post-fire seedbeds extending a few centimetres from the surface (Belnap et al. 2001, Belnap and Lange 2003, Belnap 2006). Accordingly, cryptogamic species influence seedbed conditions by controlling its physical and chemical characteristics and may play a major role by interfering or facilitating conifer seed germination and seedling establishment (Steijlen et al. 1995, Zackrisson et al. 1997, Sedia and Ehrenfeld 2003, Lavoie et al. 2007).

The physical presence of cryptogamic mats as seedbeds may act as a barrier to roots making ground contact (Brodo et al. 2001). Rhizinae of mosses and lichens excrete extracellular polysaccharides that can aggregate surface particles into a single layer that may act as a physical barrier to roots penetrating mineral soils (Belnap and Lange 2003,Johansen 1993). Mosses and lichens can alter seedbed substrate characteristics such as temperature and moisture regimes, and thereby influence seed germination and seedling growth (Belnap and Weber 2013). However, it is not clear how lichens and mosses affect substrate temperature and moisture regimes in post-fire seedbeds (Belnap et al. 2001). These authors showed both positive and negative correlations between soil moisture and cryptogamic cover. Warren (2001) argued that the effects of cryptogamic

plants on substrates moisture are site-specific and dependent on their species composition. It is unknown how a cryptogamic mat interacts with varying OM thickness of the seedbed.

Mosses can both facilitate and inhibit vascular plant establishment through a variety of mechanisms (During and van Tooren 1990, Steijlen et al. 1995). Moss mats increase soil moisture (Zackrisson et al. 1997), intercept and sequester nutrients from throughfall and litter decomposition(Chapin III et al. 1987), and supply nutrients from their senescing tissues to ectomycorrhizal hyphae (Carleton and Read 1991). Wheeler et al. (2011) showed that feather-moss seedbeds facilitate seedling regeneration in boreal forests and argued that seedbed composition may influence microhabitats, nutrients, physical structures and predation, and thereby, affect conifer regeneration. In the boreal forest, seedbed-seedling competition dominates in such a way that it can control conifer recruitment. The physical structure of moss from genus *Pleurozium* likely protects one-to three-year old seedlings from temperature extremes in the tundra ecotones, and helps in fixing atmospheric nitrogen (Steijlen et al. 1995). Similar results are reported from Swedish boreal forests where feather-mosses added nitrogen to soil in old growth forests in the absence of natural fire (Zackrisson et al. 2004, Lagerström et al. 2007). In contrast, Zackrisson et al. (1997) found that aboveground removal of feather-moss from the seedbed enhances Scots pine (*Pinus sylvestris* L.) germination and growth.

Lichens also have both positive and negative effects on tree seedling establishment and growth (During and van Tooren 1990). They produce secondary compounds, including xanthones, pulvinic acid derivatives, usnic acids, aliphatic acid, and terpinoids. Approximately 500 secondary compounds have been reported from

lichens (Brodo et al. 2001). Most of these compounds are weak phenolic acids, which are produced by the fungal partners of the lichens and accumulate on the outer walls of fungal hyphae (Lawrey 1986). Evidence suggests that these chemical compounds inhibit higher plants by interfering with seed germination and seedling establishment; as such, they are allelochemicals in the broadest sense of the term (During and van Tooren 1990, Steijlen et al. 1995, Zackrisson et al. 1997, Zamfir 2003, Sedia and Ehrenfeld 2003, Hawkes and Menges 2003). Fisher (1979) first demonstrated inhibition of *Pinus banksiana* Lamb., and *Picea glauca* Voss. germination when exposed to lichen mulch. Sedia and Ehrenfeld (2003) reported that tissue extracts of lichens strongly inhibited germination of vascular plants in post-fire succession. Stark and Hyvärinen (2003) argued that the hypothesis of lichen allelopathy against higher plants should be tested in a system where there are soil-mediated and direct effects of lichen secondary metabolites on regeneration of higher plants. Hawkes and Menges (2003) suggested testing a wide spectrum of environmental conditions in field studies on the allelopathic effect of lichens, as external stress factors such as drought or nutrients could reduce the effect of lichen mats in suppressing vascular plant germination. It is hard to separate environmental stresses and allelopathy in field condition (Inderjit and Weston 2000). Certain lichen secondary compounds have antibiotic properties and can inhibit the growth of soil fungi (Brodo et al. 2001), thereby reducing OM decomposition. Seedling growth inhibition has also been attributed to reduced mycorrhizal symbiosis due to lichen antibiotic functions (Fisher 1979, Hobbs 1985). Thick mats of lichen may also physically prevent seeds from reaching the ground or may prevent seedling growth (Zamfir 2003). Discontinuous cover of certain lichen morphotypes (crustose, squamulose) allows water, gases, and seedlings

to pass through the soil surface, whereas mosses and lichens with a more continuous cover (foliose, fruticose) often block the flow of materials to the seedbed sub-surface (Rosentreter et al. 2007).

In the boreal forest of Newfoundland, low-severity fires leave partially burned and unburned thick OM, which may amplify seedbed temperature and moisture fluctuations during summer. This residual OM may also contain moss and lichen fragments that allow rapid lichen and moss recovery after fire (Racine 1981). At the same time, pioneer crustose lichens colonize unburned OM surfaces eventually to be replaced by or associated with foliose and fruticose lichens (Bloom and Mallik 2004, 2006). As a result, early post-fire seedbeds are dominated by crustose lichen mat while late post-fire lichen seedbeds are dominated by foliose and fruticose lichen mat (Power 2000). In black spruce-*Kalmia angustifolia* L. (hereafter referred to as Kalmia) communities in eastern Canada, thick OM acts as an abiotic filter favouring Kalmia vegetative regeneration during post-fire community assembly (Siegwart Collier and Mallik 2010, Mallik et al. 2010). Mosses and lichens create unique seedbeds by altering biophysical properties, potentially interfering with post-fire seed germination and seedling establishment of vascular plants (Eckert et al.1986, Belnap et al. 2001, Belnap and Lange 2003). Thus the potential role of lichen and moss in seedling regeneration may vary depending on OM thickness and time since fire. Lichens in this system may create a physical barrier to black spruce seed germination or impose allelopathic effects on seeds and germinants, while mosses may play either a facilitative or an inhibitory role. Almost all studies in black spruce-Kalmia ecosystem highlighted Kalmia effects on post-fire regeneration with little information on the potential role of seedbeds dominated by cryptogamic mats. It is

possible that potential mechanisms of spruce regeneration failure in post-fire seedbeds in eastern Canada are mechanical obstruction, an unsuitable microenvironment in cryptogamic seedbeds (i.e. moisture and temperature regimes) and allelopathy.

The objective of this research was to investigate the potential role of cryptogamic seedbeds in black spruce seed germination and seedling establishment. This research addresses three major questions i) Do mechanical mixing of cryptogamic mats with OM and complete removal of lichen or moss mat help black spruce germination and seedling establishment in post-fire cryptogamic seedbeds? ii) How do cryptogamic seedbed type and OM thickness affect black spruce regeneration? iii) Does cryptogamic allelopathy play a role in black spruce regeneration? It was hypothesised that i) if cryptogamic mats pose a physical barrier to black spruce germination and seedling establishment, then mat-mixed and removal treatments will result in higher germination and seedling establishment than mat-intact plots regardless of OM thickness; ii) moss-dominated seedbeds support higher germination and seedling establishment than lichen-dominated seedbeds, by retaining high moisture and maintaining low surface temperatures; and iii) lichens produce higher total phenol concentration than mosses, inducing chemical inhibition, and consequently spruce germination and early growth will be greater in moss seedbeds than lichen seedbeds.

2. Materials and Methods

2.1 Study area

The study was conducted in the greater Terra Nova National Park (TNNP) ecosystem, located in the east-central and north shore eco-regions of Newfoundland, Canada (48°33' N latitude, 53°58' W longitude). Climatic conditions are both continental and maritime, due to prevailing westerly winds and close proximity to the Atlantic Ocean. Summers are brief and cool with regional mean summer temperatures averaging 12.5 °C and mean annual precipitation ranging from 1000-1300 mm (Power 2000, 2005). TNNP is nearly 80% forested and stands are similar to those of the mainland boreal forests of Canada. Forests are dominated by black spruce and balsam fir (*Abies balsamea* (L.) Mill.) mixed with white birch (*Betula papyrifera* Marsh.), trembling aspen (*Populus tremuloides* Michx.) and, to a lesser extent, larch (*Larix laricina* (du Roi) Koch) and red maple (*Acer rubrum* L.). Black spruce communities are among the most common in the Terra Nova region, occupying 52% of total forest cover. The dominant sub-canopy species associated with black spruce forests and barrens is Kalmia and black spruce-Kalmia associations occupy nearly 50 % of the land base in TNNP (Siegwart Collier and Mallik 2010). Other ericaceous shrubs such as Labrador tea (*Rhododendron groenlandicum* (Oeder) K.A. Kron and W.S. Judd), Rhododendron (*R. canadense* (L.) Torr.) and low-bush blueberry (*Vaccinium angustifolium* Aiton) are also strongly associated with these forest types (Power 2000). Pre-fire forest floor is composed of lichens (mainly *Cladonia* and *Cladina*), mosses (mainly Acrocarpous and Pleurocarpous), accumulated leaf litter and organic matter (Power 2000, 2005).

Evidence of burning from peat and lake sediment deposits and the regular occurrence of charcoal macrofossils in organic soil horizons (Power 2000) indicates a long history of fire in shaping the Terra Nova landscape. High-severity fire has historically facilitated black spruce recovery, producing even-aged stands throughout the region (Bloom and Mallik 2006). In recent years, improved fire detection and fire suppression have reduced the frequency and severity of stand replacing fires resulting in an increase in Kalmia abundance (Power, 2000).

2.2 Site selection

Three post-fire sites, Rocky Pond (RP), Spracklin Road (SR) and Terra Nova Road (TNR), burned respectively 11, 17 and 37 years ago, were selected to include a range of post-fire sites with varying fire history, organic matter thickness and cryptogamic seedbed types in conducting the *in situ* experiments (Table 1). Early and late post-fire sites dominant lichens were respectively *Cladonia cristatella* Tuck. and *Cladina stellaris,* whereas, early-and late post-fire mosses were *Polytrichum juniperinnum* and *Pleurozium schriberi* (Brid.) Mitt. (Bloom and Mallik 2006).

Table 1. General description of post-fire sites in Rocky Pond (RP), Spracklin Road (SR) and Terra Nova Road (TNR) to investigate the role of post-fire cryptogamic mats on black spruce germination and seedling establishment in an *in situ* seedbed manipulation experiment in Terra Nova National Park (TNNP), Newfoundland (modified from Kravchenko, 2012).

Site	Location	GPS coordinates	Time since fire (yrs)	Burn area (ha)	Mean OM depth ± se (cm)	OM depth range (cm)
1	Rocky Pond (RP)	N48°31'662" W53°58'967"	11	85	4.0 ± 0.5	0-20
2	Spracklin Road (SR)	N48°31'953" W54°03'091"	17	75	6.1 ± 0.7	0-10
3	Terra Nova Road (TNR)	N48°30'487" W54°07'143"	37	313	3.9 ± 0.5	0-20

2.3. Field experiments

2.3.1 Effects of OM thickness and seedbed manipulations on seed germination and seedling establishment in post-fire sites

A stratified random sampling was followed in each of the three sites for the *in situ* seeding experiment on lichen dominated seedbeds. In this study, OM thickness category (thin and thick) and seedbed manipulations treatments (intact, mixed and removed) were the two explanatory variables. Because OM thickness of a particular microsite was unknown, 12 plots with thin (0- 5cm) and 12 plots with thick (> 5cm) OM in lichen dominated seedbeds were achieved as follows. First, the locations of lichen dominated seedbeds were visually identified and marked in a reconnaissance survey by walking through a 1 ha area of the site. If a microsite met the requirement of thin (0 – 5 cm) or

thick (> 5 cm) OM depth, then it was selected for lichen cover estimation and subsequent seedbed manipulation treatment. Lichen cover was estimated by using a 50 x 50 cm quadrat placed in the middle of the plot (microsite). Generally, four seedbed components (lichen, moss, exposed duff and Kalmia litter) were found in a particular microsite (Table 2). This initial reconnaissance was followed by measuring OM depth in the microsite by taking three soil cores from around the 30 x 30 cm treatment plot that was kept undisturbed in the middle at this stage. A 40 cm long, 5.5 cm diameter Eijkelkamp split tube sampler (Hoskin Scientific, Burlington, Ontario) was used to take the soil cores. OM thickness was recorded in the field as the depth of organic matter from the surface to the most recent charcoal layer in the core (Table 3). In addition, lichen mat thickness was recorded at three places from a 10 x 10 cm sample from each micro-sites to detect any difference in seedbed substrate thickness in the three sites. These samples were brought to the laboratory and oven dried to determine their dry mass for quantifying mat bulk density.

Table 2. Mean ± standard error (se) of seedbed components cover (%) in lichen dominated seedbeds of three post-fire study sites (24 samples in each site), Rocky Pond (RP), Spracklin Road (SR) and Terra Nova Road (TNR) in TNNP.

Site	Lichen	Moss	Exposed duff	Kalmia litter
RP	79.1 ±1.9	5.3 ±2.1	8.4 ±1.1	7.5 ± 0.8
SR	87.6 ±1.1	2.9 ± 0.9	3.7 ± 0.6	6.6 ± 0.8
TNR	97.8 ± 0.6	4.2 ±1.2	0.1 ± 0.7	5.5 ± 0.6

Three seedbed manipulation treatments were applied randomly in the thin and thick seedbed types. First, any above ground vegetation was cut from an area of 50 x 50

cm using a pruner. In the middle of the plot, a 30 x 30 cm experimental plot was established, leaving 20 cm buffer around it to remove the edge effects from the uncut vegetation. The three seedbed manipulation treatments were i) mat-intact (control), ii) mat-mixed and iii) mat-removed. The mat-mixed treatment was applied by mechanically mixing the lichen mat with OM. The mat-removed treatment was applied by complete removal of the lichen mat from the top 1 cm OM, leaving the rest of the OM intact to maintain integrity of OM substrates and to mimic an immediate post-fire condition where lichen mat was absent. Seedbed manipulation treatments were replicated four times within each OM thickness (3 treatments x 2 OM thickness x 4 replicates = 24 experimental plots in each site). One hundred black spruce seeds were broadcasted in each 30 × 30 cm plot on July 2, 2014. All the experimental plots were protected from potential grazing or browsing (by rabbits and small mammals) by erecting fence made of wire mesh. Since no germination was recorded in any plot by July 23, 2014 each plot was reseeded with an additional 100 black spruce seeds on July 24, 2014.The treatment plots were examined for germination three times in the growing season (August 6, 15, and 24, 2014). Percent germination was quantified from the data collected in 2014 and the number of established seedlings was counted in those plots on July 1, 2015.

Black spruce seeds used in this study were obtained from Wooddale Provincial Tree Nursery, Grand Falls, Newfoundland. Seed viability was tested by placing 10 seeds in a Petri-dish on distilled water moist Whatman filter paper 1. This experiment was replicated five times. All seeds germinated in all five Petri-dish after 15 days showing 100 % viability.

Table 3. Mean ± se and range of estimated organic matter (OM) thicknesses of selected microsites for *in situ* seeding experiment on thin (0-5 cm) and thick (> 5 cm) OM in three post-fire sites, RP, SR and TNR in TNNP.

Site	Thin OM (cm)		Thick OM (cm)	
	Mean ± se	Thickness range	Mean ± se	Thickness range
RP	2.5 ± 0.3	1.5 - 4.8	6.3 ± 0.2	5.2 - 7.5
SP	2.7 ± 0.2	1.5 - 4.0	7.0 ± 0.4	6.0 - 10.0
TNR	2.4 ± 0.1	2.0 - 3.5	7.0 ± 0.2	6.0 - 8.5

2.3.2 Germination and seedling establishment among seedbed types

In the SR site, an additional 24 experimental plots were established in moss-dominated seedbed. Selection of lichen and moss dominated microsites, seedbed manipulation treatments and seeding methods were the same as mentioned above. The purpose of this experiment was to investigate how moss seedbeds differ from lichen seedbeds in black spruce germination and seedling establishment. To determine moisture and surface temperature difference between lichen and moss seedbeds, eight soil moisture and temperature probes (5TM, Decagon scientific, USA) were installed in the buffer area of two lichen and two moss dominated plots. In each plot, one probe was placed horizontally at 1 cm and a second at 5 cm depth on July 24, 2014. The probes were connected to a data logger to acquire data at 30 min intervals for one month (until August 24, 2014). Five OM samples were collected from each of lichen (*Cladina stellaris* Opiz.) and moss (*Polytrichum juniperinum* Hedw.) micro-sites using a ring soil sampler (height and diameter 5 cm) to measure bulk density. Bulk density (mg/cm^3) of OM samples were estimated by total oven dried weight (mg)/volume (cm^3).

2.4 Laboratory bioassay

A laboratory bioassay was designed to investigate cryptogam allelopathic effects on black spruce germination and primary growth. Macerated tissues of lichens and mosses were used for this bioassay. Ten grams fresh weight of lichens *Cladonia cristatella* and *C. stellaris*) and mosses (*Polytrichum juniperinum* and *Pleurozium schreberi*) were macerated separately in a Magic Bullet blender at short interval low speed and placed on a Petri-dish with 10 ml deionized distilled water. The reason for maceration was to remove air spaces between live moss and lichen in the Petri-dish. A layer of Whatman no. 1 filter paper was placed above the plant material. A reference treatment was prepared by placing a soft cotton layer in a Petri-dish on which a layer of Whatman no. 1 filter paper was placed. Black spruce seeds (obtained from the Wooddale Provincial Tree Nursery, Newfoundland) were surface sterilized with 30% hydrogen per oxide for 10 minutes followed by washing with distilled water (Bajaj 2012). Ten black spruce seeds were randomly placed on the filter paper as was done in the treatment plates. Each treatment was replicated five times. Subsequently, 5 ml of water was added to each dish twice during the experimental period to ensure sufficient moisture. The experiment was continued at 20 $^{\circ}$C for 15 days. The Petri-dishes were kept under florescent lights with a 12 hour day and night cycle during the experiment. The number of germinates was counted after 5 days and counting continued until the end of the experiment when root and shoot length of the seedlings were measured for each Petri-dish. Substrate pH in the Perti-dishes was measured at the end of the germination bioassay.

