Relationships between Cyanolichen Communities and Nutrient Cycling in Sub-boreal Spruce Forests

by

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ABSTRACT

Cyanolichens (lichens with cyanobacterial symbionts) form a distinct assemblage of epiphytes strongly associated with humid microclimatic conditions in inland British Columbia. Disparate abundance patterns in sub-boreal forests are examined in relation to the influence of overstorey tree species. A comparison of lichens on conifer saplings beneath five overstorey tree species revealed that saplings beneath *Populus* support a disproportionately abundant and species-rich community of cyanolichens. Cyanolichens also grew more rapidly and had lower rates of mortality beneath *Populus* than beneath conifer overstorey trees. That cyanolichens were observed beneath *Populus* in stands that were otherwise climatically unsuitable suggests that *Populus* facilitates cyanolichen communities by providing a factor that compensates for sub-optimal conditions. Chemical analyses of throughfall precipitation from beneath *Populus*, *Picea*, *Abies*, *Pseudotsuga* and *Betula* failed to explain the variation in lichen community structure. However, glucose-rich nectar, exuded from extrafloral nectaries on *Populus* leaves, may instead be supporting cyanolichen communities. The nectar accumulates during dry periods, is washed off during subsequent rain events, and may be intercepted and metabolized by cyanolichens on conifer saplings beneath mature *Populus* canopies. C-flux measurements and phospholipid fatty-acid analyses with experimental applications of $^{13}C_6$-labelled glucose revealed a strong physiological response to glucose and a rapid incorporation of exogenous-^{13}C into cyanolichen fatty-acid tissues. Field evidence further supports this hypothesis with higher rates of cyanolichen establishment observed on *Picea* branches under treatment of 2% glucose solution compared to water. The exogenous C may enable cyanolichens to become established in regions that are otherwise too dry to support them by providing a source of C despite drought-induced inactivity of the cyanobacterial partner. The abundant communities of nitrogen-fixing cyanolichens in wet, mature forests and beneath *Populus* are important to ecosystem function. The contribution of cyanolichens to N-cycling is calculated at sites with varying lichen abundances from measured rates of lichen litter deposition, decomposition and nutrient release. Cyanolichen litter biomass represents up to 11.5% of the total N-input from aboveground litterfall and is estimated to release 2.1 kg N ha^{-1} yr^{-1} of newly-fixed N that would otherwise be unavailable in these mature sub-boreal forests.

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LIST OF ABBREVIATIONS AND TERMS

ANOVA	Analysis of variance
AS	*Alectoria sarmentosa*
Bipartite cyanolichen	Lichen with cyanobacterial and fungal symbionts
Chlorolichen	Lichen with green algal and fungal symbionts
DBH	Diameter at breast height
EFN	Extrafloral nectary
k	Decomposition constant
LH	*Lobaria hallii*
LP	*Lobaria pulmonaria*
LS	*Leptogium saturninum*
MRPP	Multi-response permutation procedures
NH	*Nephroma helveticum*
NLFA	Neutral lipid fatty acid
NMS	Nonmetric multidimensional scaling
PG	*Platismatia glauca*
PLFA	Phospholipid fatty acid
SBS	Sub-boreal spruce (biogeoclimatic zone)
SBSvk	Very-wet, cool subzone of the sub-boreal spruce zone
SBSwk	Wet, cool subzone of the sub-boreal spruce zone
SD	Standard deviation
Tripartite cyanolichen	Lichen with cyanobacterial, green algal and fungal symbionts

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CHAPTER 1 INTRODUCTION

1.1 The Lichen symbiosis

Lichens are a symbiotic association between a heterotrophic fungal partner (mycobiont) and an autotrophic photobiont. The photobionts are either cyanobacteria (bipartite cyanolichens), green algae (chlorolichens), or both (tripartite cyanolichens). The fungus provides an environment in which the photobiont cells can survive in an appropriate light and moisture regime. This is particularly crucial with tree-dwelling species where most photobionts would otherwise not survive the desiccating environment of the forest canopy. The hydrophobic fungal layer also ensures adequate gas exchange at the photobiont-fungus interface and facilitates carbon and nutrient transfer between the symbionts (Honegger 1991). The fungal partner is thought to exert some level of control over cell reproduction and growth by regulating the nutrient supply (Ahmadjian 1993, Hill 1989). As the only photosynthetic cells within the lichen symbiosis, the algae and/or cyanobacteria provide fixed carbon for maintenance and growth of all partners and may supply 70-90% of assimilated carbon to the mycobiont (Smith 1980).

