Relationship between lichen species composition, secondary metabolites and soil pH, organic matter, and grain characteristics in Manitoba.

Journal:	<i>Botany</i>
Manuscript ID	cjb-2017-0176.R1
Manuscript Type:	Article
Date Submitted by the Author:	04-Dec-2017
Complete List of Authors:	Zraik, Mohanad; University of Manitoba, Biological Sciences Booth, Thomas; University of Manitoba, Biological Sciences Piercey-Normore, Michele; Memorial University of NL, Science and the Environment
Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	Cladonia, lichen species, organic matter, secondary metabolites, sand particles

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**Relationship between lichen species composition, secondary metabolites and soil pH,
organic matter, and grain characteristics in Manitoba**

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Abstract: Many lichen secondary metabolites have functions related to the environmental conditions of lichen habitats but few studies have compared soil characteristics with lichen species composition or their secondary products. The goal of this study was to investigate the relationship between soil characteristics with lichen species composition and secondary metabolites. Five locations were chosen in Manitoba, each with five sites (transect), and each transect with five quadrats (1 m x 1 m). All species were collected from each of the quadrats, presence of secondary metabolites was determined by thin layer chromatography, and soil characteristics were examined. The results revealed that round sand grains were significantly higher in southeast Manitoba than other locations corresponding with a distinct species composition. Angular grains were significantly higher in northern locations, corresponding to a different group of species. Some of the significant relationships between soil characteristics and secondary metabolites include correlations of atranorin with pH, organic matter and sand content; fumarprotocetraric acid with organic matter and sand content; and usnic acid with pH and organic matter. A better understanding of the role of lichens with respect to soil characteristics will be important for improving soil stabilization in land reclamation.

Keywords: *Cladonia*, lichen species, organic matter, sand particles, secondary metabolites.

47 **Introduction**

48 The effect of environmental conditions on individual lichen species has been shown through
49 studies where environmental variables were linked to morphological differences (Gilbert 1977;
50 Goffinet and Bayer 1997; Pintado et al. 1997; Baloch et al. 2010; Kotelko and Piercey-Normore
51 2010; Cornejo and Scheidegger 2013; Muggia et al. 2014). Some of these studies supported the
52 optimal defence theory (McKey 1974; Rhoades 1979; Asplund et al. 2010) where secondary
53 metabolite production was correlated with herbivory, and abiotic variables such as light
54 (McEvoy et al. 2006; Armaleo et al. 2008), pH (Fox and Howlett 2008; Timsina et al. 2013), and
55 humidity (Culberson and Armaleo 1992; Stocker Wörgötter 2001). However, few studies have
56 examined the relationship between substrate and the lichen phenotype or secondary metabolite
57 production (Brodo 1973; Gilbert 1977). Additionally, the substrate has been examined with
58 respect to water soluble nutrients available for lichen soil crusts (Fisher et al. 2014), substrate
59 moisture in an Alaskan tundra (Williams et al. 1978), and as an important source of nutrients by
60 fungal enzyme activity (Moiseeva 1961). The role of the soil in nutrient and moisture availability
61 corresponded with species distribution (Jun and Rozé 2005) and would be expected to depend to
62 some degree on the extent of direct contact between the soil and the lichen thallus. To our
63 knowledge, no study has examined the relationship between soil characteristics with lichen
64 species composition or secondary metabolite production.

65 Secondary metabolites produced by lichen-forming fungi comprise diverse groups of
66 metabolites (Elix and Stocker-Wörgötter 2008). A well-studied group includes the polyketides,
67 which are produced by the fungal partner in the acetyl-polymalonyl pathway. A large body of
68 literature is available on polyketide production in non-lichenized fungi (Keller and Hohn 1997;
69 Sanchez et al. 2010; Chen et al. 2014) and it is growing for the lichenized fungi (Stocker-

Wörgötter 2001; 2008). While the biosynthesis of some polyketides from non-lichenized fungi is being unravelled (e.g., Chiang et al. 2009; Campbell and Veraras 2010; Chiang et al. 2010; Weissman 2015), knowledge of the biosynthesis of the many unique compounds from lichenized fungi is lagging behind, with some progress made in usnic acid (Abdel-Hameed et al. 2016). Better knowledge of the conditions required for culturing the lichen fungus to induce polyketide production would facilitate progress on these studies. Since lichen polyketides are produced in nature, it might be expected that a better understanding of the natural conditions, including the substrate, would facilitate culturing. The potential for the application of polyketides in industry or health related fields has increased the interest in this group of compounds.

Polyketide production has been hypothesized to be associated with slow growth of the fungus. For example, it has been suggested that overwintering stages, reproduction, and other life stages may cause slow growth (Bu'Lock 1961; Calvo et al. 2001; Timsina et al. 2013). Polyketide production may also be regulated by the carbon-nutrient balance hypothesis (Bryant et al. 1983; Hyvarinen et al. 2003), which is based on the concept that an imbalance of nutrients will promote production of carbon-based secondary metabolites. The hypothesis suggests that when a plant or lichen is growing in nutrient-poor conditions, the carbon may be shunted into carbon-based secondary metabolites such as polyketides. When the plant or lichen is growing in nutrient rich and balanced conditions the carbon may be used in thallus growth rather than production of secondary metabolites. However, the hypothesis is controversial because it represents a complex model with many interacting variables (Koricheva 2002). For example, soil features and community structure may influence the nutrients available to lichens and their growth rates; therefore, they may indirectly influence production of secondary metabolites in lichens.

Some lichens are extreme stress tolerators and able to adapt to harsh conditions on sandy soils in areas where plants are absent, reducing the competition on lichens and allowing them to dominate in these locations (Botting and Fredeen 2006). Jun and Rozé (2005) found a significant relationship between the edaphic parameters (soil pH, water content, nutrient content) and the distribution of species for lichens and bryophytes in the sand dunes of the French Atlantic coast. The content of organic material and carbon also affected the occurrence of some species in sandy areas. For example, the occurrence of some species of *Cladonia*, such as *Cladonia arbuscula*, *C. mitis*, and *C. portentosa*, are favoured by the acidification of the substrate (James et al. 1977), while differences in the occurrence of *C. pocillum* and *C. pyxidata* corresponded with the pH of the soil on which they were growing (Kotelko and Piercey-Normore 2010).

The goal of this study was to investigate the relationship among secondary metabolites, lichen species composition and soil characteristics in five locations in Manitoba. The soil characteristics were examined to determine which characteristics had the largest influence on species and secondary metabolite occurrence.