2.5 Measurement of total phenols

2.5.1 Methanol extraction of cryptogamic samples

Cladonia cristatella was collected from RP*, C. stellaris* and *Pleurozium schreberi* was collected from TNR and *Polytrichum juniperinum* was collected from SR. The samples were transferred to the Biorefining Research Institute (BRI) laboratory, Lakehead University. The samples were kept in an air-dried condition at room temperature and foreign debris on the thalli was removed. Samples were ground into a powder with liquid nitrogen using a ceramic mortar and pestle and were then sieved through a 500-µm filter. Ten gram ground lichen and moss samples were mixed with 10 ml HPLC grade methanol and kept on rotary shaker at 30^{o}C temperature for 24 hours. The mixed solutions were centrifuged for 10 min at 14000 rpm.

2.5.2 Quantification of total phenols

Concentration of total phenols was determined following Inderjit and Mallik (1996), adapted from the Folin-Ciocalteu method. Gallic acid was used as a standard. Total phenols were expressed as mg/L gallic acid equivalent. Total phenol was estimated using the standard curve equation: y = 0.001x - 0.015, where y is absorbance at 765 nm and x is total phenolic content). Absorbance was obtained as follows: Five ml sample of solution (methanol extracted) was mixed with 35 ml deionized water and 2.5 ml Folin-Ciocalteau reagent, incubated for 8 min at room temperature, followed by adding 7.5 ml sodium carbonate solution then adding deionized water to bring the solution to 50 ml. The resultant solution was incubated for 2 h at room temperature. Absorbance of the sample was measured at 765 nm using a spectrophotometer (Gold S54, Biocotek, China). Total phenol concentration was determined using a standard absorbance curve of gallic

acid, plotted with the absorbance of 50, 100, 250 and 500 mg/L gallic acid standards at 765 nm (Appendix 1). Blanks were produced using deionised water and each sample was measured in triplicate.

2.6 HPLC analysis of lichen and moss secondary compounds

2.6.1 Methanol extraction of samples

Ten grams of ground and sieved samples from two lichens *Cladonia cristatella* and *Cladina stellaris* were mixed with 7 ml HPLC grade methanol and kept on a rotary shaker at 30°C for 24 hours. The mixtures were centrifuged for 10 min at 14000 rpm in a micro-centrifuge and the extract was combined into glass vials. Three ml distilled water was added to each vial to make the sample of methanol: water = 7:3 (total 10 ml).

2.6.2 Acetone extraction of samples

Ten grams of ground lichen samples were mixed with 7 ml acetone and dichloromethane (50:50) mixture and kept on a rotary shaker at 30°C for 24 h. The resultant solution was poured into a 100 ml round bottom flask through Whatman 1 filter paper. The solvent was removed using a rotary evaporator set at 58°C. After drying, the residue was dissolved in 7 ml HPLC grade methanol and then 3 ml distilled water was added to make a 10 ml (methanol **:** water = 7**:**3) solution.

2.6.3 Preparing standard solutions

Standard usnic acid sample was bought from Sigma Aldrich and 0.1 mM stock solution in HPLC grade methanol was prepared and kept in refrigerator until analysis.

2.6.4 HPLC analysis

Lichen compounds were analysed with HPLC method following Thepnuanet et al. (2013). Lichen extracts and standard lichen acids were analysed on a HP 1100 series consisting of a HP G1312A binary pump. Chemical separation was achieved on an Eclipse XDB-C18, 4.6 x 150 mm, and 5µm column. The solvent A was 1% phosphoric acid in water and the solvent B was 100% methanol. The run started on a column pre-equilibrated with 65% A and 35% B at a flow rate 1.0 ml/min. Gradient of solvent B was allowed to increase to 100% over 20 min before sample injection was started, continuing to hold for 5 min at 100% B. At the end of the run, the post time was set to 10 min to re-equilibrate the column before a new run was started. The compounds were detected at three different wavelengths (210, 256 and 280 nm), but only the result at 280 nm is reported. Results of the 210 and 256 nm runs are presented in Appendices 13 and 14 respectively. Identification of compounds was based on retention time (min).

2.7 Statistical analysis

2.7.1 Seedbed substrate difference

Differences of lichen cover (%), mat thickness (cm) and mat bulk density (mg/cm^3) among three sites were tested using Kruskal-Wallis test separately for each of the variable mentioned above. Following this, Tukey-Kramer-Nemenyi post-hoc test for independent samples was carried out. The pairwise multiple comparisons of mean ranks for independent samples were carried out using the function "*posthoc.kruskal.nemenyi.test*" in R-package "PMCMR" (Pohlert 2015). A paired t-test was used to test significant difference of bulk density of OM substrate collected under lichen and moss mats.

2.7.2 Seed germination and seedling establishment

Effects of OM thickness and lichen mat seedbed manipulation treatments on seed germination (Question 1) were investigated by fitting a linear model with percent seed germination as response variable and OM thickness and seedbed manipulation treatments as explanatory variables. Q-Q plots for the residuals are presented in Appendix 2 and estimated coefficients of the models are presented in Appendix 3. Analysis was carried out separately for three post-fire sites. Effects of OM thickness and lichen mat seedbed manipulation treatments on seedling establishment were investigated by fitting a linear model with percent seedling establishment as response variable and OM thickness and seedbed manipulation treatments as explanatory variables for early post-fire site RP and late post-fire site TNR. Normality of residuals and homogeneity of variances of the groups were tested using Shapiro-Wilk and Levene's tests. Because residual distribution was not normal in seedling establishment data of mid post-fire site SR, effects of explanatory variables were investigated by fitting Generalized Linear Model (GLM) with Poisson distributed errors. Since, all the plots of a particular treatment group (mat-mixed with thick OM) had 0-seedling establishment there was an anomalous predicted value in the result. To deal with this issue, seedling establishment data from SR site were transformed by adding a minute value (0.01) to all observations. Q-Q plots for the residuals are presented in Appendix 4 and estimated coefficients of the models are presented in Appendix 5.

Effects of seedbed type, OM thickness and seedbed manipulation treatments on seed germination (Question 2) were tested using a three-way ANOVA model for a full factorial design. Normality of residuals and homogeneity of variances of the groups were

tested using Shapiro-Wilk and Levene's tests. Diagnostic plots for this model are presented in Appendix 6 and estimated coefficients are reported in Appendix 7. Effects of seedbed type, OM thickness and seedbed manipulation treatments on seedling establishment were tested by fitting a GLM model with Poisson distributed error because residuals of linear model were not normally distributed. Diagnostic plots for this model are presented in Appendix 8 and estimated coefficients are reported in Appendix 9. ANOVA tables for all models used were derived using R function "*anova(model, test = "F")*" in R library "car" (Fox and Weisberg 2011). Tukey's HSD post-hoc was used on seed germination and establishment data to carry out pairwise multiple comparisons among treatment groups in three sites separately by using R package "multcomp" (Hothorn et al. 2007).

2.7.3 Seedling survival trend

Seedling survival data were analysed by fitting linear mixed effect model for a repeated measures design. The model was fitted with number of seedlings in each of four counts as response variable and seedbed type, OM thickness, manipulation treatments and counts (contrasts) as fixed factors. Plots were treated as random factors in the model. This analysis was done by using R package "lme4" and "lmerTest" and ANOVA table was derived by using R function "*summary(model)*". Tukey's post-hoc was used on seedling counts by interaction between seedbed type and treatments for multiple comparisons to investigate differences among the counts.

2.7.4 Moisture and temperature data

Effects of cryptogmic seedbed type and substrate depth on seedbed moisture were investigated by fitting a linear mixed effect model for a repeated measures design. The

model was fitted with average volumetric moisture content for 30-day period (25 July 2014 to 24 August 2014) as response variable and cryptogamic seedbed type (lichen and moss) and substrate depth (1 cm and 5 cm) as fixed factors. The moisture probes were treated as random factors to account for the variation in moisture condition in each day throughout the experimental period. Effects of cryptogmic seedbed type and substrate depth on diurnal fluctuation of seedbed temperature were investigated by fitting a linear mixed effect model for a repeated measures design as above. The model was fitted with temperature from 12.00 am to 11.30 pm (30-min interval) as a response variable. A randomly selected day (August 4, 2014) was analysed to investigate diurnal fluctuations. The temperature probes were treated as random factors as above. This analysis was done by using R package "lme4" and "lmerTest" and ANOVA tables were derived by using R function "*summary(model)*".

2.7.5 Laboratory bioassay

Final germination data gathered from the laboratory bioassay was analysed by Kruskal-Wallis non-parametric test because residuals of germination data failed to meet normality assumptions and treatment variance was heterogeneous (Question 3). Root length and shoot length data were analysed using one-way ANOVA. Normality of residuals (Shapiro-Wilk test) and homogeneity (Bartlett's test) of treatment groups were tested for each of the models separately. Tukey's HSD post-hoc was used for multiple comparisons among the treatments. A paired t-test was used to test significant difference of total phenol among the cryptogamic species.

3. Results

3.1 Dominant seedbed among post fire sites

There was significant difference in lichen mat cover among the three post-fire sites according to Kruskal-Wallis global test (Chi-squared = 56.20, df = 2, $p < 0.01$): TNR site had significantly higher lichen cover than RP (t-value = 10.50, $p < 0.01$) and SR (t-value = 5.67, $p < 0.01$) (Fig. 1).

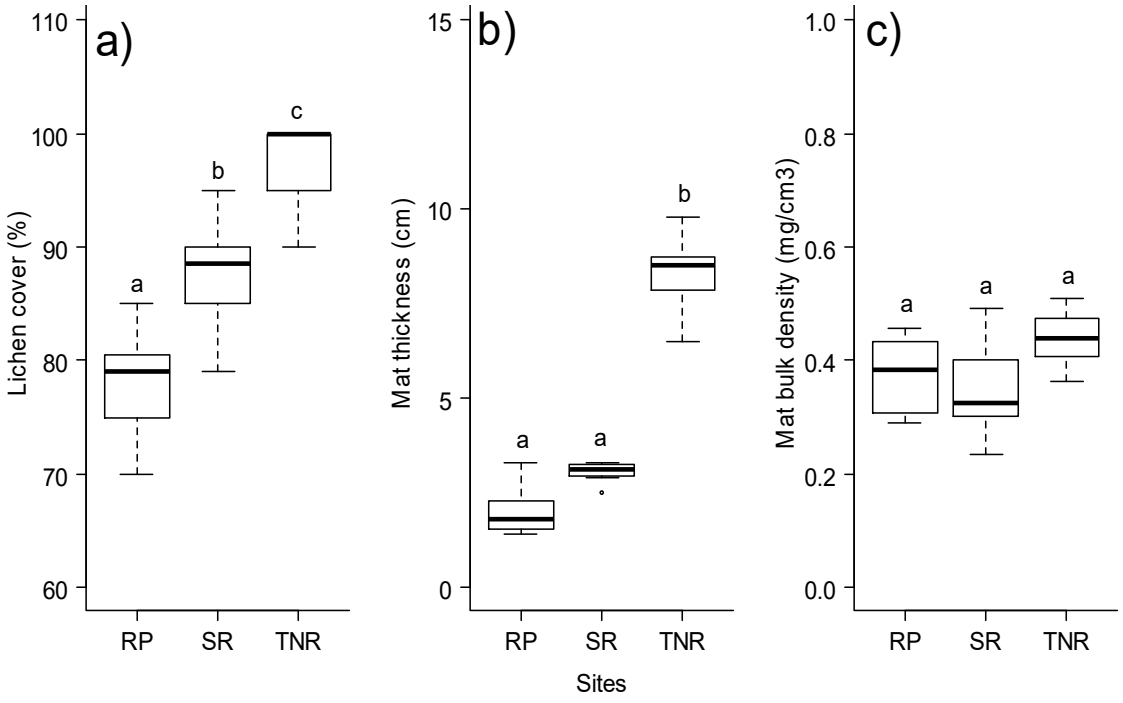

Fig. 1. Boxplots showing a) lichen cover (%), b) mat-thickness (cm) and c) mat bulk density (mg/cm^3) in Rocky Pond (RP), Spracklin Road (SR) and Terra Nova Road (TNR) in Terra Nova National Park (TNNP). Different letter(s) above the error bars denote significant difference of mean lichen cover (%) mat thickness and mat density among them based on Tukey-Kramer-Nemenyi post-hoc test. Homogeneous subsets are not significantly different.

There were significant differences in lichen mat thickness (Kruskal-Wallis chi-squared = 16.23, df = 2, $p < 0.01$), but there were no difference in mat density (Kruskal-Wallis chi-squared = 4.28, df = 2, $p = 0.11$) among the three post-fire sites. Mat thickness was higher in TNR than RP (t-value = 5.73, $p < 0.01$) and SR (t-value = 3.236, $p = 0.04$), but the difference between RP and SR was not significant (Fig. 1).

3.2 Germination and seedling establishment among post fire sites

3.2.1 Germination

Germination was very low in all three sites. Mean (± se) germination at RP, SR and TNR were 8.6 ± 1.22, 10.15 ± 0.88 and 8.54 ± 1.33 percent of seeds, respectively. There was a significant difference in germination between thin and thick OM at RP and SR, but that difference did not occur at TNR. There was significant difference in germination among the seedbed manipulation treatments at all three post-fire sites. Interactions between OM thickness and seedbed manipulation treatments were not significant for germination at any of the three post-fire sites (Table 4).

At RP, the highest germination was recorded in mat-intact plots with thick OM and lowest germination from mat-mixed plots with thin OM. However, the differences were only significant between mat-intact plots with thick OM and mat-mixed plots with thin OM (t-value = –3.99, $p < 0.05$) and between mat-removed plots with thick OM and mat-mixed plots with thin OM (t-value = 3.58, $p < 0.05$; Fig. 2). There was no difference in germination among the treatments in plots with thick and thin OM at RP. Mat-intact and mat-removed plots of thick OM produced respectively 88 and 87 % higher germination than the mat-mixed plots with thin OM at RP.

At SR, the highest seed germination was recorded from mat-mixed plots with thin OM. There was no significant difference in germination among the seedbed manipulation treatments in thick or thin OM plots. However, germination in the mat-mixed treatment with thin OM differed significantly from that in the mat-intact (t-value = 3.69, p = 0.01) and mat-removal (t-value = –4.30, p <0.01) plots with thick OM. The mat-mixed plots with thin OM had 53 % higher seed germination than mat-intact plots and 62 % higher germination than mat-removal plots with thick OM (Fig. 2). There was no significant difference between mat-mixed plots of thin and thick OM at SR.

At TNR, the highest seed germination was recorded in mat-mixed plots with thick OM. There was no difference in germination between mat-mixed plots with thin and thick OM (t-value = –1.59, p = 0.61). There was no difference in germination between mat-intact plots of thin and thick OM (t-value = 1.01, p = 0.91), but mat-mixed plots with both thin and thick OM had significantly higher germination than mat-intact plots with thin and thick OM (Fig. 2). Mat-mixed treatments with thick OM resulted in 94 and 48 % higher germination than mat-intact and mat-removal treatments. Mat-mixed plots with thin OM had 73 % higher germination than mat-intact plots, but there was no difference between mat-mixed and mat removed plots with thin OM at TNR. Germination in mat-mixed plots with thin OM was significantly higher than mat-intact plots with thick OM at TNR.

Table 4. Results of two-way ANOVA comparing black spruce germination in two classes of OM thickness and three seedbed manipulation treatments on lichen dominated seedbeds of three post-fire sites in TNNP, Newfoundland. Significant differences are marked in bold.

Sites	Sources	df	Mean Square	F-value	p-value
(1) Rocky Pond	OM thickness	1	297.51	15.14	**<0.01**
	Treatment	2	76.59	3.89	**0.03**
	OM thickness × Treatment	2	6.64	0.34	0.71
	Error	18	19.65		
(2) Spracklin Road	OM thickness	1	168.01	16.82	**<0.01**
	Treatment	2	37.64	3.77	**0.04**
	OM thickness × Treatment	2	1.32	0.13	0.88
	Error	18	9.99		
(3) Terra Nova Road	OM thickness	1	10.70	0.8	0.38
	Treatment	2	341.00	25.46	**<0.01**
	OM thickness × Treatment	2	24.80	1.85	0.19
	Error	18	13.40		

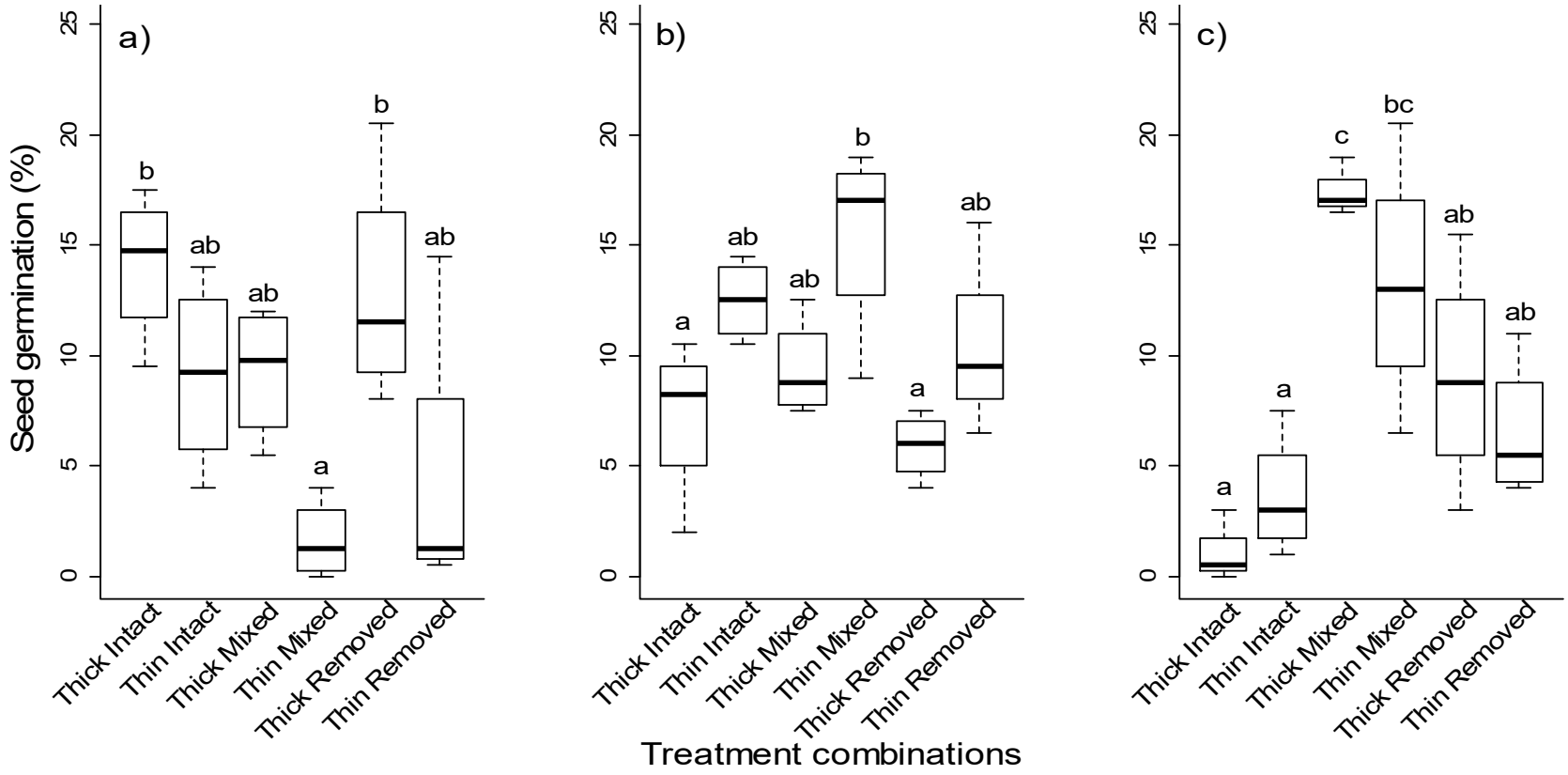

Fig. 2 Boxplots showing black spruce germination on mat-intact, mixed and removal treatments with thick and thin OM in (a) RP, (b) SR and (c) TNR. Different letters above the error bars denote significant differences of mean seed germination (%) among the treatments at $\alpha \leq 0.05$ based on Tukey's HSD post-hoc test. Homogeneous subsets are not significantly different.