Nutrient and carbon transfer occurs in chlorolichens via fungal extensions (haustoria) that penetrate the algal cell. There are no specialized tissues connecting the symbionts in bipartite cyanolichens (Honegger 1985) and mobile carbohydrates (principally glucose) are released by the photobiont into the apoplastic space between the two partners. The fixed carbon is then picked up by the hyphae of the mycobiont (Honegger 1991). That this transfer of soluble materials requires a film of moisture may be one of the physiological reasons for higher moisture requirements in cyanolichens compared with chlorolichens.

1.2 Cyanolichen habitat

Cyanolichens form a distinct assemblage of epiphytes that are strongly associated with humid microclimatic conditions (Goward and Spribille 2005) and mature forest ecosystems (Campbell and Fredeen 2004). Lichens are physiologically active only when wet, and being poikilohydric, are unable to control the degree and duration of hydration (Kappen 1988). The prevailing climate thus determines the period of physiological activity and the ability to utilize light or nutrient resources toward growth and development. Although all lichens may become completely desiccated and regain physiological activity upon rehydration, photosynthesis in cyanolichens only occurs following contact with liquid water (Lange et al. 1986). Furthermore, while chlorolichens can reach maximal activity at 50-70% thallus moisture, this only occurs in cyanolichens when thallus-water content exceeds 150% (Lange et al. 2004). Consequently, the composition of epiphytic lichen communities is often determined by climatic conditions, with drier zones being largely occupied by chlorolichens and wetter areas by cyanolichens (Sillett and Neitlich 1996; Lehmkulh 2004).

In the interior, mixed-conifer forests of British Columbia, cyanolichens are generally restricted to the lower canopy of forests more than 140 years old (Goward 1994; Campbell and Fredeen 2004) where the probability of stand-level disturbance is low (Sanborn et al. 2006). Such forests provide an irregular stand structure that allows sun-flecks and indirect light to penetrate to the lower-canopy branches that are critical as cyanolichen substrate. By contrast, younger forests are often even-aged with a single canopy layer and few gaps to allow light penetration (Benson and Coxson 2002). Under such conditions light levels in the lower canopy may be below the threshold requirements for cyanolichen growth (Gaio-Oliveira et al. 2004).

Light availability has been repeatedly demonstrated to limit lichen growth. Gauslaa et al. (2006) observed that biomass gain in *Lobaria pulmonaria* was directly proportional to the

amount of light intercepted by the lichen thallus. However, given that cyanolichens must be highly hydrated for growth, the positive influence of light will depend on moisture availability. Indeed, Dahlman and Palmqvist (2003) demonstrated that up to 66% of the variation in cyanolichen mass gain was attributable to the combination of light and water availability. Consequently, cyanolichens are often most abundant and diverse in old forests where conditions provide sufficient light for growth while preventing thallus damage due to desiccation and moisture stress (Gauslaa et al. 2006).

Lichen community composition is also strongly influenced by biotic factors, particularly relating to host or neighboring tree species. Hauck and Spribille (2005) reported higher overall epiphyte abundance and greater cyanolichen diversity on subalpine fir relative to Engelmann spruce in NW Montana. A significant interaction between tree species and soil type affecting epiphyte abundance in a sub-boreal forest makes interpreting the phorophyte effect difficult, but the results of Campbell and Fredeen (2007) also suggest preferential bipartite cyanolichen colonization on subalpine fir compared to spruce.