Methods:

Experimental design and sample collection

Field collections were made in five different locations in northern and southern Manitoba (Fig. 1), and were focused in areas with sandy soils (Table 1). The five locations have a continental climate and contain soils which vary in underlying geology (Table 1; Fig. 1; Geological Survey of Canada 2006). Samples of all species of lichens were collected from each of five quadrats (1 m x 1 m) in each of five sites (transects) in each of five locations (125 quadrats in total). The quadrats were placed every 5 m along a 25 m transect within each site and the upper right corner

of the quadrat was placed over a lichen thallus. The percent ground cover of the species recorded as guilds and other biotic (vascular plants, bryophytes, and lichens by general categories) and abiotic (bare rock, exposed soil, and vegetative debris such as twigs, cones, leaves, etc.) factors were recorded by visual estimation to the nearest 5% using a 25 cm x 25 cm grid placed within the quadrat for estimating coverage. Percent canopy cover was estimated using the same grid and held directly above the quadrat to estimate the amount of light. Specimens were air dried and returned to the lab for identification using Ahti (2000), Brodo et al. (2001), Hinds and Hinds (2007), and Kowalewska et al. (2008). In order to determine the secondary metabolites present, thin layer chromatography (TLC) was performed on all specimens using solvent A (toluene 185 mL: dioxane 45 mL: glacial acetic acid 5 mL) (Orange et al. 2001). Briefly, about 200 uL of acetone was applied to the crushed thallus samples which were left soaking for 20 minutes. Acetone extracts were applied to silica-coated glass plates, immersed within solvent A in a TLC tank until the solvent front reached the top of the plate. After drying, the plates were observed under long (365 nm) and short (255 nm) wave UV light, sprayed with 10% sulphuric acid, and heated at 80°C for 20 minutes. Standard secondary metabolites as positive controls were prepared from herbarium specimens that had the chemicals previously identified for comparison. Known Rf classes and further spot comparisons were made with characteristics in Orange et al. (2001). Frequency of occurrence for secondary metabolites was recorded.

Soil characteristics

Two soil samples (multiple representative samples and a single bulk sample) were collected from each of the 125 quadrats (25 in each of 5 locations). Multiple samples were collected from different places in the quadrat and placed within clean plastic bags (about 1kg) to represent the

surface soil in the quadrat (0 to 7 cm depth) and to determine soil characteristics. Bulk density samples were collected from the center of the quadrat using a standard bulk density cylinder (7.62 cm height X 7.62 cm diameter). Organic matter was calculated as loss of carbon on ignition by burning the soil samples at 650°F for 18 hours using a muffle furnace (Barnstead/Thermolyne). Percentage of the main soil components (sand, silt and clay), and gravel as an additional soil component, were determined by sieving the soil samples with different soil sieves using the Canadian standard sieve sizes (Fisherbrand U.S. Standard Brass Test Sieves; sieve number 10 (2.00 mm opening) to collect the silt and clay grains, sieve numbers 35 (0.50 mm opening) and 100 (0.149 mm opening) to collect the fine and coarse sand, respectively, and sieve number 200 (0.074 mm opening) for rocks and pebbles). Shape of the sand grains was also determined and divided into two main categories (angular and rounded) (Arculus et al. 2015) for 125 samples from each of 125 quadrats using a dissecting microscope (Leica MZ 6) at 10× magnification. The grain shape was determined from a soil analysis kit (Forestry Suppliers, Inc. MI). A distilled water mixture with soil was prepared for measuring the pH of the soil using a pH meter (VWR syphony SB70P). The pH measures were performed according to standard methods (Benton and Jones 1999).

Statistical analyses

Principle Coordinates Analysis (PCoA; Legendre and Legendre 1998) was used to test the similarities among lichen species composition using the R package “vegan” (Oksanen et al. 2015). The studied species were scored as present or absent. To examine whether soil characteristics influenced secondary metabolite frequency and species compositions, redundancy analyses were performed using the “rda” function in the R package “vegan”. Monte Carlo

permutation tests were performed to test the significance of redundancy analysis. The frequency of occurrence of secondary metabolites was tested by recording the number of quadrats in which each secondary metabolite was found. The soil pH levels were divided into two groups: high acidic soil (pH ranging from 4.2 to 5.7) and neutral to slightly acidic soil (pH ranging from 6.1 to 7.4) to compare frequency of secondary metabolites and lichen species. Significance tests for soil characteristics, biotic and abiotic factors, species frequency, and secondary metabolite frequency were performed using ANOVA by the JMP program 12.1.0 (64 bit), and comparison of the means was done by Tukey HSD. The frequency of secondary metabolites and species occurrence (number of quadrats in which each secondary metabolite or species was detected) was examined with location as a categorical variable. To test the relationship between soil features (organic matter, pH, and sand content) and occurrence of secondary metabolites as continuous variables, a correlation was performed using the JMP program 12.1.1. Statistical analyses were performed with the R program (R Core Development Team 2013), using a significance level of $\alpha = 0.05$ unless otherwise indicated.

Results

Soil characteristics for each of the five locations are described in Table 2. Gravel content was highest in the Sandilands Provincial Forest ($18.2 \pm 3.2\%$). The sand content in Spruce Woods Provincial Park ($92.6 \pm 0.5\%$) was significantly higher than that of the other four locations. The finest sand grains were in the Sandilands Provincial Forest and Spruce Woods Provincial Park; and the coarsest sand grains were in North Star and Athapap. The percent of rounded sand grains was significantly higher in Sandilands Provincial Forest ($82.2 \pm 2.1\%$) than other locations while angular grains were more abundant in North Star than other locations ($84.9 \pm$

2.2% though not significantly different from Sherridan). The highest Round/Angular (R/A) ratio was in Sandilands Provincial Forest. Clay and silt levels were significantly higher in Athapap ($35.8 \pm 2.0\%$) than in other locations. Organic matter was significantly higher in Sherridon and Athapap than the other locations ($28.6 \pm 5.7\%$ and $27.9 \pm 5.6\%$, respectively). The pH level was significantly higher in Spruce Woods Provincial Park than in North Star but there was no difference between the other three locations (Table 2).

The percent canopy and ground covers for the quadrats in each location are shown in Table 3. Briefly, Sherridon, and Athapap had a significantly higher percentage of canopy cover compared to Sandilands Provincial Forest and Spruce Woods Provincial Park. North Star showed a significantly higher percentage of ground cover of lichens than Athapap and Sandilands (Table 3). Bryophyte ground cover was significantly higher in Athapap. The cup-forming lichens were significantly higher in Spruce Woods Provincial Park (except North Star), while mat-forming lichens were significantly higher in Sherridon than other locations. *Stereocaulon* and crustose lichens were significantly higher in North Star than the other locations (except Sherridan), the red-fruited lichens were significantly higher in Athapap, but the foliose lichens showed no significant differences among locations. Other factors analysed included vegetative debris, which was high in Sherridan, exposed soil, which was high in Sandilands Provincial Forest, and bare rock, which was high in Sherridan and North Star.

Forty-six lichen species are reported in this study with greater than 10% frequency of occurrence (Table 4). Four species, *Cladonia arbuscula*, *C. chlorophaea*, *C. gracilis* ssp. *turbinata*, and *C. pyxidata* were the most frequently occurring species being present in 21 or 22 out of 25 total sites (transects) and were present in all five locations. Three species (*Cladonia humilis*, *C. magyarica*, and *C. sulphurina*) were found in one of the five locations (Table 4).