3.2.2 Seedling establishment

Seedling establishment was very low at all three post-fire sites (Fig. 3). The effect of OM thickness on seedling establishment was not significant at RP ($F = 3.19$, $p = 0.09$), but it was significant at SR. Seedbed manipulation treatments had a significant effect on seedling establishment at RP and TNR, but not at SR (Table 5).

At RP, the highest number of established seedlings was recorded in mat-intact plots with thin OM, where seedling establishment was significantly higher than mat-mixed ($t = –5.26$, $p < 0.01$) and mat removed plots with thin OM ($t = –5.87$, $p < 0.01$). Seedling establishment in mat-intact plots with thin OM was higher than mat-intact ($t = 3.40$, $p = 0.03$), mat-mixed ($t = –5.57$, $p <0.01$) and mat-removed ($t = –5.26$, $p < 0.01$) plots with thick OM. There was no significant difference in seedling establishment among plots with thick OM (Fig. 3).

At SR, the highest number of established seedling was recorded in mat-intact plots with thin OM. No seedlings established in mat-mixed plots with thick OM. There was no difference in seedling establishment among the control plots with thin or thick OM. However, mat-intact (control), mat-mixed and mat-removed plots with thin OM had significantly higher seedling establishment than plots with thick OM in SR ($p < 0.01$). Mat-intact plots with thick OM had 50% higher seedling establishment than that of the mat-removed plots. Mat-intact plots with thin OM had 79% higher seedling establishment than mat-intact plots with thick OM (Fig. 3).

Table 5. Results of two-way ANOVA comparing black spruce seedling establishment in two classes of OM thickness and three seedbed manipulation treatments on lichen dominated seedbeds in three post-fire sites in TNNP, Newfoundland. Significant differences are marked in bold.

Sites	Sources	df	Mean Square	F-value	p-value
(1) Rocky Pond	OM thickness	1	4.17	3.19	0.09
	Treatment	2	25.04	19.18	**< 0.01**
	OM thickness × Treatment	2	5.79	4.44	**0.03**
	Error	18	1.31		
(2) Spracklin Road	OM thickness	1	75.98	56.04	**< 0.01**
	Treatment	2	1.13	1.13	0.34
	OM thickness × Treatment	2	2.10	1.55	0.23
	Error	18	1.36		
(3) Terra Nova Road	OM thickness	1	30.37	1.94	0.18
	Treatment	2	150.04	9.60	**< 0.01**
	OM thickness × Treatment	2	21.12	1.35	0.28
	Error	18	15.62		

At TNR, the highest number of established seedlings was recorded in mat-mixed plots with thick OM, and the lowest number of seedlings was recorded in mat-intact plots with both thin and thick OM. Mat-mixed and mat removed plots with thick OM had significantly higher seedling establishment than mat-intact plots ($p < 0.01$). There was no difference in seedling establishment between mat-mixed and mat-removed plots with thick OM (Fig. 3). Mat-mixed plots with thick OM had 85 and 96% higher seedling establishment than mat-intact plots with thin and thick OM respectively.

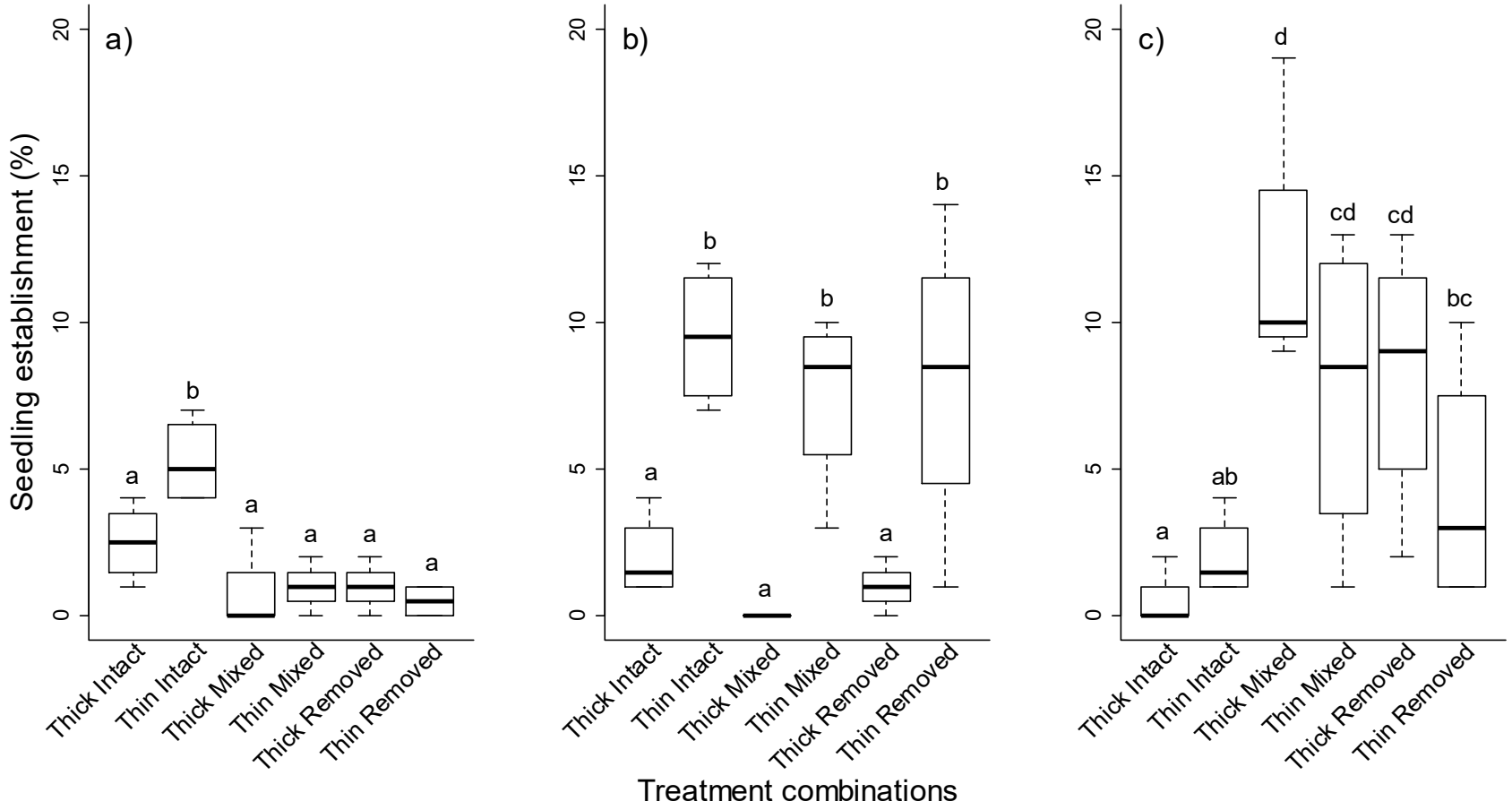

Fig. 3. Boxplots showing seedling establishment on mat-intact, mixed and removed treatments with thin and thick OM in (a) RP, (b) SR and (c) TNR. Different letters above the error bars indicate significant difference of mean seedling establishment (%) at $\alpha \leq 0.05$. Homogeneous subsets are not significantly different based on Tukey's HSD post-hoc test.

3.3 Germination and seedling establishment in lichen and moss seedbeds

3.3.1 Germination

Germination was significantly higher in moss-dominated seedbeds than lichen dominated seedbeds ($F = 8.96$, $p < 0.01$; Table 6). Germination in lichen and moss-dominated seedbed were 10.1 ± 0.88 and 13.8 ± 1.86 % respectively. Overall, germination did not differ significantly between thin and thick OM ($F = 0.004$, $p = 0.94$). However, there was a significant interaction with OM thickness and seedbed type ($F = 20.78$, $p < 0.01$) and with OM thickness and seedbed manipulation treatments ($F = 4.35$, $p = 0.02$). Germination was higher in thin OM than in thick OM on lichen-dominated seedbed. In moss-dominated seedbed, it was higher in thick OM than in thin OM. Seed germination with thin and thick OM for lichen dominated seedbed was 12.8 ± 1.14 and 7.5 ± 0.8 % respectively; for moss dominated seedbed, it was 11.0 ± 1.83 and 16.6 ± 2.87 % respectively.

Seedbed manipulation treatments also differed when seedbed type was considered as a factor in the linear model explaining germination ($F = 20.57$, $p < 0.01$). Again, the interaction between seedbed manipulation treatments and seedbed type was significant (Table 6). Germination was higher in the mat-mixed treatment on lichen dominated seedbed compared to mat-intact and removed treatments; however, these differences were not significant. On the other hand, complete removal of moss produced significantly lower germination compared to moss intact and moss mixed treatments.

Table 6. Results of three-way ANOVA comparing black spruce germination by seedbed type, OM thickness and seedbed manipulation treatments. Residuals of the model were normally distributed according to the Shapiro-Wilk test (W = 0.96, $p = 0.19$) and variances among the groups were homogeneous according to Bartlett's test (K-squared = 11.98 11, $p = 0.36$). Significant differences are marked in bold.

Source	df	Mean Square	F	p-value
Seedbed type	1	154.10	8.96	**< 0.01**
OM thickness	1	0.10	0.01	0.94
Treatment	2	353.95	20.57	**< 0.01**
Seedbed type × OM thickness	1	357.50	20.78	**< 0.01**
Seedbed type × Treatment	2	149.90	8.71	**< 0.01**
OM thickness × Treatment	2	74.95	4.36	**0.02**
Seedbed type × OM thickness × Treatment	2	96.65	5.62	**< 0.01**
Error	36	17.20		

There was no difference in germination among the seedbed manipulation treatments on lichen dominated seedbed, but in the mat-mixed treatment with thin OM there was higher germination than in mat-intact and mat-removed treatments with thick OM (Fig.4). In thick OM on moss dominated seedbed, both mat-intact and mat-mixed treatments supported higher germination than the mat-removed treatment. In thin OM on moss dominated seedbed, the mat-intact treatment resulted in significantly higher germination than the mat-removed treatment, but the difference between mat-mixed and mat-intact treatment was not significant. On moss dominated seedbed, the highest number of seeds germinated in mat-mixed treatments with thick OM.

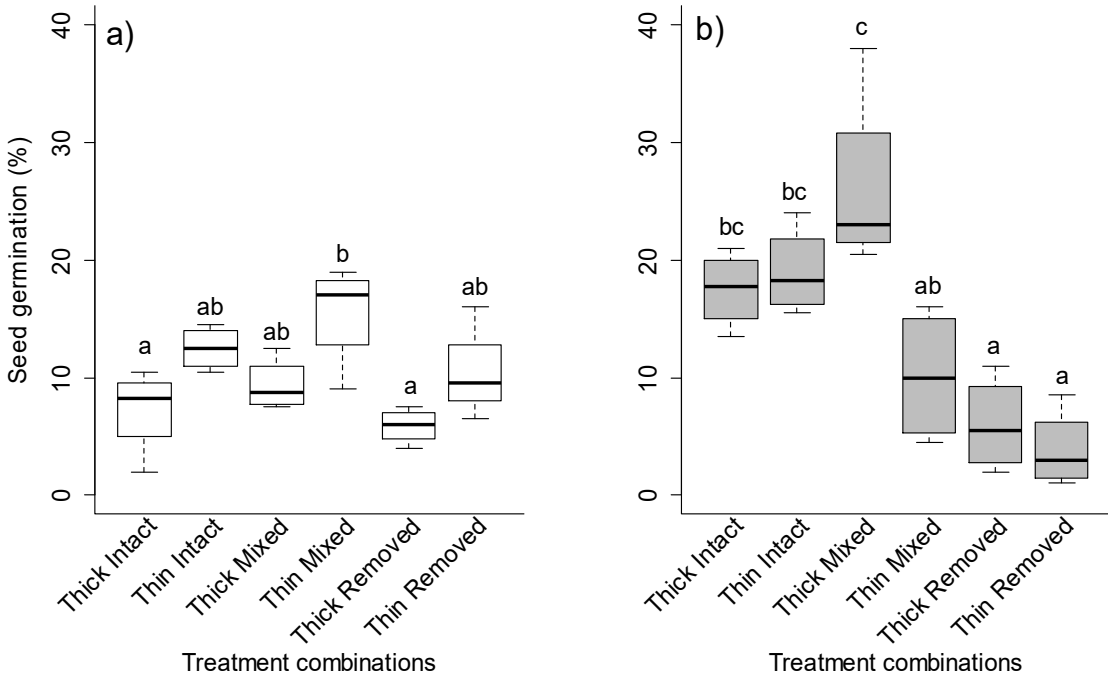

Fig. 4. Boxplots showing black spruce seed germination on mat-intact, mixed and removal treatments with both thin and thick OM on (a) lichen and (b) moss dominated seedbeds. Different letters above the error bars denote significant differences at $\alpha \leq 0.05$ determined by Tukey's HSD post-hoc test.

3.3.2 Seedling establishment on lichen and moss dominated seedbeds

Effects of cryptogamic seedbed type, OM thickness and seedbed manipulation treatments were significant on seedling establishment (Table 7). There was a significant interaction between seedbed type and OM thickness, and seedbed type and treatments, but not between OM thickness and treatments. The effect of seedbed manipulation treatments on seedling establishment was significant ($p < 0.01$). The interaction between treatment effect and seedbed type was significant, but the interaction between OM thickness and treatment was not significant (Table 5).

Table 7. Results of three-way ANOVA comparing black spruce seedling establishment by cryptogamic seedbed type, OM thickness and seedbed manipulation treatments. Significant differences are marked in bold.

Source	df	Mean square	F value	p-value
Seedbed type	1	10.58	6.34	**0.01**
OM thickness	1	7.08	4.25	**0.04**
Treatment	2	32.43	19.44	**< 0.01**
Seedbed type × OM thickness	1	57.87	34.69	**< 0.01**
Seedbed type × Treatment	2	15.24	9.14	**< 0.01**
OM thickness × Treatment	2	3.57	2.14	0.13
Seedbed type ×OM thickness × Treatment	2	10.61	6.36	**< 0.01**
Error	35	1.67		

In lichen dominated seedbeds, the mat-intact plots on thin OM had the highest seedling establishment where 70.37% of total germinated seedlings were established after one year. There was no difference in seedling establishment among the treatments with

thin OM, but mat-intact and mixed plots with thin OM had significantly higher seedling establishment than plots with thick OM on lichen-dominated seedbed (Fig. 5). There was a significant difference between thin (79 % higher seedling establishment) and thick OM in seedling establishment in mat-intact plots. The mat-mixed plots with thin OM had 47 % seedling establishment while no seedling was established in mat-mixed plots with thick OM. There was no difference in seedling establishment in mat-removed plots between thin and thick OM on lichen-dominated seedbeds (Fig. 5).

On moss dominated seedbeds, the highest seedling establishment was recorded in mat-intact plots with thick OM, where 81% of total germinated seedlings were established, a rate 19 % higher than that on mat-intact plots with thin OM. There was no significant difference in mat-intact plots between thin and thick OM in terms of number of seedlings established (Fig. 5). In mat-mixed plots with thin and thick OM, 13 and 41% of total germinated seedlings were established. The mat-removed plots with thin and thick OM had 39 and 67 % of total germinated seedlings. The mat-intact plots with thin OM on moss dominated seedbeds had 19 and 83 % higher established seedlings than those of thick OM on lichen dominated seedbeds. On the other hand, the mat-intact plots with thick OM on moss dominated seedbed had 33 and 87 % more established seedlings than those with thin and thick OM on lichen dominated seedbeds.