Phorophyte preferences may be particularly evident when cyanolichen communities are compared between coniferous and deciduous trees. The structural differences between coniferous and deciduous tree canopies create disparate patterns of rain and light interception. Epiphytes beneath a broadleaf canopy experience greater seasonal fluctuation in rainfall and light and may be exposed to higher irradiance than under dense coniferous canopies. In addition, needles and leaves determine, through the amount and composition of litter, the nutrient dynamics of the stand (Prescott 2002). Broadleaf trees may result in localized regions of nutrient enrichment and increased base-cation saturation compared to conifers within the same ecosystem (Fujinuma et al. 2005). Whether due to climatic or chemical factors, deciduous trees, particularly in the

Populus and *Salix* genera, support a unique, species-rich community of epiphytic lichens. In Scandinavia (Kuusinen 1994; Gauslaa 1995; Hedenås and Ericson 2000), Estonia (Jüriado et al. 2003) and the United Kingdom (Ellis and Coppins 2007) *Populus* trunks provide habitat for cyanobacterial lichens that are otherwise absent in the predominantly conifer forest. In British Columbia, cyanolichens are often asymmetrically distributed on conifer branches with the heaviest loading in the direction of a neighbouring *Populus balsamifera* (Goward and Arsenault 2000). Such differences in lichen community structure between phorophytes have been attributed to inhibition by high manganese concentration (Hauck 2003), and promotion by increased calcium concentration, decreased acidity (Gauslaa 1995; Goward and Arsenault 2000), and increased phosphorus concentrations (Benner and Vitousek 2007). However, neither the identity, nor the concentrations of chemicals that are influencing lichen communities are well understood. Thus the composition of epiphyte communities beneath different tree species is examined here with reference to climatic and chemical site factors to provide a deeper understanding of the habitat requirements of cyanolichens in sub-boreal forests of British Columbia.

1.3 Contribution of cyanolichens to forest nutrient cycling

Decay of, and nutrient release from, nitrogen-fixing lichens may be important to ecosystem N-cycling. In late-seral forest ecosystems, where atmospheric inputs are as low as 0.8 kg N ha^{-1} yr^{-1} (Hope 2001), N is often considered a growth-limiting element. Symbiotic N-fixing organisms are less abundant in older forests and so N is tightly cycled (Davidson et al. 1992) and largely supplied by decomposing forest litter (Sollins et al. 1980). Nitrogen-fixing cyanolichen biomass, on the other hand, increases with forest age. McCune (1994) reported epiphytic lichen loadings of 2250 kg ha^{-1} in wet-coastal forests and Campbell and Fredeen (Campbell and Fredeen 2004) recorded 1400 kg ha^{-1} in wet-interior forests of British Columbia. While these

biomass loadings are small relative to vascular plants which can exceed 1500 Mg ha^{-1} in interior forests (Fredeen et al. 2005), N contribution from cyanolichens may represent approximately 50% of the total N input (Denison 1979). The quantity of in situ cyanolichen-N has been calculated for wet interior forests of British Columbia (Campbell and Fredeen 2007), but these data provide only limited insight into the role of lichens in N-cycling. Thus the rates of cyanolichen litter deposition, decay, and nutrient release are examined here to provide an accurate estimate of cyanolichen-N contributions to nutrient cycling.

1.4 Research approach and objectives

This research can be divided into two components; an investigation of the ecology of cyanolichens with reference to climatic and chemical site factors, and an evaluation of the contribution of cyanolichens to nutrient cycling.

The first component begins with a description of the lichen communities in sub-boreal spruce forests and an evaluation of lichen community structure across site-types and between overstorey tree species (Chapter 2). A strong positive association between cyanolichens and overstorey *Populus* led to an examination of the climatic conditions (temperature, relative humidity and light availability) and the chemistry of throughfall precipitation as potential factors influencing lichen community composition. Chapter 3 documents differences in rates of growth and mortality for two cyanolichen species across site-types and between tree species. Finding no climatic or chemical differences between tree species that could account for the abundant and species-rich cyanolichen communities observed beneath *Populus,* Chapter 4 evaluates whether the proliferation of cyanolichens beneath *Populus* is due to facilitation by an exogenous source of labile carbon.