Secondary metabolites with more than 20% frequency of occurrence among the five locations are shown in Table 5. Zeorin, barbatic acid, and usnic acid were significantly high in Sherridon and North Star. Squamatic acid and thamnolic acid were significantly high in North Star. The presence of atranorin was significantly high in Spruce Woods Provincial Park. Fumarprotocetraric acid and perlatolic acid were recorded in high frequency in all the five locations and there were no significant differences among them.

The relationship between location and each of the soil characteristics is shown in Fig. 2. In this PCoA, the horizontal axis explains 51.4% of the variation in the data, and the vertical axis accounts for an additional 21.6%. Spruce Woods Provincial Park clustered at the positive end of the horizontal axis with higher sand content and fine sand, while Athapap, North Star, and Sherridon clustered at the negative end of the horizontal axis. Sandilands Provincial Forest clustered at the positive end of the horizontal axis with rounded sand grains. The PCoA showed three main groups (Sandilands Provincial Forest, North Star, and Spruce Woods Provincial Park) which were separated based on the soil characteristics (Fig. 2). Athapap and Sherridan had more variable soil characteristics.

A highly significant relationship between the secondary metabolites and the soil characteristics is shown by a Redundancy Analysis (Monte Carlo permutation test, $p = 0.001$) (Fig. 3). The RDA axis 1 accounted for 62.2% of the variation in the data and axis 2 accounted for an additional 22.8% of the variation. Some of the trends show that fumarprotocetraric acid and atranorin were more commonly produced when lichens were growing in sandy fine soils. Grayanic acid, thamnolic acid and merochlorophaeic acid were weakly associated with more neutral pH levels (Fig. 3). Usnic acid, zeorin, and barbatic acid were associated with angular

sand grains (Fig. 3). The sand content was negatively correlated with organic matter (Pearson's product-moment correlation $r = -0.89\%$ $p = 0.001$).

A significant relationship was found between species and soil characteristics (Fig. 4). The RDA axis 1 accounts for 44.81% of the variation and axis 2 accounts for an additional 19.93% of the variation in the data. The trends show that *Peltigera rufescens* was associated with round sand grains while *Cladonia deformis*, *C. phyllophora*, *C. stellaris*, *Stereocaulon tomentosum*, and *Vulpicida pinastri* were more associated with the angular sand grains. It also suggested that *C. cariosa* is associated with soil pH levels and *C. humilis* and *C. magyarica* are associated with fine sand and total amount of sand (Fig. 4).

The correlation between soil characteristics and secondary metabolites showed strong relationships. There were strong negative correlations between zeorin, usnic acid, barbatic acid and squamatic acid with pH of the soil but the correlation between atranorin and pH was strongly positive (Table 6). The correlation with organic matter was strongly positive with zeorin and usnic acid, but it was strongly negative with atranorin and fumarprotocetraric acid. The correlations between percent sand content and each of atranorin and fumarprotocetraric acid were strongly positive (Table 6).

The correlation between soil characteristics and species occurrence also showed strong relationships (Table 7). Some species (*C. deformis*, *C. amaurocraea*, *C. gracilis* spp. *turbinata*, *C. stellaris*, *Stereocaulon tomentosum*, and *Vulpicida pinastri*) showed a strong negative relationship with pH. A strong positive relationship was present between two species (*C. humilis*, *C. magyarica*) and the percent sand content in soil (Table 7).

Discussion

Soil characteristics may explain some species distributions

The significant differences among the five locations were explained by differences in the soil characteristics and partially by species composition. This finding suggests that some species may not rely on the substrate for nutrients and moisture and have a wide tolerance for substrate characteristics while other species may be linked to soil characteristics. For example, *Peltigera rufescens*, *Cladonia cariosa*, *C. humilis*, *C. scabruiscula*, and *C. magyarica* segregated with rounded sand grains (Fig. 2 and Table 2). Furthermore, *Arctoparmelia centrifuga*, *Stereocaulon tomentosum*, *C. deformis*, *C. stellaris*, *C. uncialis* and *C. amaurocraea* correlated more strongly with angular sand grains. The angular shape of the sand grains may minimize the spaces between sand grains by fitting together but the round shape may allow for more space between grains and therefore more water and air movement between grains (Lipiec et al. 2016). The shape of sand grains has been shown to affect the compaction, compressibility and shear stress of soils and therefore also the aeration, water holding capacity, and temperature regulation (Horn 2011; de Bono and McDowell 2015). The thick mat of rhizines diagnostic of *P. rufescens* and the persistent basal squamules in *C. cariosa*, *C. humilis*, and *C. magyarica* would help to stabilize sand better than tall podetia with disintegrating basal squamules such as *Cladonia gracilis* ssp. *turbinata*, *C. cornuta*, *C. amaurocraea* or *C. uncialis*. This hypothesis would need further testing.

Cladonia phyllophora and *C. gracilis* ssp. *turbinata* are common boreal species (Brodo et al. 2001) which look very similar and were both present in all five locations suggesting they may be tolerant of a wide range of soil conditions. They may receive their nutrients from a combination of the soil (especially when they are young with squamules attached directly to the

soil) and the atmosphere (especially as they age as the squamules disintegrate). *Cladonia*
phyllophora has no cortex near the top of a tall podetium and a black mottled base whereas *C.*
gracilis ssp. *turbinata* has a smooth cortex giving it an olive color throughout the length of a tall
podetium. The similar morphological features in these species, where both species produce tall
podetia and the primary basal squamules disintegrate with time, suggests that the direct
connection with the soil by the primary squamules may be severed after the podetia have been
produced. It would stand to reason that after squamule disintegration, the colony of tall podetia
must rely predominantly on an atmospheric source of nutrients and moisture or splash from the
substrate or adjacent mosses. Few studies have been conducted on the source of nutrients;
however, the substrate has been shown to be correlated with lichen growth for water soluble
nutrients in lichen soil crusts (Fisher et al. 2014), in an Alaskan tundra (Williams et al. 1978),
and an important source of nutrients through enzyme activity (Moiseeva 1961). While early
squamule growth of these species may penetrate the soil particles and offer some stabilization
properties for disturbed soils, the later podetial growth may be more independent of the soil
characteristics.