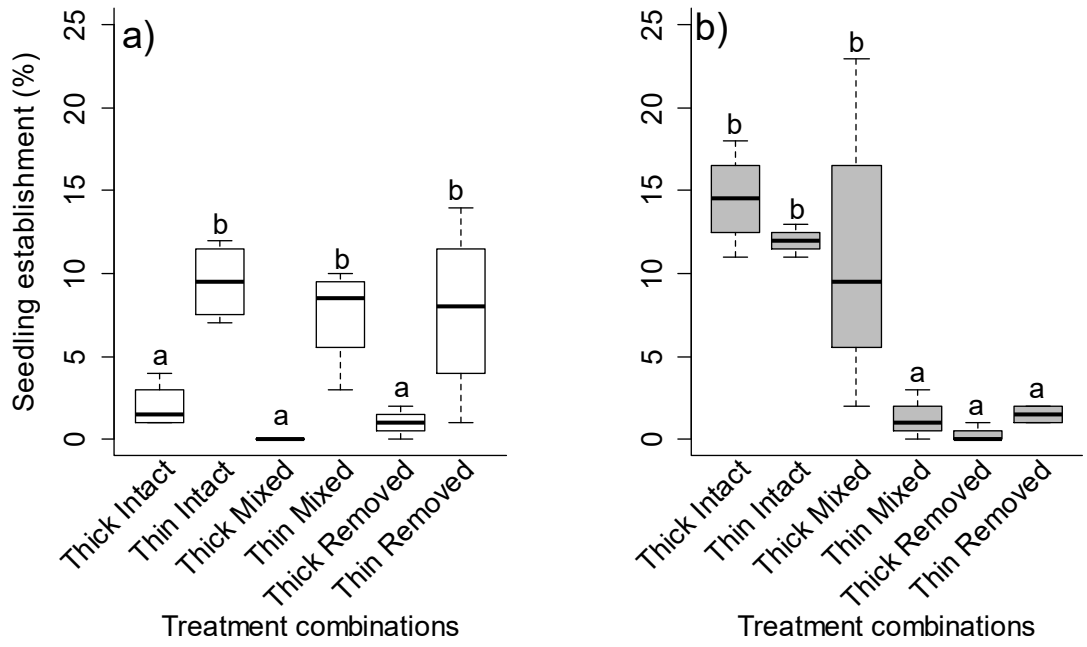

Fig. 5. Boxplots showing seedling establishment on mat-intact, mixed and removed treatments with thin and thick OM in (a) lichen and (b) moss seedbeds. Different letter(s) above the error bars denote significant difference at $\alpha \leq 0.05$. Homogeneous subsets are not significantly different based on Tukey's HSD post-hoc test.

3.3.3 Seedling survival and establishment trends in lichen and moss dominated seedbeds

There was no difference in the number of seedlings surviving (July 01, 2015) between mat-intact and mat-mixed ($z = -0.95$, $p = 1.0$), mat-intact and mat-removed ($z = -0.64$, $p = 1.0$) and mat-mixed and mat-removed plots ($z = -0.39$, $p = 1.0$) on lichen-dominated seedbeds. However, there was an increase in the number of seedlings from first count to third count (end of the first growing season) in all treatments (mat-intact $z = 5.31$, $p < 0.01$, mat-mixed: $z = 7.93$, $p < 0.01$, mat-removed: $z = 5.11$, $p < 0.01$). The number of overwintering seedlings declined in all treatment plots of lichen-dominated

seedbeds, but the difference between the third (pre-winter) and final count (post-winter) was only significant in mat-mixed treatments, meaning that overwinter mortality was comparatively higher in mat-mixed treatments on lichen dominated seedbeds (mat-intact: $z = -2.7$, $p = 0.72$, mat-mixed: -5.76, $p < 0.01$, mat-removed: $z = -2.49$, $p = 0.62$; Fig. 6a).

The lowest germination and seedling establishment occurred in mat-removed plots on moss dominated seedbed and it did not differ across the counts (Fig. 6b). There were significant differences in the number of winter-survived seedlings between mat-intact and mat-removed ($z = -4.331$, $p = 0.001$). There were no differences between mat-intact and mat-mixed ($z = -2.45$, $p = 0.32$) and between mat-mixed and mat-removed plots ($z = -1.87$, $p = 0.73$) in moss-dominated seedbeds (Fig. 7b). The number of seedlings increased significantly from the first count to second count in mat-intact ($z = 7.81$, $p < 0.01$) and mat-mixed ($z = 9.08$, $p < 0.01$) plots. However, there was a significant decline in the number of seedlings from the second count to third count (end of the first growing season) in mat-mixed plots ($z = -6.01$, $p < 0.01$) but not in mat-intact plots ($z = -2.04$, $p = 0.90$). There was no difference in the number of seedlings survived between third count (pre-winter) to fourth count (post-winter) in all treatment plots (mat-intact: $z = -1.02$, $p = 1.0$; mat-mixed: $z = -2.0$, $p = 0.92$; mat-removed: $z = -1.50$, $p = 0.99$) (Fig. 6). In other words, overwinter seedling survival rates were similar regardless of treatments in moss-dominated seedbeds, suggesting that seedling survival mechanisms and mortality in the first year are fully responsible for the difference (Fig. 6b).

Table 8. Results of repeated-measures analysis of variance showing difference of number of seedling survived by seedbed type, OM thickness and seedbed manipulation treatments among four counts (contrasts) in Spracklin Road, TNNP.

Response		Df	Mean Square	F-value	Pr (>F)
Number of seedlings	*Between subjects*				
	Seedbed	1	1680.3	15.3	**< 0.01**
	OM	1	7.5	0.07	0.79
	Treatment	2	2449.8	22.25	**< 0.01**
	Seedbed × OM	1	2324.1	21.12	**< 0.01**
	Seedbed × Treatment	2	1086.8	9.87	**< 0.01**
	OM × Treatment	2	643.5	5.84	**< 0.01**
	Seedbed × OM × Treatment	2	492.6	4.47	**0.01**
	Error-1	36	110.1		
	Within subjects				
	Contrasts	3	2082.5	96.47	**< 0.01**
	Seedbed × Contrasts	3	844.6	39.12	**< 0.01**
	OM × Contrasts	3	96.4	4.46	**< 0.01**
	Treatment × Contrasts	6	179.4	8.31	**< 0.01**
	Seedbed × OM × Contrasts	3	447.7	20.74	**< 0.01**
	Seedbed × Treatment × Contrasts	6	197.8	9.17	**< 0.01**
	OM × Treatment × Contrasts	6	82.6	3.82	**< 0.01**
	Seedbed × OM × Treatment × Contrasts	6	40.8	1.88	0.08
	Error-2	108	21.6		

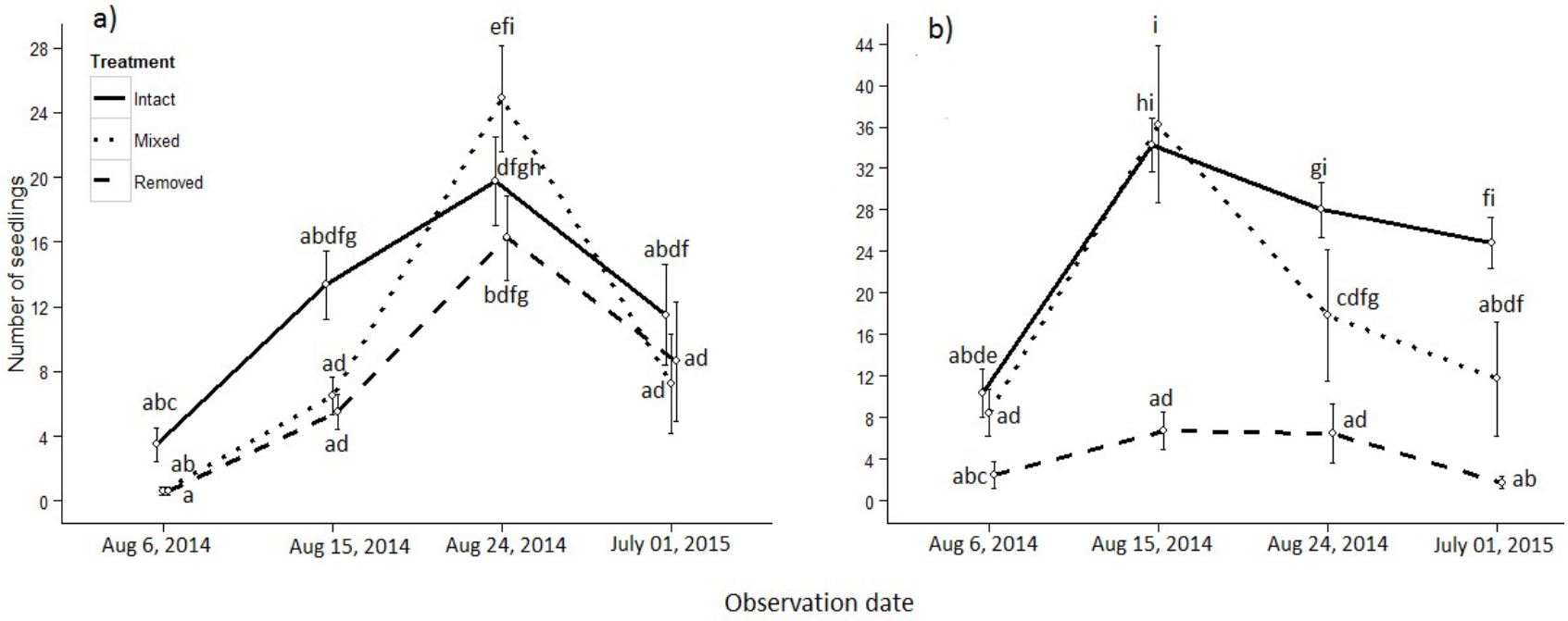

Fig. 6. Mean (± se) number of survived seedlings among the seedbed manipulation treatments in a) lichen and b) moss seedbeds across four repeated counts in Spracklin Road site. Different letter (s) above the error bars denote significant difference at $\alpha = 0.05$. Homogenous subsets are not significantly different based on Tukey's HSD multiple comparison on number of seedlings by 3-way interaction of seedbed type, treatments and contrasts. See Table 8 for statistical differences.

3.3.4 Bulk density of OM under lichen and moss seedbed

Bulk density of OM collected under moss mats was significantly higher than that under lichen mats ($t = -3.9901$, $p = 0.016$). Mean (± se) bulk density of organic matter collected from lichen and moss dominated seedbeds were 0.82 ± 0.06 and 2.09 ± 0.32 mg/ cm^3 respectively.

3.5 Moisture and temperature regimes in lichen and moss dominated seedbeds

3.5.1 Seedbed moisture

Seedbed moisture was different among seedbed types (lichen and moss) and between OM depths (1 and 5 cm) (Table 9). Seedbed moisture over the first growing season (July 25 to August 24, 2014) was higher in moss-dominated seedbed than lichen dominated seedbed at 1cm depth. In lichen dominated seedbed, soil moisture was below 10% over the period and it did not exceed that level even on rainy days (Aug 10 - 15 and Aug 20 - 24, 2014) (Fig. 7). In moss dominated seedbed, even during the extreme dry period, soil moisture was consistently above 10% (Aug 3- 8 and Aug 16 - 18, 2014) (Figs. 7 and 8). Soil moisture patterns at 5 cm depth in both lichen and moss dominated seedbeds were almost similar throughout the growing season (Fig. 7). On lichen-dominated seedbed, soil moisture was significantly lower at 1 cm than at 5 cm depth during the first growing season throughout rainy and dry periods (Fig. 7). On moss-dominated seedbeds, surface soil moisture at 1 cm depth was not always significantly lower than that at 5 cm depth.

Table 9. Results of repeated-measures ANOVA showing differences in moisture and temperature by seedbed type and substrate depth. Significant differences are marked in bold.

Response		df	Mean Square	F-value	p-value
(a) Moisture	*Between subjects*				
	Seedbed type	1	3713.0	8.1	**0.04**
	Substrate depth	1	7443.0	16.1	**0.01**
	Seedbed type × Substrate depth	1	2107.0	4.6	0.09
	Error-1	4	461.0		
	Within Subjects				
	Date	30	44.4	24.1	**< 0.01**
	Seedbed type × Date	30	4.8	2.62	**< 0.01**
	Substrate depth × Date	30	18.5	10.0	**< 0.01**
	Seedbed type × Substrate depth × Date	30	4.6	2.6	**< 0.01**
	Error-2	120	1.8		
(b) Temperature	*Between subjects*				
	Seedbed type	1	1745.5	46.4	**< 0.01**
	Substrate depth	1	84.8	2.3	0.21
	Seedbed type × Substrate depth	1	60.7	1.6	0.27
	Error-1	4	37.6		
	Within subjects				
	Time	47	94.2	17.5	**< 0.01**
	Seedbed type × Time	47	34.7	6.6	**< 0.01**
	Substrate depth × Time	47	24.5	4.5	**< 0.01**
	Seedbed type × Substrate depth × Time	47	2.3	0.4	0.99
	Error-2	188	5.3		

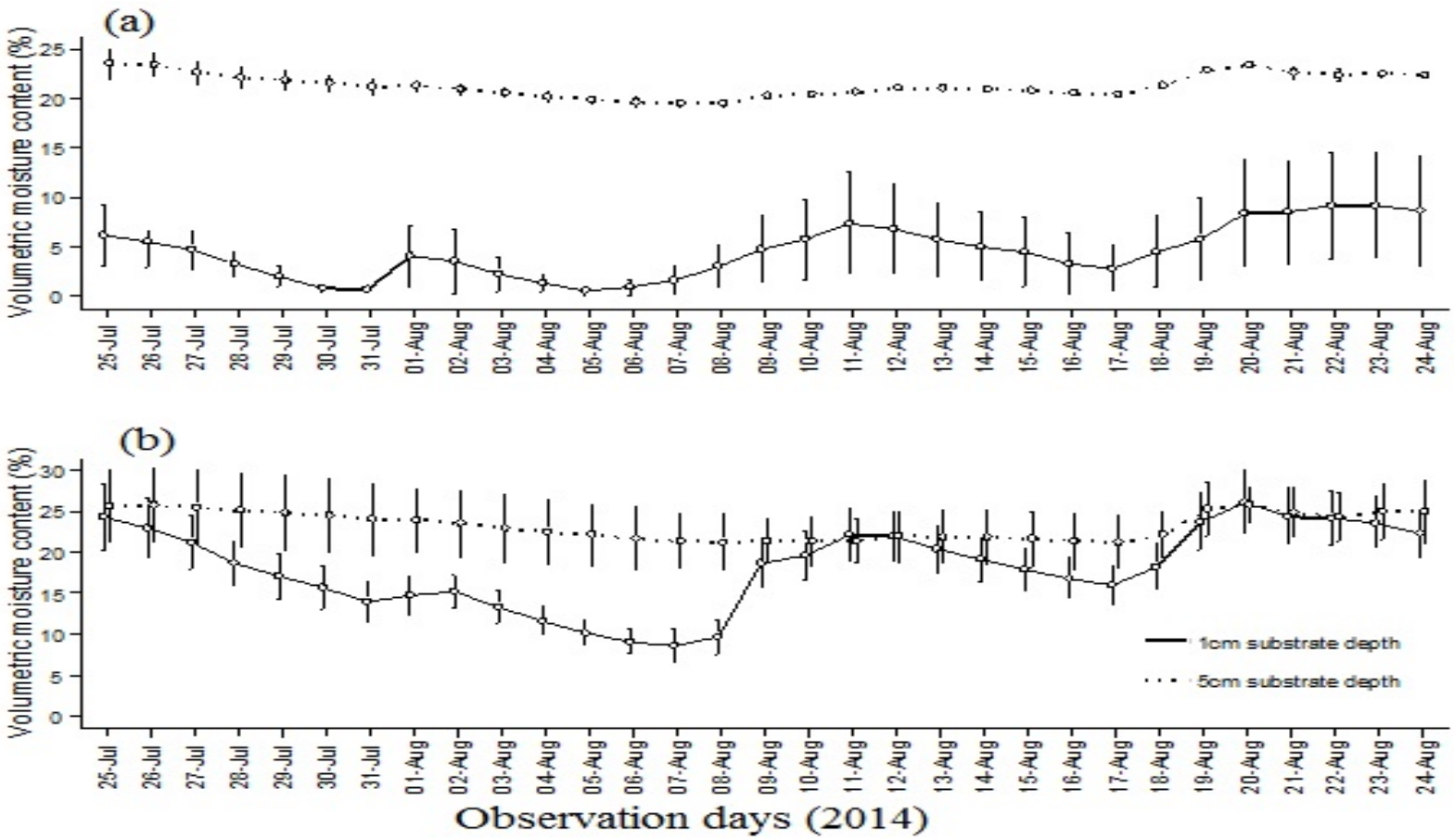

Fig. 7. Volumetric moisture content at 1 cm and 5 cm depths in (a) lichen dominated and (b) moss dominated seedbeds. Data points represent the mean of two readings ± se.

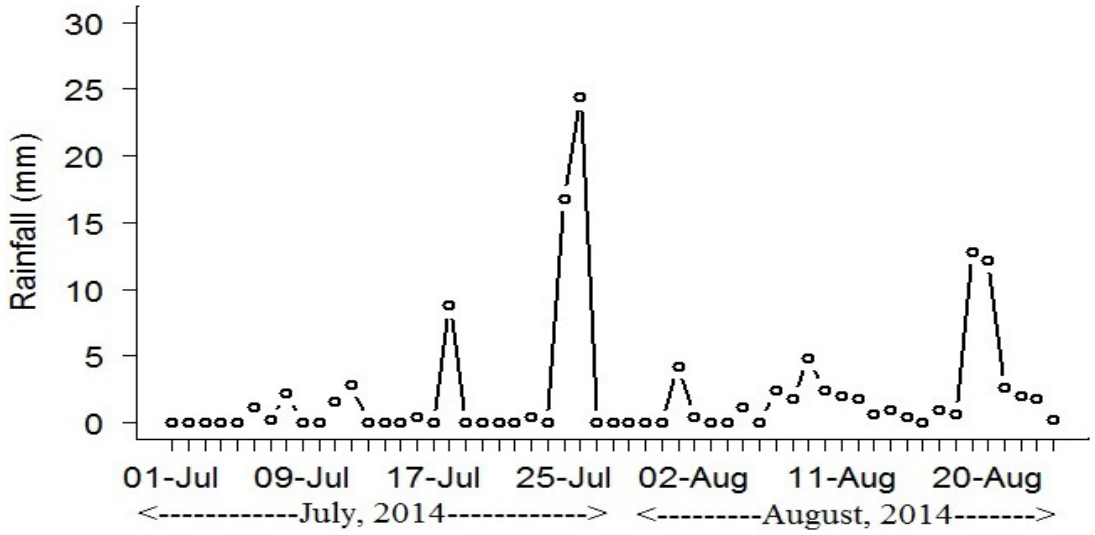

Fig. 8. Daily rainfall patterns in July and August, 2014 in TNNP, Newfoundland.

3.5.2 Seedbed temperature

There were significant differences in seedbed temperatures between lichen and moss dominated seedbeds for the recorded period. Surface soil temperature at 1 cm depth was significantly higher than that at 5 cm depth during daytime (Table 9). There was a greater fluctuation of diurnal temperature in lichen seedbed than moss seedbed (Figs. 9a, b). Although there was very little diurnal temperature fluctuation at 5 cm depth in moss seedbed, it was substantially and consistently higher at 1 cm depth. The maximum temperature range recorded in moss seedbed was 20 to 25°C at around noon, while that in lichen seedbed was 35 to 40°C at noon on August 4, 2014. The maximum temperature at 5 cm depth was about 25°C at noon, while that at 1 cm was between 35 and 40°C at noon on August 4, 2014 (Figs. 9a,b).