The second component evaluates the role of nitrogen-fixing cyanolichens in N-cycling in sub-boreal spruce forests. Rates of lichen litterfall, decomposition and nutrient release are analysed in Chapter 5 to predict the contribution of cyanolichen litter to ecosystem N. The objectives and working hypotheses for each chapter are as follows:

CHAPTER 2. To evaluate the extent to which variation in lichen communities on understorey conifer saplings is related to neighbouring, overstorey tree species and to identify patterns in microclimate and precipitation chemistry to which this variation might be related.

Hypotheses:

1. There is a strong positive spatial-association between the epiphytic cyanolichens observed on conifer saplings and overstorey *Populus* that occur intermittently in the mixed-conifer forest.
2. Overstorey *Populus* trees sustain patches of high cyanolichen diversity on understrorey conifer saplings in stands that are less favourable to cyanolichen establishment or growth.
3. Patterns in cyanolichen community species-richness and abundance on understorey conifer saplings are related to the chemistry of throughfall precipitation beneath different overstorey tree species.

CHAPTER 3. To evaluate the influence of overstorey tree species, relative to regional climatic conditions, on the rate of cyanolichen growth.

Hypotheses:

1. Cyanolichen thalli transplanted beneath *Populus* canopies will have faster growth rates than thalli transplanted beneath the canopy of overstorey *Picea* or *Pseudotsuga* regardless of site-type.
2. Tripartite cyanolichen species will have a faster growth rates than bipartite cyanolichen species regardless of overstory tree-species.

CHAPTER 4. To examine the possibility that cyanolichen communities proliferate beneath overstorey *Populus* under sub-optimal climatic conditions due to facilitation by exogenous-glucose from *Populus* extrafloral nectaries.

Hypotheses:

1. Cyanolichen net photosynthesis will decrease and nitrogen fixation will markedly increase in response to exogenous glucose.
2. Exogenous glucose will be readily taken up and assimilated into lichen fatty-acid tissues.
3. The rate of cyanolichen establishment will significantly increase with the provision of exogenous glucose.

CHAPTER 5. To examine rates of epiphytic lichen litter deposition, decay and nutrient release and to evaluate the contribution of cyanolichens to forest N-cycling.

Hypotheses:

1. Cyanolichen litter will decompose faster and release N and P faster than chlorolichens, resulting in a convergence of N and P concentrations in lichen litter as decomposition proceeds.
2. Decomposing cyanolichen litter will rapidly release N and thereby provide a source of N for forest ecosystem functioning.

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CHAPTER 2 The Influence of overstorey *Populus* on epiphytic lichens[1]

2.1 Introduction

Cyanolichens (lichens with cyanobacterial symbionts) form a distinct assemblage of epiphytes strongly associated with humid microclimatic conditions (Goward 1994; Arsenault and Goward 2000) and mature forest ecosystems (McCune 1993; Campbell and Fredeen 2004). Unlike chlorolichens (lichens with green-algal symbionts), cyanolichens require contact with liquid water to become physiologically active (Lange et al. 1993). Consequently, the composition of epiphytic lichen communities is often determined by climatic conditions, with drier zones being largely occupied by chlorolichens and wetter areas by cyanolichens (Sillett and Neitlich 1996; Lehmkulh 2004). Cyanolichens are also abundant and diverse in later-seral forests (McCune 1993; Campbell and Fredeen 2004) where the discontinuous canopy structure allows greater penetration of indirect light through to the more humid lower-canopy (Benson and Coxson 2002).