High levels of organic matter may reduce the pH level of the soil, making the soil more
acidic (Sparks 2003). Acidic soils seem to be more favourable for *C. amaurocraea*, *C.*
arbuscula, and *C. deformis*, while *C. cariosa*, *C. pocillum* and *C. magyarica* are known to grow
in calcareous substrata (Culberson 1969; Wetherbee 1969; Brodo et al. 2001). While *C. pocillum*
was limited to the less acidic soils in Athapap and Spruce Woods Provincial Park (Table 4), *C.*
pyxidata was more widespread showing no correlation with pH (Table 7) suggesting that acidic
substrata may have been available or the species can tolerate less acidic soils. Species such as *C.*
chlorophaea and *C. rangiferina* were found in both acidic and neutral soils suggesting they may

also be more tolerant of pH changes in the substrata than some other species. While some chemotypes of the *C. chlorophaea* complex prefer only calcareous substrata such as *C. cryptochlorophara*, *C. grayi*, *C. pocillum* (Gilbert 1977; Piercey-Normore 2006) and *C. cariosa* (Culberson 1969), *Cladonia merochlorophaea* and *C. pyxidata*, are known to grow on acidic substrates (Brodo et al. 2001). Patches of soil or substrata with higher pH levels even in a location characterized by low pH levels may explain the presence of these species. For example, *C. merochlorophaea* was reported to grow directly on moss over calcareous soil where the moss provided the acidic substrate for the lichen (Piercey-Normore 2005; 2006; 2008).

Soil characteristics and the carbon-nutrient balance hypothesis

The carbon-nutrient balance (CNB) hypothesis (Bryant et al. 1983; Hyvarinen et al. 2003) was not supported by the results of this study. Lichens such as *Cladonia arbuscula* and *C. rangiferina* are mat-forming lichens since they grow acropetally forming a carpet of upright thalli in which the crustose primary thallus disintegrates over time and the upright thallus often grows well above the soil layer. The formation of this mass of vegetative thallus suggests that more carbon was needed to produce a larger mass of thallus (Crittenden 2000). If the CNB hypothesis was supported, the larger mass would have corresponded with fewer secondary metabolites, but this was not the case. Some species with this growth form were more abundant in locations with high organic matter in the soil such as *C. uncialis*, *C. stellaris*, and *C. amaurocraea*, which were predominantly in Sherridan and North Star. However, other species such as *C. arbuscula* and *C. rangiferina* were present in 4 or 5 of the locations suggesting they could tolerate a wider range of organic matter in the soil. Only *C. arbuscula*, of these mat-forming species, was present in Athapap, which also had significantly higher levels of organic matter than other locations. The

322 larger amount of carbon in the soil with more organic matter may allow for more vegetative
323 growth in these mat-forming species, but all species produced similar numbers of carbon based
324 secondary metabolites. While the numbers of secondary metabolites in this study showed no
325 support for the carbon-nutrient balance hypothesis, it may be hypothesized that the quantity of
326 each secondary metabolite may show more support for the hypothesis.

327

328 *Soil features may influence the occurrence of some secondary metabolites*

329 The distribution of usnic acid and atranorin, but not fumarprotocetraric acid, may be explained
330 by soil characteristics. The presence of usnic acid was significantly higher in the more acidic
331 sites of Sherridon and North Star (Table 6), where usnic acid may help the lichen tolerate acidic
332 substrata (Hauck et al. 2009a). Usnic acid may also serve as an anti-herbivorous substance
333 (Nimis and Skert 2006) or as a sun-screening metabolite (Hauck 2008). Lichen species that
334 produce usnic acid were more dominant on acidic substrata such as *Cladonia amaurocraea*, *C.*
335 *arbuscula*, *C. cristatella*, *C. deformis*, *C. stellaris*, and *C. uncialis* (Table 3; Hauck 2009b;
336 Doering et al. 2016). On the other hand, production of atranorin and fumarprotocetraric acid may
337 also be affected by sand acidity levels. The presence of atranorin in lichen species in the less
338 acidic sites, such as Spruce Woods Provincial Park and to a lesser extent Athapap (Table 5), may
339 reflect the higher pH levels in the habitat rather than light or humidity levels in the environment.
340 If light or humidity played a role in the distribution of thalli containing atranorin, the canopy
341 cover should influence their distribution. However, Spruce Woods Provincial Park had the
342 lowest canopy cover and Athapap had the highest canopy cover (Table 3), which does not
343 support the light regulating properties of atranorin to make light available for the photobiont

(Melo et al. 2011). However, shrub or herbaceous canopy layers may have played a role in the amount of light reaching the lichens and therefore the presence of atranorin in the lichen thallus.

The pH of the soil alone does not seem to influence the type of secondary metabolite produced by lichens in this study. Species containing fumarprotocetraric acid were reported to be more common in acidic sites such as on peat soils (Purvis et al. 1992) or on the bark of conifers (Gauslaa et al. 1998) and the production of fumarprotocetraric acid was thought to increase the tolerance of lichens for acidic substrates (Hauck 2008; Hauck 2009a). However, fumarprotocetraric acid was present in all five locations with no significant difference in frequency of occurrence (Table 5). Culberson et al. (1977) reported a correlation with the amount of fumarprotocetraric acid production in the *C. chlorophaea* group with distance from the ocean in North Carolina. The frequency of occurrence of 13 other fumarprotocetraric acid-containing species varied among locations with different pH levels suggesting that pH was not affecting the production of this compound.

Conclusion

The physical and chemical components of the soil substrate have been previously assumed to be important factors in species assemblages but this is one of the first studies to closely examine soil characteristics relative to species and secondary metabolite occurrence. The finding that many of the species, with some exceptions, were not influenced by soil characteristics, supports the concept that lichens receive their nutrients and moisture from atmospheric sources. However, the few species that showed relationships with soil, highlight valuable implications for stabilizing soils and provide further hypotheses for testing. The carbon-nutrient balance hypothesis was not supported in this study since all species produced some secondary metabolites even when carbon

was present in the soil. However, the quantification, rather than occurrence, of the secondary metabolites in lichen species may be used in future studies to test the carbon-nutrient balance hypothesis more directly. The sand content and shape of the soil particles seemed to have the greatest effect on species composition and the occurrence of some secondary metabolites in this study, but the correlations provide insights that need to be further tested. These novel findings are partially exploratory and draw upon relationships among variables that have not previously been examined.

Acknowledgements

The authors thank J. Doering and C. Deduke for assistance in the field and statistical analysis. This project was supported by a Faculty of Science award to MZ, GETS to MPN for MZ, and an NSERC Discovery Grant to MPN, and collection permits from Manitoba Conservation and Parks Canada.

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Table 1. General characteristics (rows) of the locations (columns) of collection sites (transects) including latitude and longitude, prevalent parent bedrock material, maximum and minimum yearly temperatures, and location descriptions with dominant vegetation.