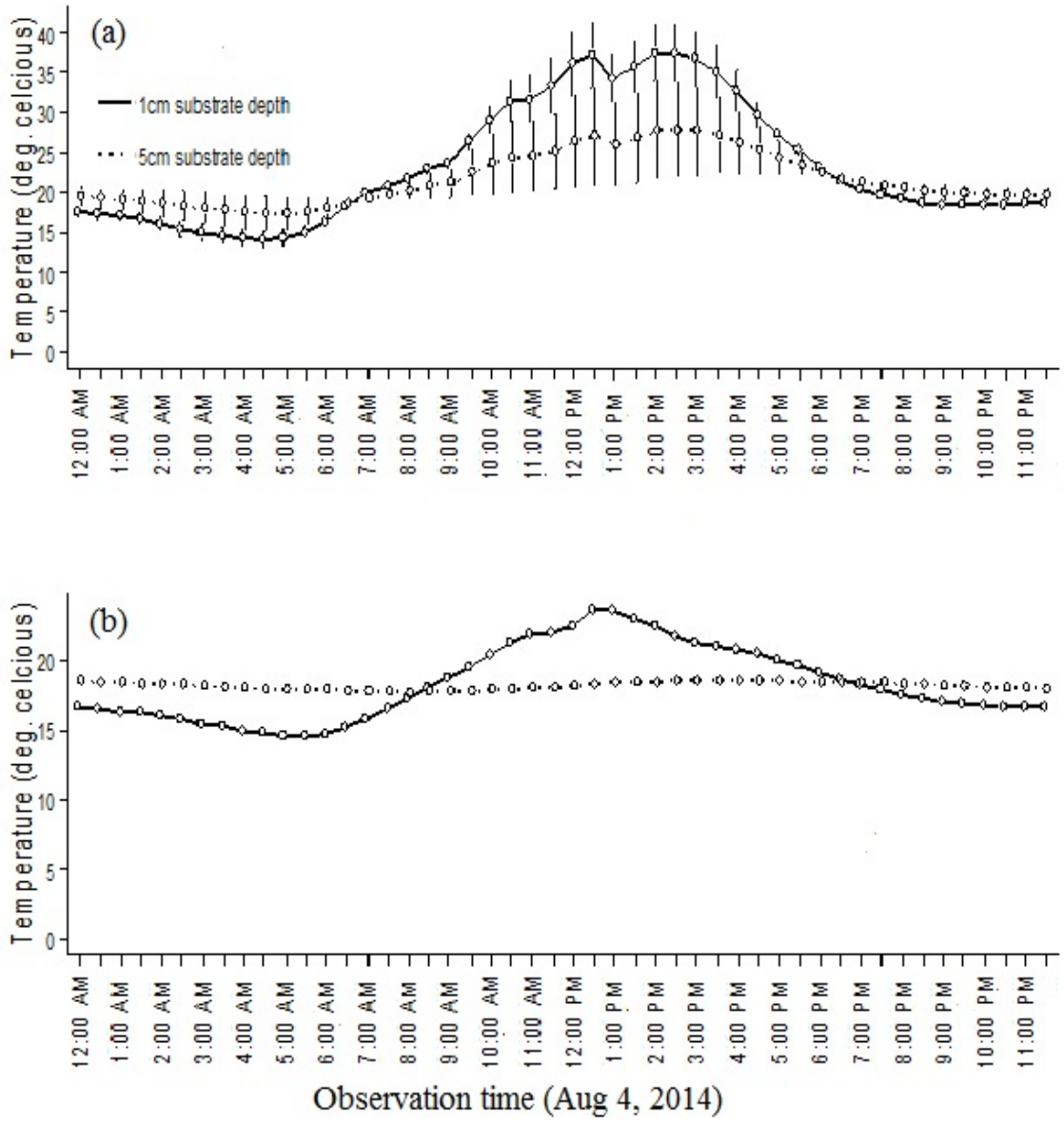

Fig. 9. Diurnal temperature (^{O}C) fluctuations between 1 and 5 cm depths in (a) lichen and (b) moss dominated seedbeds. Data points represent the mean of two readings ± se.

3.6 Laboratory bioassay

3.6.1 Germination

Black spruce germination differed among the cryptogamic species (Kruskal-Wallis chi-square d = 14.48, $p < 0.01$, df = 4). Petri-dishes containing the lichen *Cladonia cristatella* had significantly lower germination than all other treatments including control. Total seed germination (mean ± se) with this lichen was 7.60 ± 0.40. There was no significant difference in germination comparing the lichen *C. stellaris*, the mosses *Polytitrichum juniperinum* and *Pleurozium scheberi*, and the reference seedbed (Fig. 10). Total seed germination with both *P. juniperinum* and *P. schreberi* was 10 ± 0.0 and that in lichen *C. stellaris* was 9.4 ± 0.68.

3.6.2 Seedling root and shoot length

Seedling root length was significantly different among the cryptogamic species (F = 16.96, df = 4, p = <0.01, Appendix 10). Root length of seedling germinated in lichen *C. cristatella* was significantly lower than the other treatments including control (Fig. 11). While mean root length in control was 20.1 mm ± 0.69, it was 14.05 mm ± 2.0 in *C. cristatella*. In contrast, black spruce root lengths were higher in both moss treatments compared to lichens and the reference. Root length of seedlings grown with the mosses *P. schreberi* and *P. juniperinum* were 26.04 mm ± 0.64 and 25.39 mm ± 0.64 respectively and 14.05 mm ± 2.0, 20.76 mm ± 0.98 and 20.1 mm ± 0.69 with the lichens *C. cristatella, C. stellaris* and the control respectively.

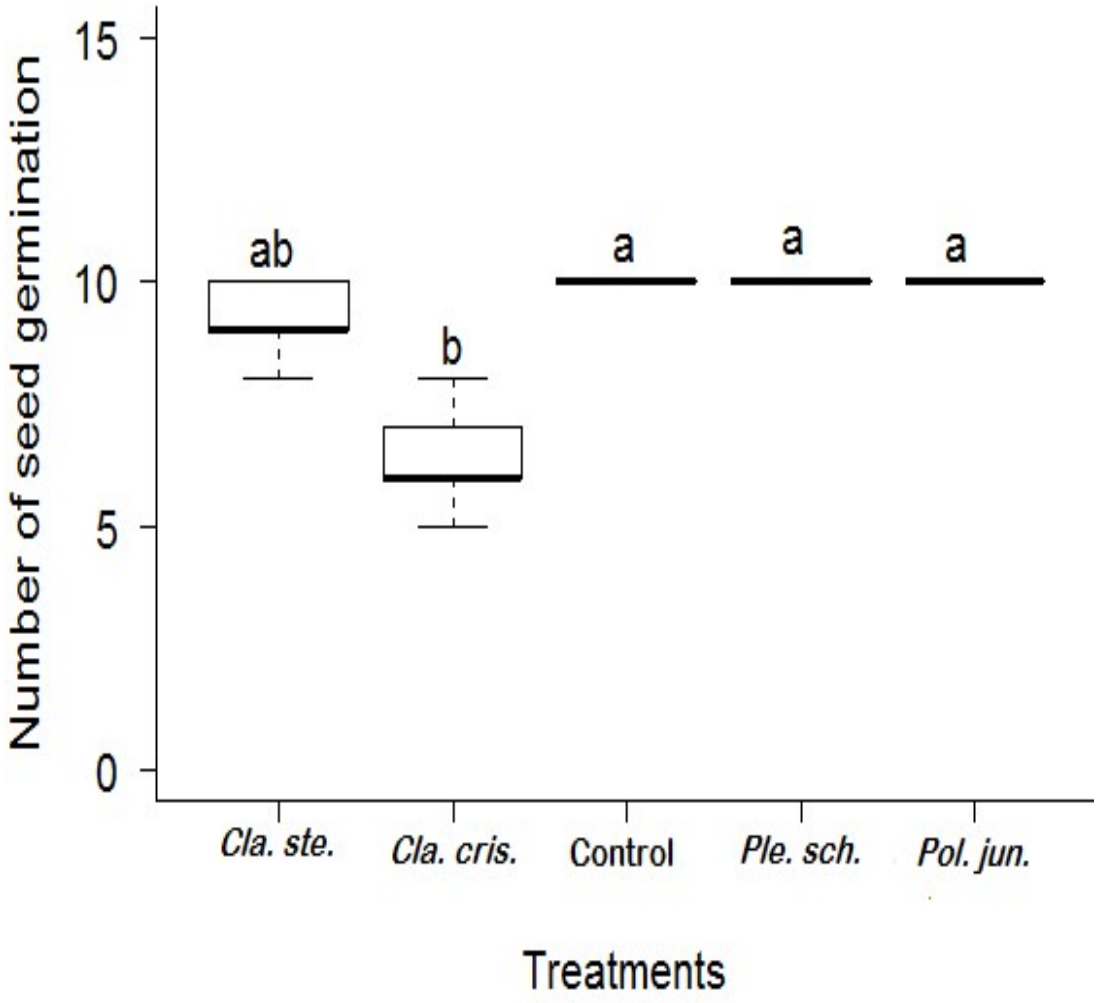

Fig. 10. Mean ± se seed germination in lichen and moss species in laboratory bioassay where 10 seeds were placed in a Petri-dish and each treatment had five replicates. The x-axis abbreviations *Cla.ste*. *Cla. cris*., *Ple. sch*., and *Pol. jun*. denote *Cladina stellaris*, *C. cristatella*, *Pleurozium schreberi* and *Polytrichum junniperinum* respectively. Different letters above boxes denote significant differences among the treatments. Homogeneous subsets are not significantly different based on Kruskal-Wallis multiple comparison at α = 0.05.

Similarly, seedling shoot length was significantly different due to cryptogamic species treatments ($F = 4.851$, $p < 0.01$, $df = 4$, Appendix 10). Shoot length of black spruce seedlings grown with lichen *C. cristatella* was significantly lower than in the reference and moss treatments (Fig. 11). Mean shoot length of seedlings grown with the lichen *C. cristatella* was 24.37 mm ± 1.52, and that grown with *P. schreberi, P. juniperinum* and in the reference treatment were 29.16 mm ± 0.60, 29.22 mm ± 0.89 and 30.01 mm ± 0.91 respectively.

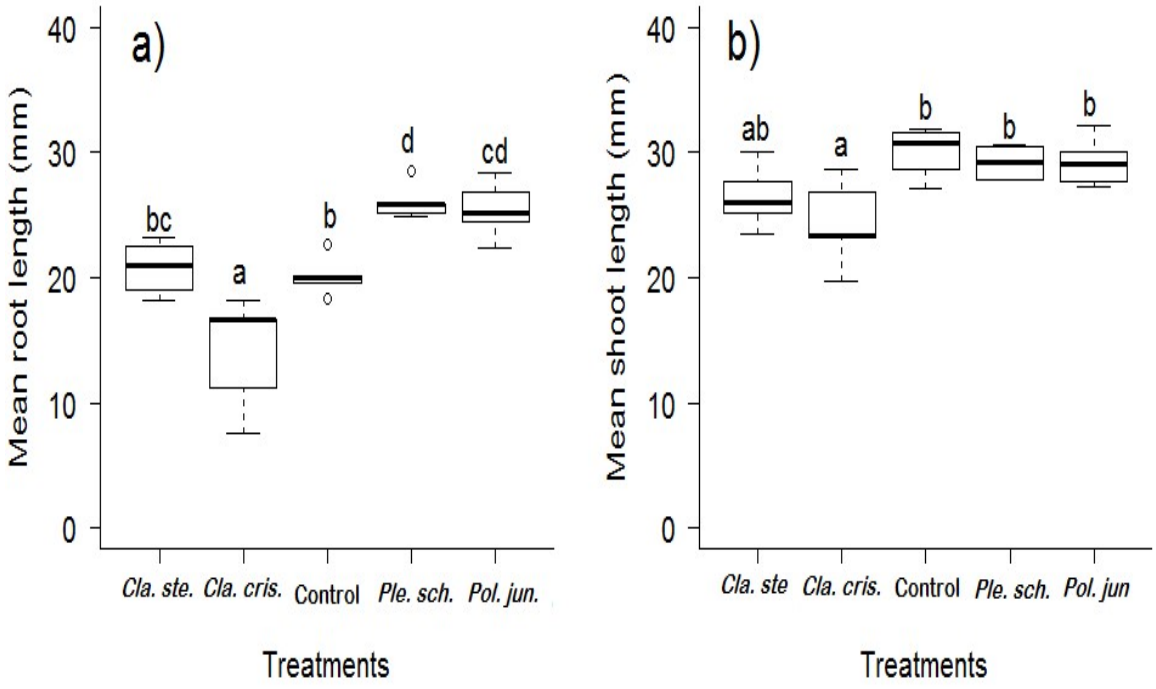

Fig. 11. Boxplot showing difference of a) root length (mm) and b) shoot length (mm) of seedling growing on selected cryptogamic species in laboratory bioassay where *Cla. ste.*, *Cla. cris.*, *Ple. sch.* and *Pol. jun.* denote *Cladina stellaris*, *C. cristatella*, *Pleurozium schreberi* and *Polytrichum juniperinum* respectively. Different letter(s) above the error bares denote significant difference at $\alpha \leq 0.05$ level of Tukey's HSD post-hoc test.

3.7 Species specific pH and total phenol

The Petri-dishes treated with *Cladonia cristatella* were more acidic with a mean pH 4.00 ± 0.06 compared to the Petri-dishes treated with *C. stellaris* (4.03 ± 0.09), *P. schreberi* (4.10 ± 0.06) and *P. juniperinum* (5.07 ± 0.03). Total phenol content varied from 81 to 187 mg/L among the cryptogam species. The maximum total phenolic content was wtih the lichen *C. cristatella* (187 mg/L GAE). Multiple comparisons of means using t-tests revealed that the differences were significant among *C. stellaris* ($p < 0.001$), *P. schreberi* ($p < 0.001$) and *P. juniperinum* ($p < 0.001$). Minimum phenolic content was with *P. schreberi* (81 mg/L GAE). Total phenols in the *C. stellaris* and *P. juniperinum* treatments were 118 and 108 mg/L GAE respectively (Table 10).

Table 10. Total phenolic contents (mean ± se) of lichen and moss species. se= standard error and sd = standard deviation.

Cryptogamic species		Mean ± se total phenol (GAE)	sd
Moss	*Pleurozium shreberi*	81 ± 1.73	3
	Polytrichum juniperinum	108 ± 1.73	3
Lichen	*Cladina stellaris*	118 ± 0.58	1
	Cladonia cristatella	187 ± 2.31	4

3.8 HPLC analysis

Usnic acid was used as standard stock solution because usnic acid is a widely cited allelopathic phenolic acid found in different lichen species. Presence of usnic acid from selected lichen samples were identified based on retention time for major peaks (Thepnuan et al. 2013). Usnic acid standard solution produced a peak at 22.623 min (area 1424.99 mAU*s and peak height 244.69 mAU) (Fig. 14). Methanol extraction of *C. cristatella* produced two major peaks at 22.501 and 23.082 min and the acetone-dichloromethane extraction produced one additional major peak at 3.814 min (area = 1793.71 mAU*s, height = 323.135 mAU) (Fig. 12). Since usnic acid produced peak at 22.623 min, which is close to the first major peak of *C. cristatella*, the presence of usnic acid was assumed in this lichen. Similarly, methanol extraction of *C. stellaris* produced a peak at 22.505 min (area = 2902.61mAU*s, height = 517.19) (Appendix 12). Similarly since usnic acid produced peak at the same retention time its presence in *C. stellaris* was suggested based on similar retention time in the HPLC chromatogram to usnic acid (Fig.13). Peak area and height in *C. stellaris* was higher than that of *C. cristatella* but they both produced peak at the same time (Appendix 12). Representative chromatograms of HPLC for *C. cristatella*, *C. stellaris* and the standard solution of usnic acid at wavelengths 210 nm and 256 nm are given in Appendices 13, 14 and 15 respectively.

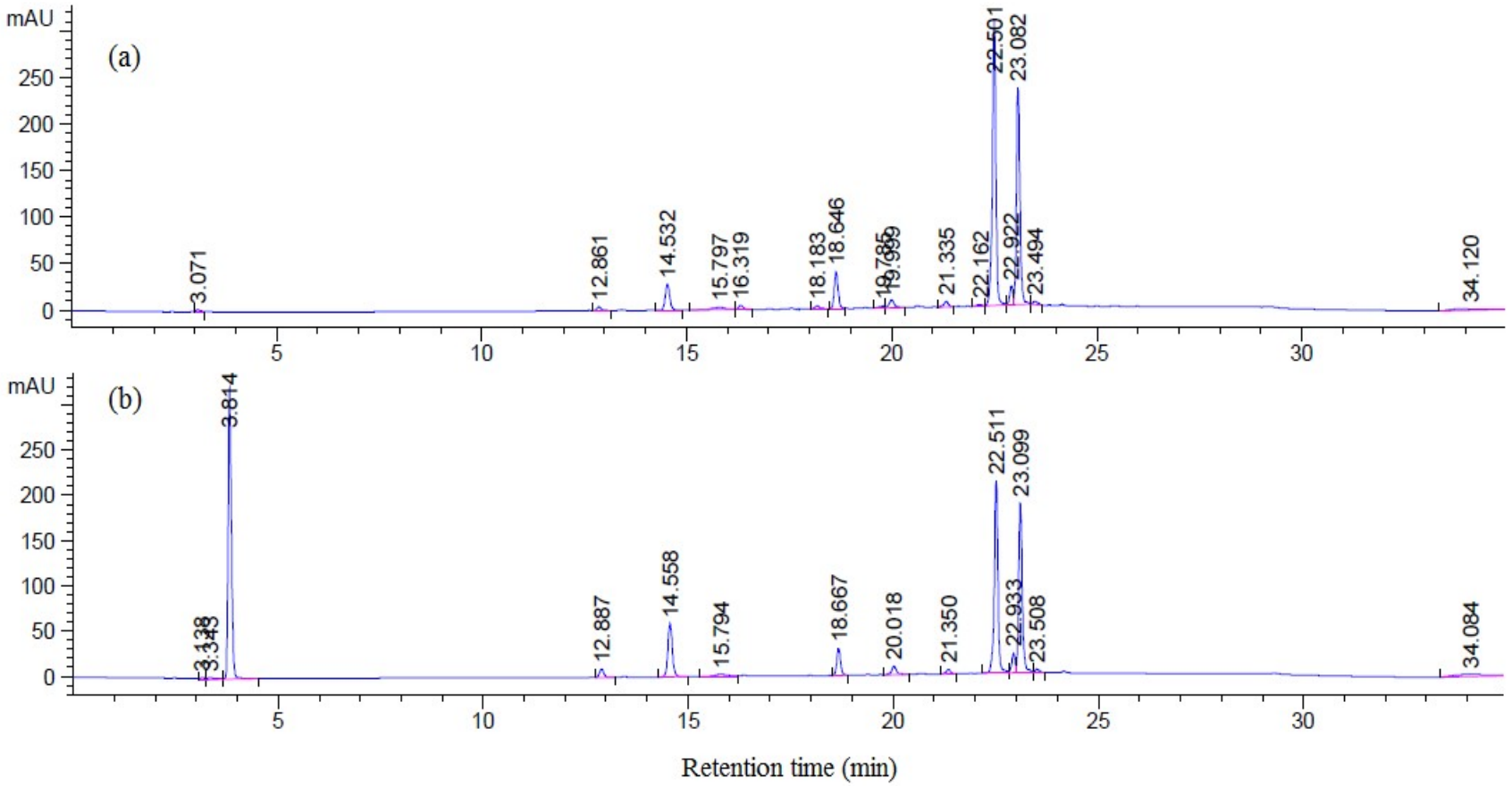

Fig. 12. Representative chromatograms of *Cladonia cristatella* (a) methanol extraction and (b) acetone-dichloromethane extraction at 280 nm wavelength.