Characteristics	Sherridon Road	North Star Road	Athapap Road	Spruce Woods Provincial Park	Sandilands Provincial Forest
Latitude and longitude	N54° 77' 72.9" W101° 47' 37.5"	N54° 69' 75.4" W101° 68' 12.3"	N54° 51' 58.8" W101° 53' 19"	N49° 43' 45.4" W99° 17' 29.2"	N49° 44' 31.9" W96° 39' 22.8"
Parent material	Granite	Gneiss	Calcaric regosols	Aeolian regosols	Glacial till regosols
Temp (°C) max/min	23.8/-24.7	23.8/-24.7	24/-28.9	26/-24.3	26/-23.2
Location description and dominant plant species	Precambrian Shield. Patches of soil in crevices of bedrock and among outcrops. Aspen, birch, white spruce, Jack pine, willow and alder.	Precambrian Shield. Patches of soil in crevices of bedrock and among outcrops. Aspen, birch, white spruce, Jack pine, willow and alder.	Shallow regosol on limestone bedrock. Mixed forest with aspen, white spruce, fir, white birch, and alder.	Active and stabilized sand dunes. Grasses and bluffs of white spruce, aspen, and juniper in depressions. Oaks on high ridges.	Rolling forest and sandy till. Jack pine, aspen, and white spruce interspersed with low lying black spruce stands.

Table 2. Soil characteristics in five sites (transects) across five locations in this study. Values are mean $\pm$ standard error derived from five one-m² quadrats from each of five transects for each of five locations. Soil characteristics include gravel, sand, fine sand, coarse sand, rounded grain, angular grain, organic matter (in percentage of fraction of total weight), ratios of round to angular grains (R/A), and pH levels. R/A ratios are calculated as mean $\pm$ standard error over the individual values of R/A for the 25 plots sampled in each of the locations. Bold numbers represent significant values at the given probability levels in the first column for each soil characteristic. Upper case letters indicate significant differences within rows at $p < 0.05$ using Tukey’s HSD test.

Soil Characteristics	Sherridon Road	North Star Road	Athapap Road	Spruce Woods Provincial Park	Sandilands Provincial Forest
Gravel ($p = 0.0001$)	11.7 $\pm$ 1.7 ^{AB}	9.8 $\pm$ 0.9 ^B	8.9 $\pm$ 1.1 ^B	6.4 $\pm$ 0.5 ^B	18.2$\pm$3.2^A
Sand ($p < 0.0001$)	65.8 $\pm$ 3.9 ^C	60.2 $\pm$ 1.6 ^{C D}	54.8 $\pm$ 2.6 ^D	92.6$\pm$0.5^A	78.7 $\pm$ 3.2 ^B
Fine sand ($p < 0.0001$)	61.6 $\pm$ 3.8 ^B	39.2 $\pm$ 1.4 ^C	46.3 $\pm$ 0.8 ^C	89.3$\pm$1.4^A	87.1$\pm$1.7^A
Coarse sand ($p < 0.0001$)	38.4 $\pm$ 3.8 ^B	60.2$\pm$1.4^A	53.6$\pm$0.8^A	10.7 $\pm$ 1.4 ^C	12.9 $\pm$ 1.7 ^C
Rounded grain (R) ($p < 0.0001$)	28.7 $\pm$ 4.0 ^D	15.2 $\pm$ 2.1 ^E	57.6 $\pm$ 3.3 ^B	40.0 $\pm$ 1.2 ^C	82.8$\pm$2.1^A
Angular grain (A) ($p < 0.0001$)	71.3 $\pm$ 5.4 ^{AB}	84.9$\pm$2.2^A	42.5 $\pm$ 4.6 ^C	60.1 $\pm$ 1.0 ^B	17.1 $\pm$ 3.4 ^D
R/A ratio ($p < 0.0001$)	0.62 $\pm$ 0.2 ^B	0.19 $\pm$ 0.03 ^B	1.77 $\pm$ 0.2 ^B	0.68 $\pm$ 0.03 ^B	8.22$\pm$1.6^A
Clay and silt ($p < 0.0001$)	18.2 $\pm$ 3.4 ^C	27.3 $\pm$ 1.7 ^B	35.8$\pm$2.0^A	0.8 $\pm$ 0.1 ^D	2.7 $\pm$ 0.4 ^D
Organic matter ($p = 0.001$)	28.6$\pm$5.7^A	17.9 $\pm$ 3.5 ^B	27.9$\pm$5.6^A	1.7 $\pm$ 0.3 ^C	5.4 $\pm$ 1.1 ^C
pH level ($p = 0.001$)	5.54 $\pm$ 0.4 ^{BC}	4.98 $\pm$ 0.3 ^C	6.62 $\pm$ 0.1 ^{AB}	7.00$\pm$0.2^A	6.38 $\pm$ 0.2 ^{AB}

Table 3. Mean percentage of quadrat cover including canopy, vascular plants, vegetative debris, bryophytes, lichens (total and by general categories), unvegetated soil, and bare rock over five one square meter quadrats for each of five sites (transects) in each of the locations. Bold numbers represent significant values at the given probabilities for each cover characteristic. Upper case letters indicate significant differences within rows at $p < 0.05$ using Tukey's HSD test.

Cover	Location	Sherridon Road	North Star Road	Athapap Road	Spruce Woods Provincial Park	Sandilands Provincial Forest
Canopy cover ($p < 0.0001$)		36±7.2^A	28±5.7 ^{AB}	34.4±6.1^A	3.8±2.5 ^C	11.6±4 ^{BC}
Vascular plants ($p = 0.1000$)		4.9±1.4 ^A	15.1±3.8 ^A	17.1±3.3 ^A	10.6±3 ^A	12.6±4.3 ^A
Vegetative debris ($p = 0.0030$)		26.5±5.8^A	14.4±4.1 ^{AB}	11.8±2.4 ^B	7.0±1.9 ^B	11.2±2.5 ^B
Bryophytes ($p < 0.0010$)		10.0±2.4 ^B	2.0±0.6 ^B	29.0±5.4^A	6.4±2.4 ^B	7.3±1.7 ^B
Lichens total ($p = 0.0070$)		49.8±4.8 ^{AB}	56.64±5.9^A	35.8±4.9 ^B	46.3±4.3 ^{AB}	34±4.5 ^B
Cup lichens ($p < 0.0001$)		18.7±5 ^B	30.6±5 ^{AB}	16.4±4.5 ^B	44.3±4.4^A	20.4±3.3 ^B
Red-fruited lichens ($p < 0.0001$)		0.28±0.09 ^B	0.44±0.1 ^B	14.1±4.4^A	0.2±0.08 ^B	0.42±0.9 ^B
Crustose lichens ($p < 0.0001$)		0.00± ^B	13.8±3.7^A	1.1±0.6 ^B	0.00 ^B	0.00 ^B
<i>Stereocaulon</i> ($p < 0.0001$)		1.1±0.6 ^{AB}	3.4±1.3^A	0.2±0.13 ^B	0.00 ^B	0.00 ^B
Mat-forming lichens ($p < 0.0001$)		29.3±4.8^A	6.7±2.3 ^B	1.3±0.6 ^B	1.3±1 ^B	11.2±3.6 ^B
Foliose lichens ($p = 0.052$)		0.16±0.07 ^A	1.3±0.67 ^A	2.7±1 ^A	0.5±0.4 ^A	2±0.8 ^A
Exposed soil ($p = 0.0005$)		0.12±0.07 ^B	1.8±0.8 ^B	2.6±1.2 ^B	4.6±1.2 ^{AB}	10.3±3.1^A
Bare rock ($p = 0.0011$)		5.4±0.6^A	5.1±1.4^A	0.72±0.6 ^{AB}	0.00 ^B	0.00 ^B

Table 4. Frequency of occurrence (means $\pm$ standard error (SE)) of lichen species¹ over 25 one-square meter quadrats per site (transect) in each of the five locations. Bold numbers are significant at the given probabilities for each species as shown in the first column. Upper case letters indicate significant differences within rows at $p < 0.05$ using Tukey's HSD test.

Location	Sherridon	North Star	Athapap	Spruce Woods	Sandilands
<i>Arctoparmelia centrifuga</i> ($p < 0.0001$)	0.00 ^C	0.64$\pm$0.09^A	0.36 $\pm$ 0.09 ^B	0.000 ^C	0.00 ^C
<i>Cladonia amaurocraea</i> ($p < 0.0001$)	0.40$\pm$0.10^A	0.24$\pm$0.08^A	0.00 ^B	0.00 ^B	0.00 ^B
<i>Cladonia arbuscula</i> ($p < 0.0001$)	1.00$\pm$0.0^A	0.64 $\pm$ 0.09 ^B	0.44 $\pm$ 0.1 ^{BC}	0.16 $\pm$ 0.08 ^C	0.52 $\pm$ 0.10 ^{BC}
<i>Cladonia cariosa</i> ($p < 0.0001$)	0.00 ^B	0.00 ^B	0.56$\pm$0.10^A	0.36$\pm$0.09^A	0.04 $\pm$ 0.04 ^B
<i>Cladonia chlorophaea</i> ($p = 0.0196$)	0.52$\pm$0.10^{AB}	0.68$\pm$0.09^A	0.68$\pm$0.09^A	0.28 $\pm$ 0.09 ^B	0.44 $\pm$ 0.10 ^{AB}
<i>Cladonia cornuta</i> ($p = 0.0450$)	0.48$\pm$0.10^A	0.28 $\pm$ 0.09 ^{AB}	0.12 $\pm$ 0.06 ^B	0.32 $\pm$ 0.09 ^{AB}	0.28 $\pm$ 0.09 ^{AB}
<i>Cladonia crispata</i> ($p = 0.0650$)	0.12 $\pm$ 0.06 ^{AB}	0.16 $\pm$ 0.07 ^A	0.00 ^A	0.04 $\pm$ 0.04 ^A	0.00 ^A
<i>Cladonia cristatella</i> ($p = 0.0010$)	0.24$\pm$0.08^{AB}	0.44$\pm$0.10^A	0.04$\pm$0.04^A	0.04 $\pm$ 0.04 ^B	0.28 $\pm$ 0.09 ^{AB}
<i>Cladonia decorticata</i> ($p = 0.0494$)	0.00 ^A	0.04 $\pm$ 0.04 ^A	0.00 ^A	0.12 $\pm$ 0.07 ^A	0.04 $\pm$ 0.04 ^A
<i>Cladonia deformis</i> ($p < 0.0001$)	0.40$\pm$0.10^A	0.56$\pm$0.10^A	0.00 ^B	0.00 ^B	0.00 ^B
<i>Cladonia gracilis</i> spp. <i>turbinata</i> ($p < 0.0001$)	0.76$\pm$0.08^A	0.68$\pm$0.09^A	0.52$\pm$0.10^A	0.12 $\pm$ 0.06 ^B	0.48$\pm$0.10^A
<i>Cladonia grayi</i> ($p = 0.3144$)	0.12 $\pm$ 0.06 ^A	0.04 $\pm$ 0.04 ^A	0.00 ^A	0.04 $\pm$ 0.04 ^A	0.12 $\pm$ 0.06 ^A
<i>Cladonia humilis</i> ($p < 0.0001$)	0.00 ^B	0.00 ^B	0.00 ^B	0.44$\pm$0.10^A	0.000 ^B
<i>Cladonia magyarica</i> ($p < 0.0001$)	0.00 ^B	0.00 ^B	0.00 ^B	0.96$\pm$0.04^A	0.000 ^B
<i>Cladonia merochlorophaea</i> ($p = 0.038$)	0.16 $\pm$ 0.07 ^A	0.00 ^A	0.00 ^A	0.00 ^A	0.12 $\pm$ 0.07 ^A
<i>Cladonia multiformis</i> ($p = 0.0008$)	0.00 ^B	0.08 $\pm$ 0.05 ^B	0.16 $\pm$ 0.07 ^{AB}	0.00 ^B	0.32$\pm$0.09^A
<i>Cladonia phyllophora</i> ($p = 0.0331$)	0.20 $\pm$ 0.08 ^{AB}	0.16 $\pm$ 0.07 ^{AB}	0.08 $\pm$ 0.05 ^B	0.04 $\pm$ 0.04 ^B	0.28$\pm$0.09^A
<i>Cladonia pocillum</i> ($p = 0.0660$)	0.00 ^A	0.00 ^A	0.04 $\pm$ 0.04 ^A	0.12 $\pm$ 0.07 ^A	0.00 ^A
<i>Cladonia pyxidata</i> ($p = 0.0004$)	0.16 $\pm$ 0.07 ^B	0.64$\pm$0.09^A	0.60$\pm$0.1^A	0.36 $\pm$ 0.09 ^{AB}	0.68$\pm$0.09^A

<i>Cladonia rangiferina</i> ($p < 0.0001$)	0.44±0.10^A	0.16±0.07 ^{ABC}	0.00 ^C	0.04±0.04 ^{BC}	0.32±0.09 ^{AB}
<i>Cladonia scabruiscula</i> ($p = 0.0024$)	0.00 ^B	0.04±0.04 ^{AB}	0.04±0.04 ^{AB}	0.28±0.09^A	0.24±0.08 ^{AB}
<i>Cladonia stellaris</i> ($p < 0.0001$)	0.44±0.10^A	0.28±0.09^A	0.00 ^B	0.00 ^B	0.00 ^B
<i>Cladonia stygia</i> ($p = 0.1876$)	0.12±0.10 ^A	0.04±0.06 ^A	0.00 ^A	0.00 ^A	0.04±0.04 ^A
<i>Cladonia sulphurina</i> ($p = 0.0138$)	0.00 ^B	0.12±0.06^A	0.00 ^B	0.00 ^B	0.00 ^B
<i>Cladonia uncialis</i> ($p < 0.0001$)	0.40±0.10^A	0.12±0.06 ^B	0.00 ^B	0.00 ^B	0.00 ^B
<i>Cladonia verticillata</i> ($p = 0.0494$)	0.04±0.04 ^A	0.00 ^A	0.00 ^A	0.04±0.04 ^A	0.16±0.07 ^A
<i>Peltigera rufescens</i> ($p < 0.0001$)	0.08±0.05 ^B	0.04±0.04 ^B	0.56±0.10^A	0.20±0.08 ^B	0.28±0.09^{AB}
<i>Sterocaulon tomentosum</i> ($p < 0.0001$)	0.04±0.04 ^B	0.32±0.09^A	0.00 ^B	0.00 ^B	0.00 ^C
<i>Umbilicaria deusta</i> ($p < 0.0001$)	0.04±0.04 ^B	0.28±0.09^A	0.00 ^B	0.00 ^B	0.00 ^C
<i>Vulpicida pinistari</i> ($p < 0.0001$)	0.28±0.09^{AB}	0.40±0.10^A	0.08±0.05 ^{BC}	0.00 ^C	0.00 ^B
<i>Xanthoparmelia cumberlandia</i> ($p = 0.0365$)	0.04±0.04 ^{AB}	0.20±0.08^A	0.04±0.04 ^{AB}	0.00 ^B	0.04±0.04 ^C