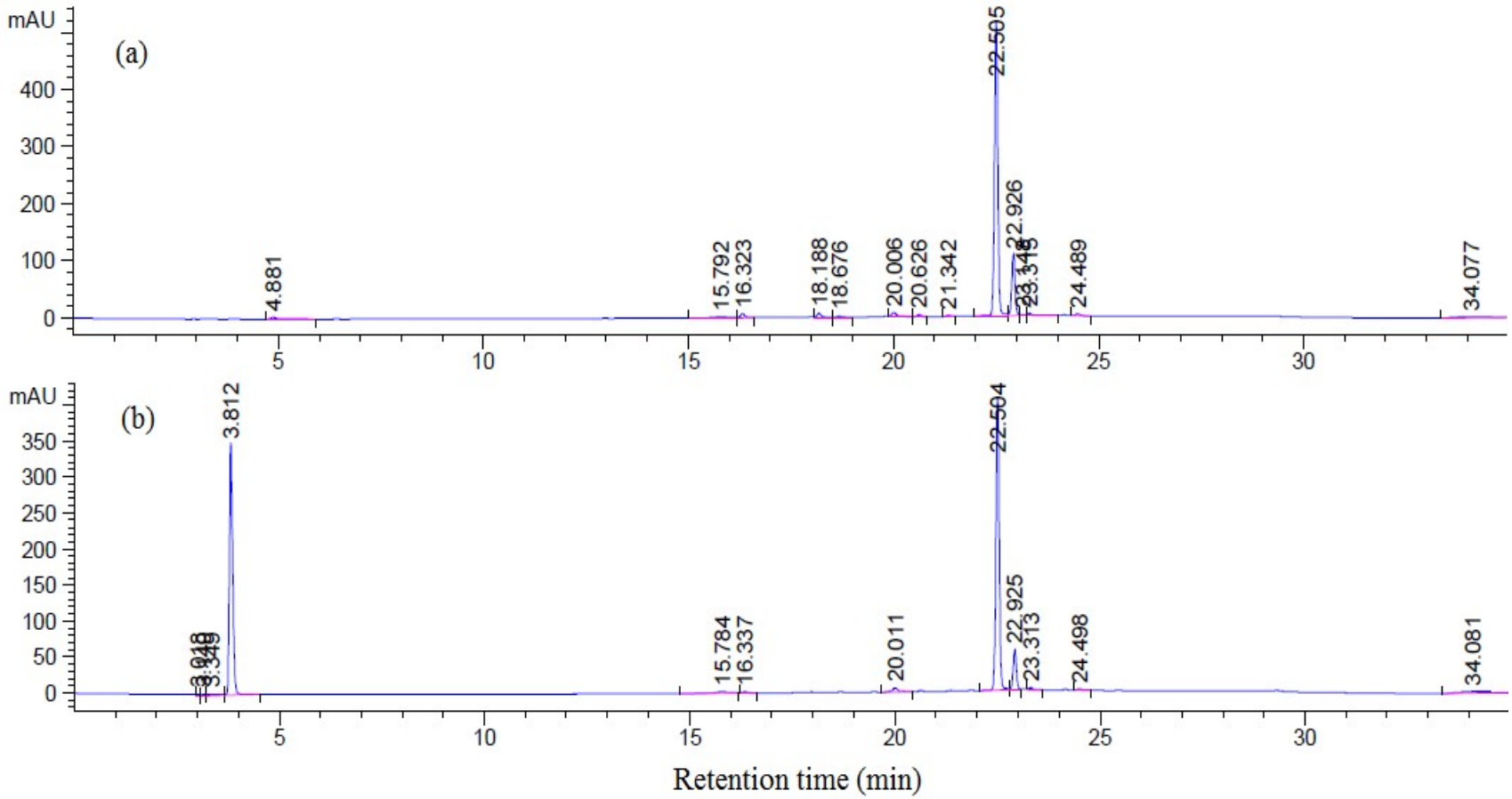
(a)
mAU
4.881
15.792
16.323
18.188
18.676
20.006
20.626
21.342
22.505
22.926
24.489
34.077
(b)
mAU
3.812
15.784
16.337
20.011
22.504
22.925
23.313
24.498
34.081
Retention time (min)

Fig. 13. Representative chromatograms of *Cladina stellaris* methanol extraction (a) and acetone-dichloromethane extraction (b) at 280 nm wavelength.

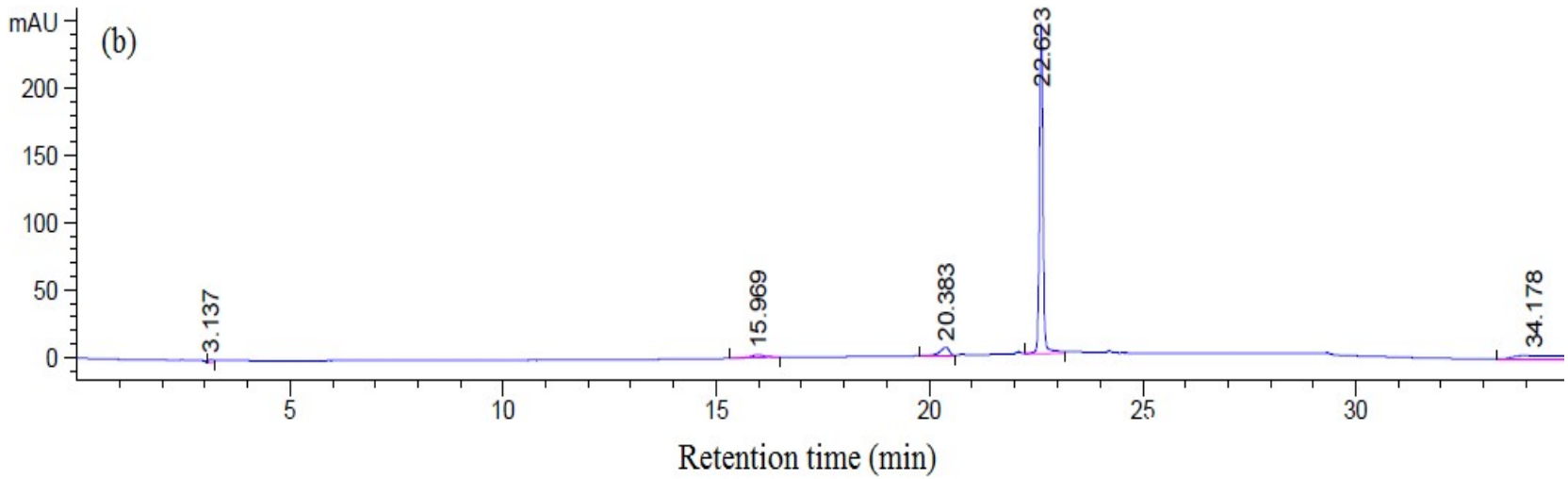

Fig. 14. Representative chromatogram of standard solution of usnic acid at 280 nm wavelength.

4. Discussion

4.1 Effects of seedbed manipulation treatments on seed germination and seedling establishment

I hypothesized that if lichen mat is a physical barrier to black spruce germination and seedling establishment, then mixing the mat with associated OM or removing the mat completely would result in higher germination and seedling establishment. Results showed lichen mat-intact plots with thick OM had higher seed germination but mat-intact plots with thin OM had higher seedling establishment in the early post-fire site. Mat-mixed plots with thin OM had the lowest germination and all other treatments except mat-intact plots with thick OM had lower seedling establishment. In general, seedbed manipulation treatments did not improve black spruce germination and seedling establishment in the early and mid post-fire sites (RP and SR). Thus, OM thickness seems to be the primary factor controlling seedling establishment in early and mid post-fire sites. Mat intact microsites consisting of cracks and fissures might have provided favourable germination and establishment conditions while the mat manipulation treatments (mat mixing and mat removal) eliminated the favourable microsite condition. Several authors suggested that surface cracks and fissures on unburned lichen mats provide favourable conditions for seedling establishment (Sirois 1995, Cowels 1982, Power 2005). The possible reason for successful seedling establishment in the cracks of lichen mats might be that surface cracks provide safe sites (*sensu* Harper 1977) with favourable soil moisture and temperature and reduced OM depth to mineral soil. Thus, germinated seedlings within cracks had a greater chance of reaching mineral soil than

those on the surface. Mechanical mixing of lichen mat with OM and removal of lichen mat in early and mid post-fire sites eliminated the cracks and fissures thereby resulting in lower seed germination. Kayes (2008) also reported lower germination in similarly altered plots in early post-fire sites in SW Oregon, USA. Studying in the same area Siegwart Collier and Mallik (2010) found that thin residual OM (≤ 2 cm) had more naturally regenerated spruce seedlings than those with thicker residual OM. Kravchenko (2012) however, suggested 0 - 5 cm OM as suitable seedbeds for spruce regeneration.

Contrary to the results of early and mid post-fire sites (RP and SR), seedling establishment was higher in mat mixed plots in the late post-fires site (TNR). This difference can be explained by the difference in lichen mat density and thickness. Lichen mat thickness in the late post-fire site was higher than that of early and mid post-fire sites. Thick lichen mats of intact plots of the late post-fire site might have impeded seed contact with mineral soil, while mat-mixed plots would have eliminated this physical barrier and resulted in higher germination. However, the mat-removal treatment eliminated ground cover, rendering seeds vulnerable to direct sunlight and desiccation due to loss of seedbed moisture. Removal of ground cover also exposed the seedlings to potential herbivore damage. Mallik and Kravchenko (2016) reported higher herbivore damage of planted spruce seedlings in scarified and micro-site mulching plots than undisturbed control plots. Extreme surface temperature due to exposed OM surface in post-fire sites was also suggested by Johnstone and Kasischke (2005). They argued that the black charred OM adsorb and retain heat from the sunlight causing excessive moisture loss. This might be the case in mat removed and mat mixed plots.

In this study, most germination occurred during the rainy period of August 15 to 24, 2014. However, during this time in late post-fire site (TNR), I found no difference in seedling establishment between thin and thick OM seedbed. In this site seed germination and seedling establishment were significantly lower in mat intact plots than mat mixed plots regardless of OM depth. Removal of the physical barrier of the thick lichen mat in this site might have enhanced germination and establishment. Several authors reported improved growth of planted black spruce in artificially created safe sites by mixing OM with part of mineral soil (Walker and Mallik 2008, Mallik and Kravchenko 2016).

4.2 Moss facilitation

Seed germination and seedling establishment was higher in moss-dominated seedbed than lichen dominated seedbed. Wheeler et al. (2011) reported a similar results showing higher seed germination and seedling growth in Pleurocarpous moss seedbed compared to bare soil and *Cladonia* lichen seedbed in tundra region. In early post-fire sites of Terra Nova National Park, the dominant mosses are mostly acrocarpous including *Polytrichum juniperinum* and *P. commune* and dominant lichens are foliose and fruticose morphotypes including different species of *Cladonia* and *Cladina*. This research suggests that moss facilitation was mostly mediated by high soil moisture retention and low surface temperature. Wheeler et al. (2011) suggested similar mechanism of bryophyte facilitation in conifer regeneration from tundra regions. Moss facilitation could also be argued in light of the lack of physical barrier of *P. juniperinum* moss. The growth form of this and other acrocarpous mosses are erect consisting of single stems as such that they do not form continuous mats unlike pleurocarpus mosses. The dense stems of acrocarpous mosses have belowground rhizinies that binds seedbed substrates together

and thereby increase bulk density, which help moisture retention (Brodo et al. 2001). High moisture retention due to high bulk density and cooler surface temperature due to their high shoot density creates favourable seedbed condition for germination and seedling establishment. Bloom (2003) reported higher soil respiration under *Kalmia*-moss community than *Kalmia*-lichen community that indicates higher microbial activity in the former. Higher microbial activity generally enhances decomposition of substrates under moss mat than lichen mat (Sedia and Ehrenfeld 2006). Higher moisture availability may facilitate higher microbial activity and thereby increase substrate bulk density.

Although seed germination was similar in moss intact and moss mixed plots, seedling establishment was higher in moss intact plots. In this study, seedbed moisture content was almost similar between moss intact and moss mixed plots, however, seedling establishment was lower in moss mixed plots. This can be explained by comparing juvenile seedling mortality between the two treatments due to predation and frost heaving (Mallik and Kravchenko 2016). Many seedlings were found partly or wholly uprooted that eventually died during the third count in the first growing season (August 24, 2014) in moss mixed plots despite moist seedbed. Seedling predation by slugs is a possibility under this condition. Seedling establishment can also be prevented in due to frost heaving in mat mixed plots (Allen 1929; Mallik and Kravchenko 2016). In dry weather, *Cladonia* lichen can be very dry, but with occasional morning dew they can absorb water and expand when the germinating cotyledons are often caught by *Cladonia* thalli. The contraction of lichen thalli due to drying may be sufficient to pull some seedlings partly or wholly from the ground. This lifting of seedlings was noticed one week after of germination. This phenomenon was most common in mat-mixed treatments of both

lichen and moss dominated seedbed. In addition, decapitated seedlings were found beneath the cut standing stems. I also found clipped off uprooted stems lying on the ground in or outside the plot boundary in both mat-mixed and mat-removed plots of lichen and moss seedbeds but absent in lichen intact and moss intact plots.

To exclude potential small mammal and insect damage, I designed a plot erecting about 10 cm high plastic boundary using bottom cut plastic pot and seeded with 100 black spruce seeds in mineral soil. I watered the plot daily to maintain sufficient soil moisture and I found 72 seeds were germinated. During the following week, I found large number of damaged seedlings as described above. Only 23 seedlings had cotyledons and needles but the others were clipped off from the collar region of both standing and uprooted stems. Power (2005) reported one individual seedling (out of 69) clipped off by an unknown insect in Terra Nova National Park (cited in Moss and Hermanutz 2009). He suggested that, ants (*Formica fusca* L.) and unidentified beetles were potential juvenile seedling predators in this area. Ants are known to be seed predators rather than seedling predators (Beattie and Culver 1983). Nystrand (1998) identified curculinoid beetles as conifer seedling predators and Nystrand and Granström (1997) observed curculinoid beetle consuming Scots pine (*Pinus sylvestris* L.) seedlings in laboratory experiment. In a latter study Nystrand and Granström (2000) identified two species of carabid beetles to consume Scot pine seedlings in a field experiment in Swedish boreal forest. Moss and Hermanutz (2009) collected ants and beetles from Terra Nova National Park area and argued that they could be potential damaging agents to juvenile seedlings. Additional research on ants and beetles as juvenile seedling predator is recommended. However, such seedling predation was absent in moss intact plots and thereby, it facilitates seedling

establishment through protecting from seedling predation. Wheeler et al. (2011) reported that feather moss protect spruce seedlings from predators during the first two to three years of recruitment.

Seed germination and seedlings establishment in moss removal plots were lower than moss intact and moss mixed plots. Moisture and temperature data showed that moisture content was higher when moss was present in the seedbed and it suggests lower moisture in mat removal plots due to moss mat removing exposed the black surface of the duff, which might have lost moisture faster than moss intact and mixed plots (Johnstone and Kasischke 2005). Wheeler et al. (2011) also demonstrated that mat removal did not help spruce regeneration in arctic tundra ecotone.

4.3 Lichen inhibition

In situ field experiment revealed that lichen dominated seedbed resulted lower germination and seedling establishment than moss dominated seedbed. The potential mechanism of this germination and seeding establishment inhibition might be due to seedbed moisture, temperature, and chemical effects. Lichen dominated seedbeds had lower moisture and surface temperature than moss-dominated seedbeds. Furthermore, diurnal temperature fluctuation was higher in lichen than moss seedbed. Zamfir (2000) demonstrated that, *Filipendula ulmaria* and *Arenaria montana* had lower seedling emergence in both thick and thin lichen mat than on bare soil in dry treatment, but in moist treatment their emergence on thin lichen mat was not different from bare soil. Thus, in relatively dry environment even a thin lichen cover may inhibit seedling emergence of conifers.

The possible reason for low moisture in lichen seedbed might be its low bulk. The OM under lichen mats was composed of unburned lichens and forest floor litter that are spongy, porous and devoid of soil particle. Unlike forest floor, this unburned and partially burned OM persists for a long time in the seedbed. Sedia and Ehrenfeld (2005, 2006) showed that lichen tissues and all plant materials under lichen mat decompose more slowly than moss tissue because microbial activity is affected more by groundcover rather than OM. Therefore, nutrient and moisture retention remain relatively unchanged under lichen mat for a long time. In addition, the dark color of OM adsorbs more heat from the sun causing higher surface temperature (maximum temperature reached at around 40°C at noon) than moss seedbed. Greater cryptogam biomass and cover cause greater evaporative loss of moisture from the seedbed surface (Groeneveld et al. 2007). Additionally, lichen fruiting body "Podetia" that cover the OM surface can make the surface water repellent.