[†]Only those species with >10% frequency of occurrence are included in the table. The species, *Cetraria islandica*, *Cladonia acuminata*, *C. borealis*, *C. botrytes*, *C. cenotea*, *C. coniocraea*, *C. fimbriata*, *C. gracilis* ssp. *gracilis*, *C. mitis*, *C. pleurota*, *C. subulata*, *Nephroma helveticum*, *Parmelia saxatilis*, *Sterocaulon grande*, and *U. muehlenbergii*, were found at frequencies <10% and are not included in the table.

Table 5. Frequency of occurrence (means $\pm$ standard error (SE)) of secondary metabolites¹ over 25 one-square meter quadrats per site (transect) for each of five locations in Manitoba. Bold numbers are significant at the given probabilities for each species as listed in the first column. Upper case letters indicate significant differences within rows at $p < 0.05$ using Tukey's HSD test.

Location	Sherridon Mean $\pm$ SE	North Star Mean $\pm$ SE	Athapap Mean $\pm$ SE	Spruce Woods Mean $\pm$ SE	Sandilands Mean $\pm$ SE
Lichen acids					
Zeorin ($p < 0.0001$)	0.48$\pm$0.1^A	0.68$\pm$0.09^A	0.00 ^B	0.00 ^B	0.00 ^B
Barbatic acid ($p < 0.0001$)	0.52$\pm$0.1^A	0.48$\pm$0.1^A	0.04 $\pm$ 0.04 ^B	0.04 $\pm$ 0.04 ^B	0.32 $\pm$ 0.09 ^{AB}
Usnic acid ($p < 0.0001$)	0.96$\pm$0.04^A	0.92$\pm$0.05^A	0.2 $\pm$ 0.08 ^C	0.2 $\pm$ 0.08 ^C	0.6 $\pm$ 0.1 ^B
Thamnolic acid ($p = 0.0138$)	0.00 ^B	0.12$\pm$0.06^A	0.00 ^B	0.00 ^B	0.00 ^B
Squamatic acid ($p = 0.0042$)	0.2 $\pm$ 0.08 ^{AB}	0.28$\pm$0.09^A	0.04 $\pm$ 0.04 ^B	0.00 ^B	0.04 $\pm$ 0.04 ^B
Perlatolic acid ($p = 0.2757$)	0.16 $\pm$ 0.07 ^A	0.24 $\pm$ 0.08 ^A	0.04 $\pm$ 0.04 ^A	0.16 $\pm$ 0.07 ^A	0.08 $\pm$ 0.07 ^A
Fumarprotocetraric acid ($p = 0.2479$)	1.00 $\pm$ 0.00 ^A	0.92 $\pm$ 0.05 ^A	0.96 $\pm$ 0.04 ^A	1.00 $\pm$ 0.00 ^A	1.00 $\pm$ 0.00 ^A
Atranorin ($p < 0.0001$)	0.36 $\pm$ 0.08 ^{BC}	0.32 $\pm$ 0.08 ^C	0.68 $\pm$ 0.08 ^{AB}	0.96$\pm$0.08^A	0.4 $\pm$ 0.08 ^{BC}
Grayanic acid ($p = 0.3144$)	0.12 $\pm$ 0.06 ^A	0.04 $\pm$ 0.04 ^A	0.00 ^A	0.04 $\pm$ 0.04 ^A	0.12 $\pm$ 0.06 ^A
Merochlorophaeic acid ($p = 0.016$)	0.16 $\pm$ 0.07 ^A	0.00 ^A	0.00 ^A	0.04 $\pm$ 0.04 ^A	0.2 $\pm$ 0.08 ^A

¹Only those metabolites with >20% occurrence are included in the table. Metabolites <20% occurrence include didymic, gyrophoric, stictic, norstictic, psoromic, salazinic, pinastric, lobaric, alectoronic, and vulpinic acids and are not included in the table.

Table 6: Pearson correlation between three main soil characteristics (pH, organic matter, and sand content) and the number of occurrence of secondary metabolites (n). Bold values are significant at $p < 0.05$.

Secondary metabolite	<i>n</i>	pH			Organic matter (%)			Sand content (%)		
		<i>R</i> ²	<i>F</i>	<i>P</i>	<i>R</i> ²	<i>F</i>	<i>P</i>	<i>R</i> ²	<i>F</i>	<i>P</i>
Atranorin	141	15.48	22.53	<0.0001	-7.30	9.63	0.002	11.58	16.10	0.0001
Barbatic acid	36	-14.11	20.21	<0.0001	2.72	3.44	0.070	9.78e ⁻⁵	0.01	0.910
Fumarprotocetraric acid	432	2.40	3.05	0.083	-11.00	15.20	0.0002	10.30	14.18	0.0003
Grayanic acid	8	0.58	0.72	0.390	1.30	1.60	0.210	2.99	3.79	0.054
Merochlorophaeic acid	13	0.07	0.09	0.760	0.27	0.34	0.560	0.01	0.02	0.890
Perlatolic acid	20	1.50	1.88	0.170	0.07	0.09	0.760	0.07	0.09	0.760
Squamatic acid	15	-3.21	4.08	0.045	0.17	0.21	0.650	0.09	0.11	0.740
Thamnolic acid	3	0.15	0.19	0.670	1.50	1.88	0.170	0.02	2.80	0.090
Usnic acid	185	-25.24	41.50	<0.0001	3.60	4.67	0.032	2.40	3.04	0.080
Zeorin	46	-12.27	17.20	<0.0001	3.30	4.19	0.042	0.42	0.52	0.470

Table 7: Pearson correlation between three main soil characteristics (pH, organic matter, and sand content) and the frequency of occurrence of lichen species (*n*). Bold values are significant at *p* = 0.05.