4.4 Laboratory bioassay

The laboratory bioassay revealed that total black spruce germination, seedling root and shoot length were significantly lower with the lichen *C. cristatella* than the lichen *C. stellaris* and the mosses *P. juniperinum* and *P. screberi*. This lower germination, root and shoot length of seedlings in *C. cristatella* indicates potential allelopathic effects of *C. cristatella* on spruce seed germination and early seedling growth. This result also resembles that of Pyatt (1967), who found that adverse effect of foliose ground lichen, *Peltigera canina* (L) Willd. on grasses was based on a direct effect of lichen secondary metabolites on root formation and elongation. Lawrey (1977) showed with an *in vitro* experiment that, lichen extracts and lichen products inhibit spore

germination of several mosses. Gardner (1981) showed that eight tested lichenic acids inhibit spore germination and sporeling growth of *Funaria hygrometrica*. Similarly, Gardner and Mueller (1981) observed toxic effects of *Cladonia foliacea* on bryophytes. Since lichen allelochemicals negatively affect bryophytes, they might also affect vascular plants. Several *in vitro* studies suggest that the competitive relationships between terricolous lichens and vascular plants depend on allelopathic interference (Lawrey 1986). In this study, I found no difference in total germination, root and shoot length of spruce seedlings among *Cladina stellaris, Polytrichum juniperinum, Pleurozium schiberi* and control suggesting the absence of allelopathy in two mosses and the lichen *Cladina stellaris*. Kytöviita and Stark (2009) also found no allelopathic effect from *C. stellaris* on pine seedlings. Castells et al. (2005) found no allelopathic inhibition of mosses *Sphagnum* spp. and *Hylocomium splendens* on white spruce (*Picea glauca* (Moench) Voss) germination and root-shoot growth. In contrast, Michel et al. (2011) found that water extracts of 12 bryophyte species stimulated radical growth of vascular plants at low concentration (1%) but at high concentration it was inhibited (5 - 10 %). In this study, total phenol content of *C. cristatella* was highest among the four cryptogams. The pH of *C. cristatella* treated medium was lower as well compared to *C. stellaris, Polytrichum juniperinum, Pleurozium schiberi*. Since germination, seedling root and shoot length were lower only in *C. cristatella* lichen, it is possible that the presence of usnic acid found in this lichen might have inhibited germination and seedling growth of black spruce.

5. Conclusions

This research demonstrates that cryptogamic mats act as a physical barrier to seed germination and seedling growth in older burns but not in recent burns (RP and SR). It appears that post-fire moss dominated seedbeds facilitate black spruce regeneration by improving moisture retention and maintaining low surface temperatures, while lichen mats inhibit seedling regeneration by creating physically and chemically inhospitable seedbeds. While thick OM and undisturbed micro-sites control post-fire seedling regeneration in lichen-dominated seedbed, thin OM makes comparatively better seedbed condition in RP and SR sites. The difference between lichen and moss mats with respect to spruce seed germination and seedling establishment is best explained by the altered seedbed surface microenvironment such as moisture retention and temperature regime during seed germination and initial seedling establishment. Based on the result from TNR site, one can argue that the degree of lichen inhibition increases with the increasing time since fire with increased lichen mat density, OM thickness and accumulation of total phenol. This study also demonstrates the possible chemical inhibition of allelochemical usnic acid on black spruce seed germination. On the other hand, moss dominated seedbeds provide facilitation to spruce seedling regeneration. Mechanical mixing of lichen mats by eliminating the physical and chemical barrier may create a better seedbed for black spruce regeneration in late post fire sites. However, mat mixing may enhance seedling damage due to frost heaving and animal browsing.

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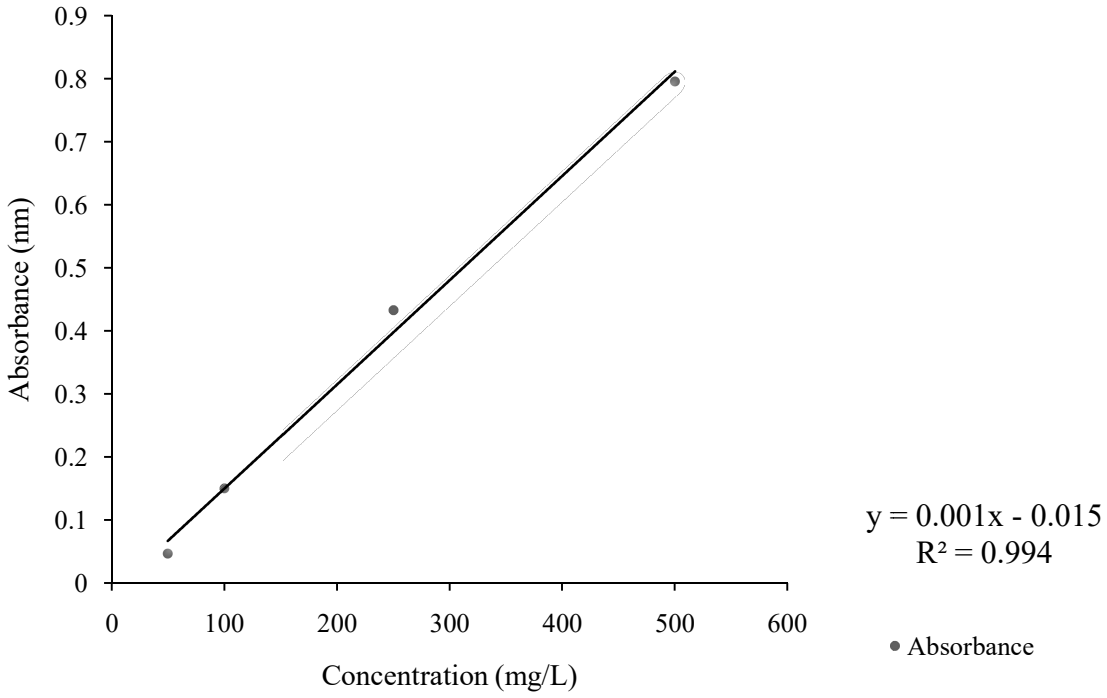
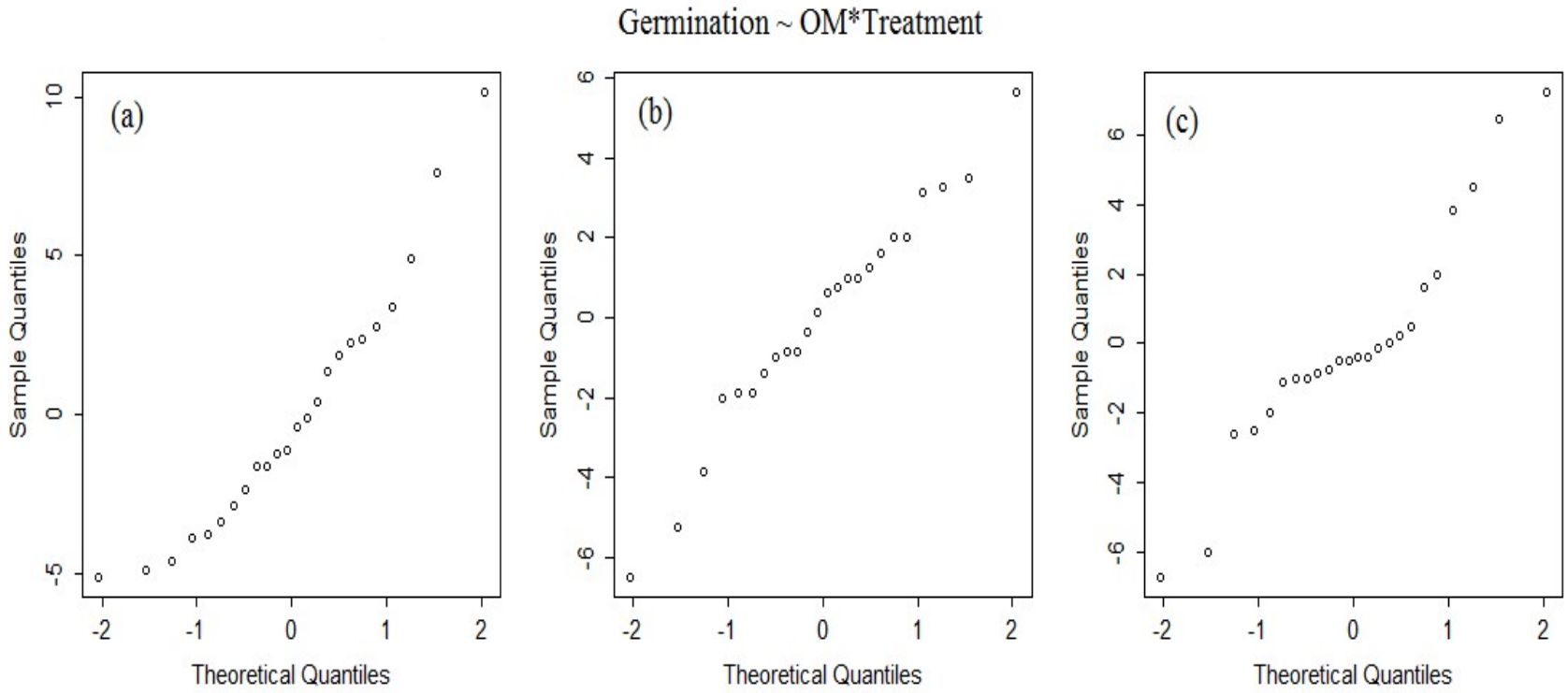
7. Appendices

Appendix 1. Standard curve for Gallic acid concentrations in Folin-Ciocaltue method of quantifying total phenol from cryptogamic species



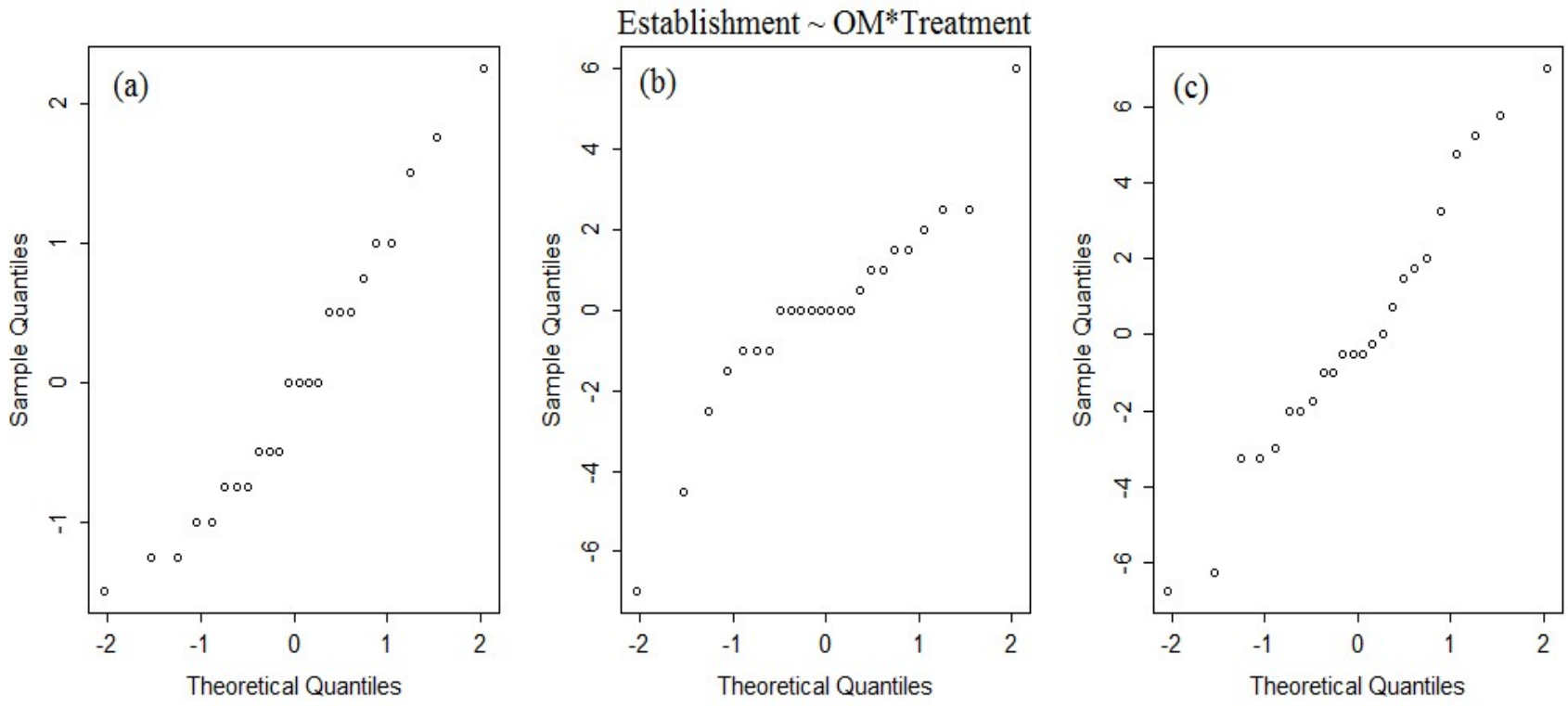
Appendix 2. Residuals Q-Q plots for linear model on seed germination by OM thickness and seedbed manipulation treatments in (a) Rocky Pond, (b) Spracklin Road and (c) Terra Nova Road separately.



Appendix 3. Results of estimated coefficients and their significance test derived from linear model on seed germination by OM thickness and seedbed manipulations treatments in (a) RP, (b) SR and (c) TNR, TNNP, Newfoundland. Significant *p*-values are marked bold.

Sites	Coefficients	Estimate	St. error	t - value	p- value	R^2
(a) RP	Intercept	14.12	2.21	6.37	**< 0.01**	
	OMThin	-5.00	3.13	-1.59	0.12	
	TreatmentMixed	-4.87	3.13	-1.55	0.13	0.57
	TreatmentRemoval	-1.25	3.13	-0.39	0.69	
	OMThin × TreatmentMixed	-2.62	4.43	-0.59	0.56	
	OMThin × TreatmentRemoval	-3.50	4.43	-0.79	0.44	
(b) SR	Intercept	7.25	1.58	4.58	**<0.01**	
	OMThin	5.25	2.23	2.34	**0.03**	
	TreatmentMixed	2.12	2.23	0.95	0.35	0.58
	TreatmentRemoval	-1.37	2.23	-0.61	0.54	
	OMThin × TreatmentMixed	0.87	3.16	0.27	0.78	
	OMThin × TreatmentRemoval	-0.75	3.16	-0.23	0.81	
(c) TNR	Intercept	1.00	1.83	0.54	0.59	
	OMThin	2.62	2.58	1.01	0.32	
	TreatmentMixed	16.37	2.58	6.32	**<0.01**	0.75
	TreatmentRemoval	8.00	2.58	3.09	**<0.01**	
	OMThin × TreatmentMixed	-6.75	3.660	-1.84	0.08	
	OMThin × TreatmentRemoval	-5.12	3.660	-1.40	0.17	

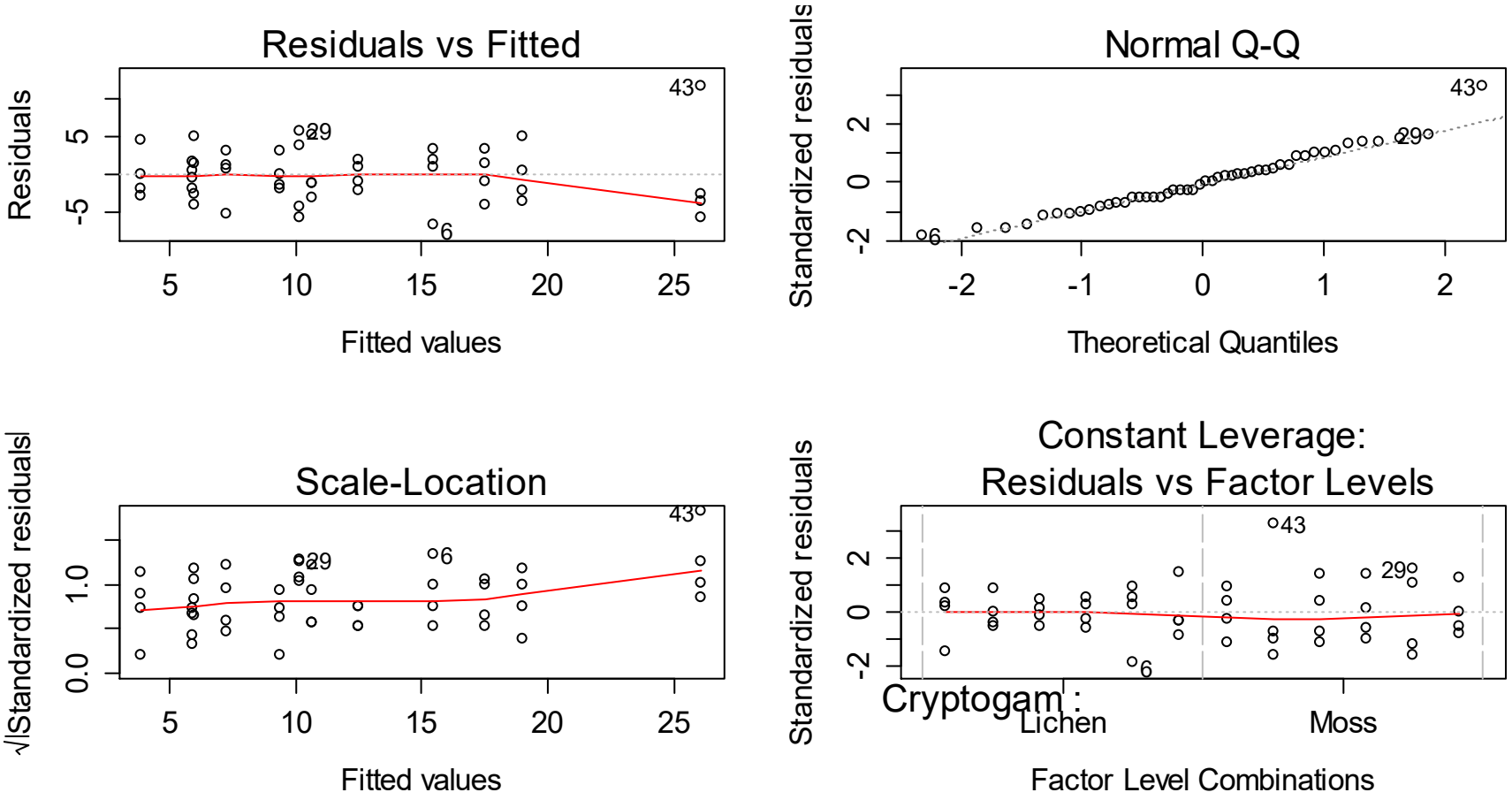
Appendix 4. Residuals Q-Q plots for linear model on seedling establishment by OM thickness and seedbed manipulation treatments in (a) Rocky Pond, (b) Spracklin Road and (c) Terra Nova Road separately.



Appendix 5. Results of estimated coefficients and their significance test derived from Generalized Linear Model on seedling establishment by OM thickness and seedbed manipulations treatments. Significant p-values are marked bold.

Sites	Coefficients	Estimate	St. error	t - value	p- value	R^2
(a) RP	Intercept	2.50	0.57	4.37	**< 0.01**	
	OMThin	2.75	0.80	3.40	**< 0.01**	
	TreatmentMixed	-1.75	0.80	-2.16	**0.04**	0.74
	TreatmentRemoval	-1.50	0.80	-1.85	0.07	
	OMThin × TreatmentMixed	-2.50	1.14	-2.18	**0.04**	
	OMThin × TreatmentRemoval	-3.25	1.14	-2.84	**0.01**	
(b) SR	Coefficients	Estimate	St. error	z-value	p-value	AIC
	Intercept	0.69	0.35	1.96	**0.04**	
	OMThin	1.55	0.38	4.00	**<0.01**	
	TreatmentMixed	-2.07	1.06	-1.96	**0.04**	104.77
	TreatmentRemoval	-0.69	0.61	-1.13	0.25	
	OMThin × TreatmentMixed	1.84	1.08	1.69	0.09	
	OMThin × TreatmentRemoval	0.52	0.65	0.79	0.42	
(c) TNR	Coefficients	Estimate	St. error	t - value	p- value	R^2
	Intercept	0.50	1.97	0.25	0.80	
	OMThin	1.50	2.79	0.53	0.59	
	TreatmentMixed	11.50	2.79	4.11	**<0.01**	0.57
	TreatmentRemoval	7.750	2.79	2.77	**0.01**	
	OMThin × TreatmentMixed	-5.75	3.95	-1.45	0.16	
	OMThin × TreatmentRemoval	-5.50	3.95	-1.39	0.18	

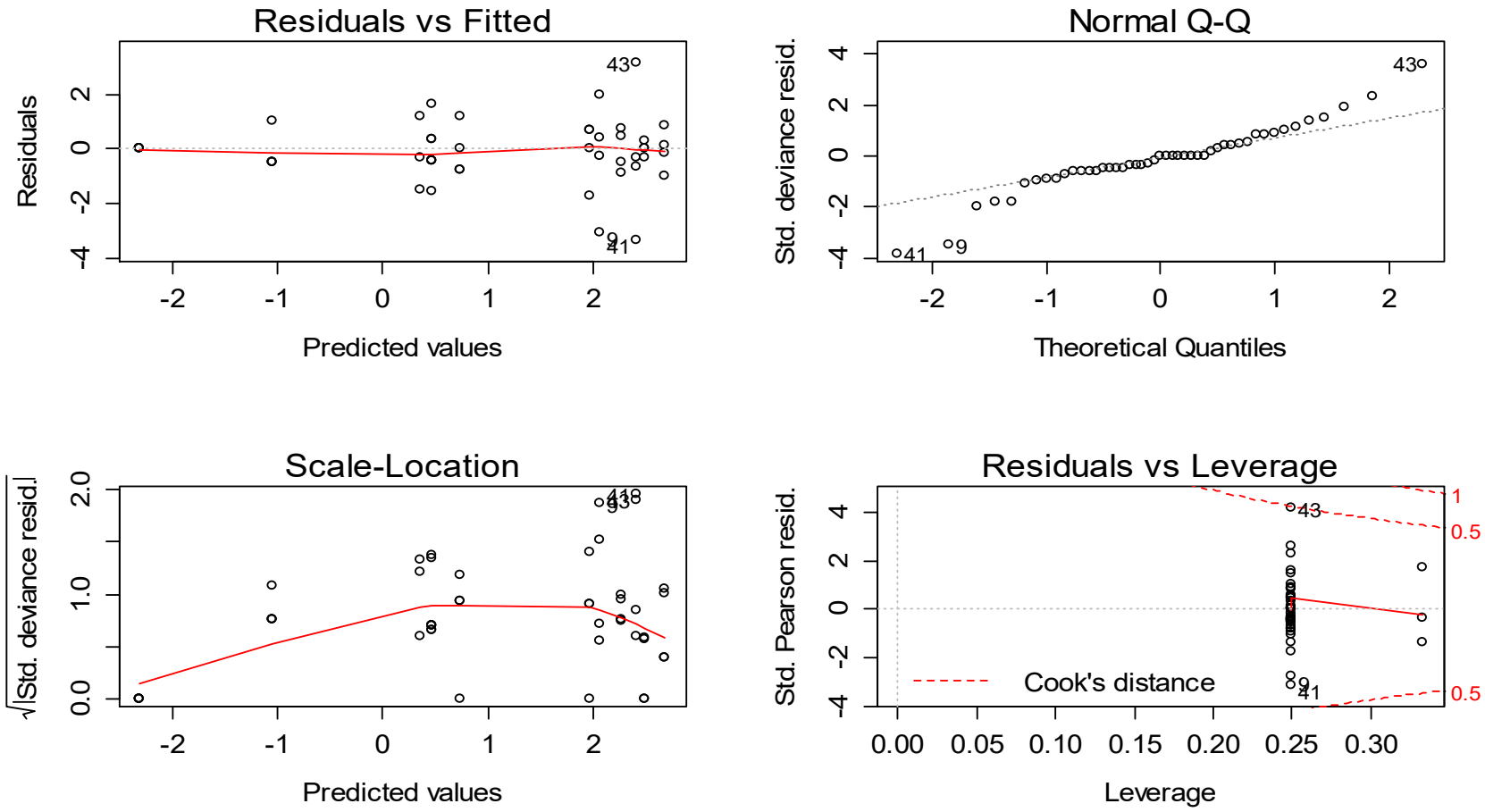
Appendix 6. Diagnostic plots of linear model on seed germination by seedbed type, OM thickness and seedbed manipulation treatments



Appendix 7. Results of estimated coefficients and their significance test derived from linear model on seed germination by seedbed type, OM thickness and manipulations treatments (multiple $R^2 = 0.75$). Significant *p*-values are marked bold.

Coefficients	Estimate	Std. error	t- value	p-value
Intercept	7.25	2.07	3.49	**<0.01**
CryptogamMoss	10.25	2.93	3.49	**<0.01**
OMThin	5.25	2.93	1.79	0.08
TreatmentMixed	2.12	2.93	0.72	0.47
TreatmentRemoval	-1.37	2.93	-0.46	0.64
CryptogamMoss×OMThin	-3.75	4.14	-0.90	0.37
CryptogamMoss × TreatmentMixed	6.50	4.14	1.56	0.12
CryptogamMoss × TreatmentRemoval	-10.12	4.14	-2.44	**0.01**
OMThin × TreatmentMixed	0.87	4.14	0.21	0.83
OMThin × TreatmentRemoval	-0.50	4.41	-0.12	0.90
CryptogamMoss × OMThin × TreatmentMixed	-18.37	5.86	-3.013	**<0.01**
CryptogamMoss × OMThin × TreatmentRemoval	-3.12	5.86	-0.53	0.59

Appendix 8. Diagnostic plots of GLM model on seedling establishment by seedbed type, OM thickness and seedbed manipulation treatments.



Appendix 9. Results of estimated coefficients and their significance test derived from Generalized Linear Model (GLM) on seedling establishment by seedbed type, OM thickness and manipulations treatments (AIC = 218.65). Significant *p*-values are marked in bold.

Coefficients	Estimate	Std. error	z- value	p-value
Intercept	0.69	0.35	1.96	**0.04**
CryptogamMoss	1.98	0.37	5.25	**<0.01**
OMThin	1.55	0.38	4.01	**<0.01**
TreatmentMixed	-2.07	1.06	-1.96	**0.04**
TreatmentRemoval	-0.28	0.54	-0.53	0.59
CryptogamMoss×OMThin	-1.74	0.43	-4.01	**<0.01**
CryptogamMoss × TreatmentMixed	1.80	1.07	1.67	0.09
CryptogamMoss × TreatmentRemoval	-3.77	1.14	-3.29	**<0.01**
OMThin × TreatmentMixed	1.77	1.08	1.62	0.10
OMThin × TreatmentRemoval	0.08	0.59	0.14	0.88
CryptogamMoss × OMThin × TreatmentMixed	-3.69	1.22	-3.01	**<0.01**
CryptogamMoss × OMThin × TreatmentRemoval	1.89	1.24	1.52	0.12

Appendix 10. Results of One-way ANOVA of seedling root length (mm) in laboratory bioassay by cryptogam species collected from post-fire sites, TNNP, Newfoundland. Residuals were normally distributed (W = 0.95, $p = 0.28$) and variances among the treatments were homogeneous (Bartlett's K square = 6.65, $p = 0.15$). Significant *p*-values are marked bold.

Source	df	Sum of Squares	Mean Square	F	p-value
Treatments	4	467.9	117.0	16.7	**< 0.01**
Error	20	137.9	6.9		

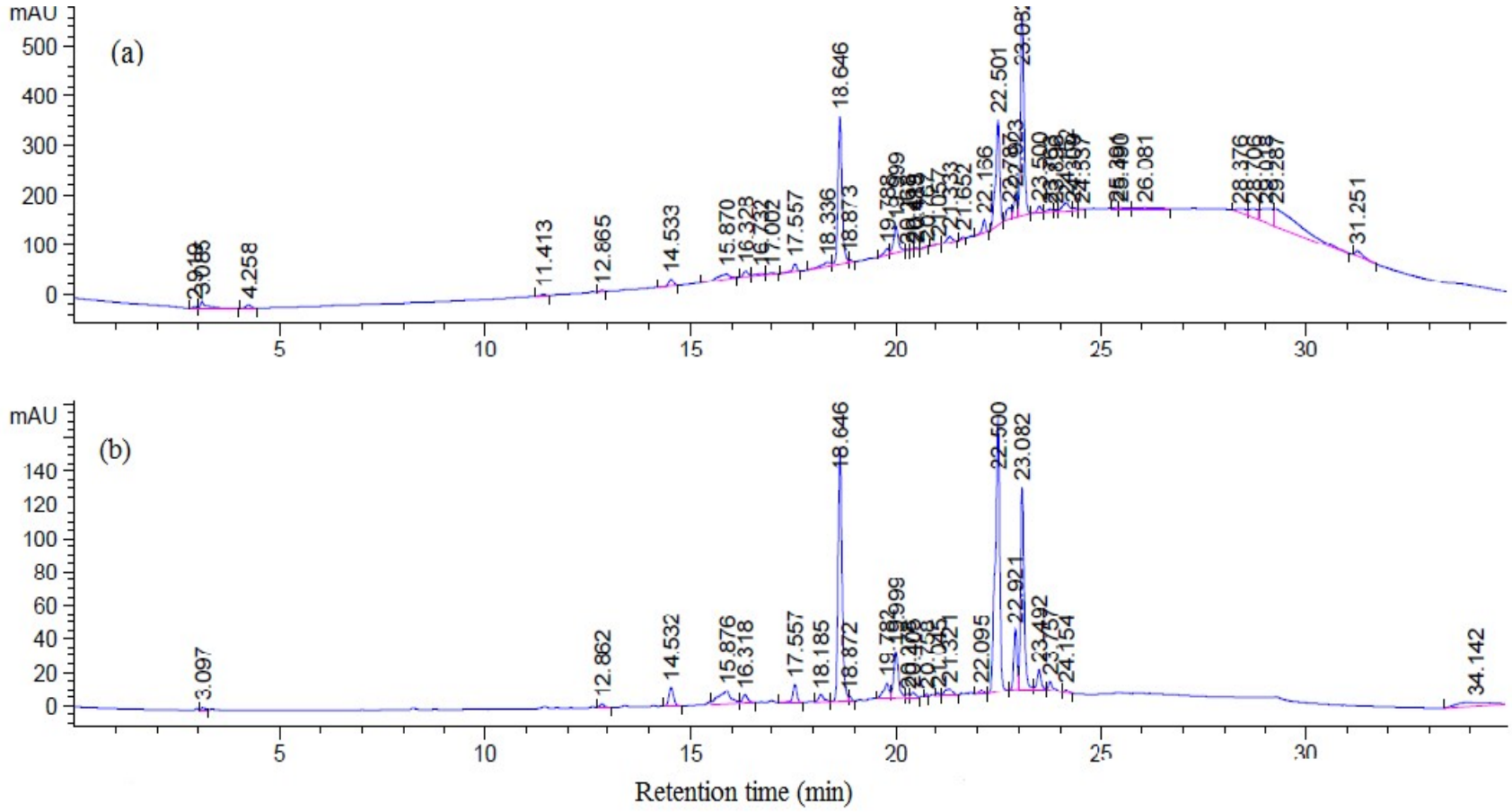
Appendix 11. Results of One-way ANOVA of total seedling shoot length (mm) in laboratory bioassay by cryptogam species collected from post-fire sites, TNNP, Newfoundland. Residuals were normally distributed (W = 0.09, $p = 0.94$) and variances among the treatments were homogeneous (Bartlett's K square = 3.48, $p = 0.48$). Significant *p*-values are marked bold.

Source	df	Sum of Squares	Mean Square	F	p-value
Treatments	4	111.0	27.7	4.8	**< 0.01**
Error	20	114.4	5.7		

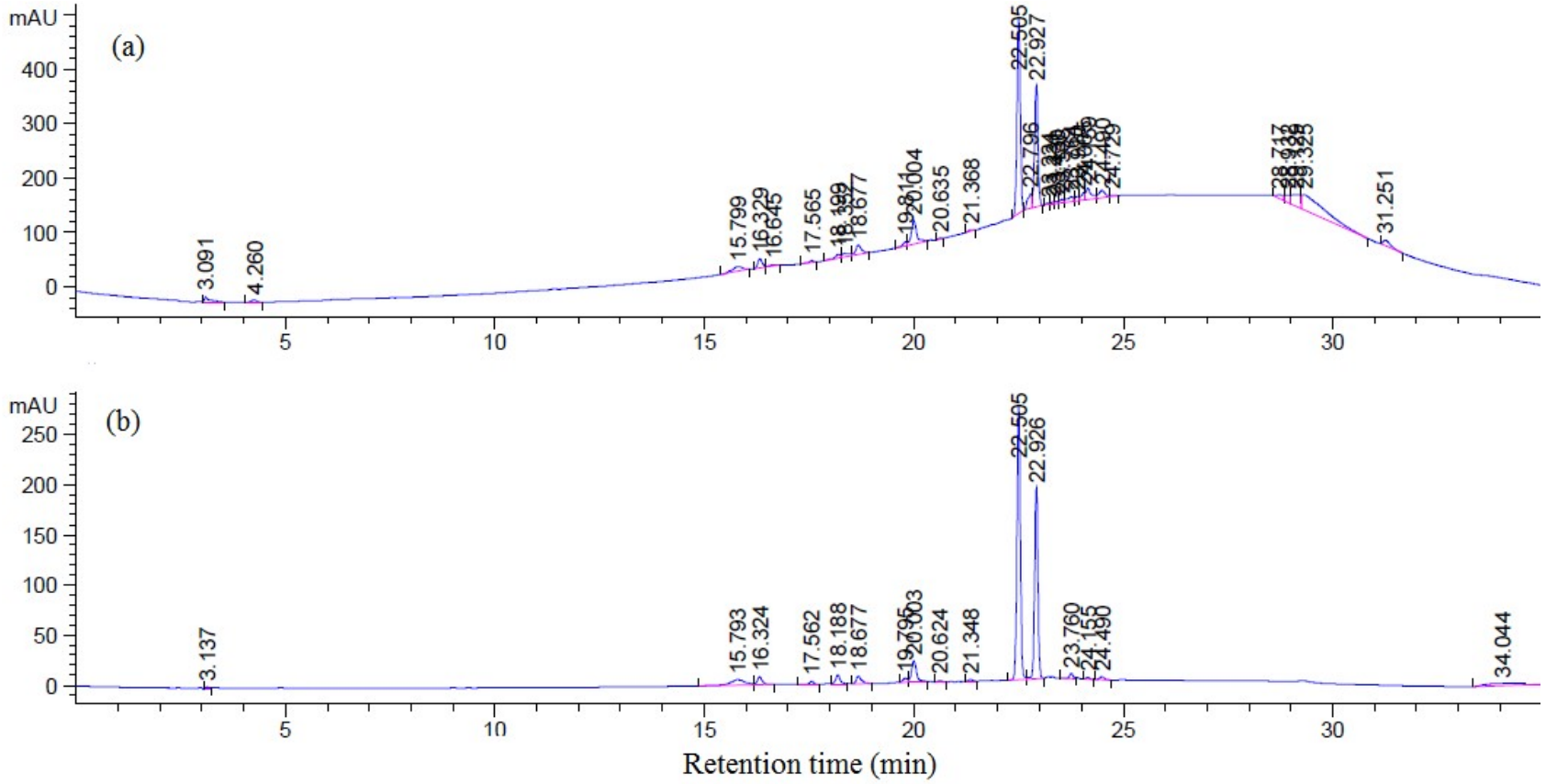
Appendix 12. Peak summary of HPLC chromatograms of lichen *Cladonia cristatella* and *C. stellaris*

Species	Methanol extraction									Acetone-dichloromethane extraction								
	Peak #	RetTime	[min]	Width	[min]	Area	mAU*s	Height	mAU	Area %	Peak #	RetTime	[min]	Width	[min]	Area	mAU*s	Height	mAU	Area %
Cladonia	12	22.50		0.09		1844.14		307.40		42.98	3	3.38		0.08		1793.71		323.13		32.96
cristatella	14	23.08		0.09		1392.11		233.10		32.45	10	22.51		0.09		1311.28		212.60		24.09
	-	-		-		-		-		-	12	23.09		0.09		1144.68		187.92		21.03
Cladina	9	22.51		0.08		2902.61		517.10		72.07	4	3.81		0.08		1958.65		351.29		40.22
stellaris	10	22.93		0.08		599.48		106.70		14.88	8	22.50		0.08		2274.19		406.93		46.70

Appendix 13. Representative chromatograms of HPLC analysis using methanol extraction of lichen *Cladonia cristatella* at (a) 210 nm and (b) 256 nm wavelengths.



Appendix 14. Representative chromatograms of HPLC analysis using methanol extraction of lichen *Cladina stellaris* at (a) 210 nm and (b) 256 nm wavelengths.



Appendix 15. Representative chromatograms of HPLC analysis of standard solution usnic acid at (a) 210 nm and (b) 256 nm wavelengths.