Species	<i>N</i>	pH			Organic matter (%)			Sand content (%)		
		<i>R</i> ²	<i>F</i>	<i>P</i>	<i>R</i> ²	<i>F</i>	<i>P</i>	<i>R</i> ²	<i>F</i>	<i>P</i>
<i>Arctoparmelia centrifuga</i>	25	23.5	7.0847	-0.0139*	43.1	17.4422	+0.0004**	16.9	4.7060	-0.0406*
<i>Cladonia amaurocraea</i>	16	40.2	15.4861	-0.0007**	22.5	6.6996	+0.0164*	4.8	1.1611	-0.2924
<i>Cladonia arbuscula</i>	70	24.3	7.4047	-0.0122*	16.7	4.6230	+0.0423*	13.2	3.4800	+0.0749
<i>Cladonia cariosa</i>	24	16.5	4.5638	+0.0435*	2.9	0.6901	-0.4147	0.29	0.0671	+0.7979
<i>Cladonia chlorophaea</i>	65	3.4	0.8168	-0.3755	34.6	12.1679	+0.0020**	23.5	7.1006	-0.0138*
<i>Cladonia cornuta</i>	37	1.03	0.2406	-0.6284	1.2	0.2907	-0.5950	1.4	0.3320	+0.5701
<i>Cladonia crispata</i>	8	13.5	3.5995	-0.0704	0.28	0.0656	+0.8001	0.07	0.0175	+0.8960
<i>Cladonia cristatella</i>	26	19.5	5.6053	-0.0267*	2.8	0.6652	+0.4231	0.05	0.0020	+0.9645
<i>Cladonia decorticata</i>	6	5.7	1.4035	+0.2482	3.7	0.8741	-0.3595	6.1	1.4948	+0.2339
<i>Cladonia deformis</i>	24	45.9	19.5178	-0.0002**	3.8	0.9173	+0.3481	1.2	0.2874	-0.5970
<i>Cladonia gracilis</i> spp. <i>turbinata</i>	66	42.7	17.1879	-0.0004**	23.2	6.9482	+0.0148*	14.3	3.8526	-0.0169*
<i>Cladonia grayi</i>	8	9.2	2.3289	+0.1406	4.1	0.9832	-0.3317	5.4	1.3291	+0.2608
<i>Cladonia humilis</i>	11	18.5	5.2081	+0.0321	15.0	4.0656	-0.0556	37.7	13.9474	+0.0011**
<i>Cladonia magyarica</i>	24	24.5	7.4652	+0.0119*	16.7	4.6328	-0.0421*	42	16.6716	+0.0005**
<i>Cladonia merochlorophaea</i>	9	0.8	0.1852	+0.6709	1.4	0.5679	-0.5679	0.3	0.0735	+0.7887
<i>Cladonia multiformis</i>	14	0.01	0.0033	-0.9545	0.53	0.1247	-0.7272	1.2	0.2831	+0.5998
<i>Cladonia phyllophora</i>	11	23.4	7.0359	-0.0142*	1.2	0.3001	+0.5891	0.9	0.2111	-0.6502
<i>Cladonia pocillum</i>	4	15.9	4.3614	+0.0480*	1.7	0.4048	-0.5309	3.7	0.8806	+0.3578
<i>Cladonia pyxidata</i>	61	0.29	0.0687	+0.7955	6.1	1.5001	+0.2331	1.5	0.3497	-0.5601
<i>Cladonia rangiferina</i>	24	2.1	0.4928	-0.4897	0.01	0.0027	+0.9588	1.2	0.2915	+0.5945
<i>Cladonia scabruiscula</i>	15	2.9	0.6834	+0.4169	8.3	2.0876	-0.1620	16.7	4.6027	+0.0427*
<i>Cladonia stellaris</i>	17	29.7	9.7325	-0.0048**	3.7	0.8956	+0.3538	1.2	0.2988	-0.5899
<i>Cladonia stygia</i>	5	6.9	1.7141	-0.2034	4.2	1.0196	+0.3231	1.1	0.2586	-0.6159
<i>Cladonia uncialis</i>	14	2.9	0.6786	-0.4185	1.9	0.4546	+0.5069	1.7	0.4096	-0.5285
<i>Cladonia verticillata</i>	6	0.01	0.0033	+0.9547	8.7	2.2149	-0.1503	11.7	3.0625	+0.0934
<i>Peltigera rufescens</i>	29	11.07	2.8648	+0.1040	4.1	0.9922	+0.3296	5.07	1.2226	-0.2803
<i>Sterocaulon tomentosum</i>	31	31.4	10.5529	-0.0035**	20.3	5.8556	+0.0238*	8.29	2.0792	-0.1628
<i>Umbilicaria deusta</i>	9	22.6	6.7020	-0.0164*	23.1	6.9313	+0.0149*	4.2	1.0273	-0.3213
<i>Vulpicida pinistari</i>	19	43.1	17.4429	-0.0004**	13.1	3.4801	+0.0749	2.8	0.6744	-0.4199
<i>Xanthoparmelia cumberlandia</i>	8	4.1	0.9856	-0.3311	12.4	3.2572	+0.0842	6.2	1.5151	-0.2308

* Significance was determined at *p* < 0.05

** Bonferonni correction of *p* < 0.0005 (0.05/*X*)

Figure captions

Figure 1. Map of Manitoba, Canada, showing locations of collection sites (transects) in this study including the Sandilands Provincial Forest, Spruce Woods Provincial Park, Athapap Road, Sherridon Road, and North Star Road.

Figure 2. Principle Coordinates Analysis relating soil characteristics including gravel, sand (total, angular fraction, rounded fraction and grain size), silt and clay, organic matter, and pH in each of the five sites (transects) in each of five locations (NS = North Star Road; S = Sherridon Road; A = Athapap Road; SW = Spruce Woods Provincial Park; SA = Sandilands Provincial Forest). The horizontal axis explains 51.4% of the variation in the data and the vertical axis explains 21.6% of the variation.

Figure 3. Redundancy analysis of the Sherridon (S), North Star (NS), Athapap (A), Spruce Woods (SW), and Sandilands (SA) locations and percent frequencies of the secondary metabolites, i.e. atranorin (At), barbatic acid (Bar), fumarprotocetraric acid (Fum), grayanic acid (Gra), merochlorophaeic acid (Mer), perlatolic acid (Per), squamatic acid (Sq), thamnolic acid (Thm), usnic acid (Us), and zeorin (Zeo), as constrained by soil features (silt/clay, sand (total, fine and coarse), gravel, organic matter, soil particle shape (angular and rounded), and pH value). The secondary metabolites (didymic, gyrophoric, lobaric, norstictic, pinastric, salazinic, stictic,

and vulpinic acids) were not found to be at significant levels in any of the five locations or were at frequencies $< 20\%$ and are not included in the figure. The first RDA axis accounts for 62.2% of the total variation and the second axis for 22.8% of the variation.

Figure 4. Redundancy analysis of sites and lichen species (see Table 5 for species abbreviations) constrained by soil features (silt/clay, sand (total, fine and coarse), gravel, organic matter, soil particle shape (angular and rounded), and pH value). The collection locations are abbreviated as in Fig. 3. The first RDA axis accounts for 44.81% and the second axis for 19.93% of the variation in the data.

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Figure 1

Figure 2

Figure 4