Cyanolichen community composition is also strongly influenced by biotic factors, particularly relating to host or neighbouring canopy tree species. For example, higher overall epiphyte abundance and greater cyanolichen diversity has been observed on mature *Abies lasiocarpa* trees compared to mature *Picea* trees in east-central British Columbia (Campbell and Fredeen 2007) and in Montana (Hauck and Spribille 2005). Hauck and Spribille (2005) attributed these differences to the inhibitory effect of higher manganese concentrations in *Picea* bark. Enriched cyanolichen communities have also been noted on the trunks of *Populus* and *Salix* compared to those of surrounding conifer trees (Kuusinen 1994; Gauslaa 1995; Hedenås and Ericson 2000; Jüriado et al. 2003; Ellis and Coppins 2007). The higher pH of *Populus* bark has

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been cited as an important factor promoting cyanolichens in acid-affected European and Scandinavian forests (Gauslaa 1995).

Most comparisons of epiphytic lichen communities between particular tree species relate to observations on the trunks and branches of the host trees themselves and make no note of the potential influence of surrounding trees. Goward and Arsenault (2000) provide an exception, reporting an asymmetrical distribution of epiphytic cyanolichens on temperate conifers, with the heaviest loading in the direction of a neighbouring *Populus balsamifera* ssp. *trichocarpa*. These observations, coupled with accounts of unique cyanolichen flora on *Populus* trunks as mentioned above, suggest that cyanolichen communities may benefit from the chemical environment created by *Populus* trees. More specifically, as suggested by Goward and Arsenault (2000) and Gauslaa (1995), cyanolichens may be responding to an increase in pH resulting from higher base cation concentrations. However, neither the identity, nor the concentration of chemicals that are required for the development of cyanolichen communities are well understood.

The objectives of this study were to evaluate the extent to which variation in lichen communities on understorey conifer saplings is related to neighbouring, overstorey tree species and to identify patterns in microclimate and precipitation chemistry to which this variation might be related. Specifically, the study was designed to test the following hypotheses: (1) there is a strong, positive spatial association between the epiphytic cyanolichens observed on conifer saplings and overstorey *Populus* trees that intermittently occur in the mixed-conifer forests, (2) overstorey *Populus* trees sustain patches of high cyanolichen diversity on understorey conifer saplings in stands that are less favourable to cyanolichen growth, (3) patterns in cyanolichen community species-richness and abundance on understorey conifer saplings are related to the chemistry of throughfall precipitation beneath different overstorey tree species.

2.2 Methods and materials

2.2.1 Study area

The study area was located north-east of Prince George, British Columbia, in old-growth (mean tree age >240 years) forests of the Sub-Boreal Spruce (SBS) biogeoclimatic zone (Meidinger and Pojar 1991). These forests are characterized by cool, moist summers and cold, snowy winters. Three mature, conifer-leading forest site-types representing different levels of moisture and light deficiencies for lichen growth were selected for study within the wet Sub-boreal spruce zone (Fig. 2.1). For each site-type, three replicate stands (nine in total, hereafter referred to as study sites) were randomly chosen for sampling from a pool of candidate sites meeting the criteria of showing no evidence of recent disturbance, and having intermittent occurrence of deciduous trees in an otherwise conifer-dominated forest canopy.

The three 'Aleza' sites were located in the wet, cool subzone (SBS wk) in the Aleza Lake Research Forest at 680 m in elevation. The Aleza sites receive approximately 897 mm in annual precipitation (Murphy 1996) and so were considered the most moisture-deficient of the three site-types. The three 'Herrick' sites were located along the Herrick Forest Service Road approximately 40 km north-east of the Aleza sites in the very-wet, cool SBS subzone (SBS vk) at an elevation of 850 m. Selective logging approximately 100 years ago at the Herrick sites resulted in a relatively closed overstorey canopy. Thus, Herrick sites were considered the most light-deficient of the three site-types. Finally, the three 'Fraser' sites (680 m elevation) were located in an ecotonal region between the two aforementioned site-types and neither moisture nor light were considered deficient. Annual precipitation at both the Fraser and Herrick sites was approximately 964 mm (Murphy 1996).

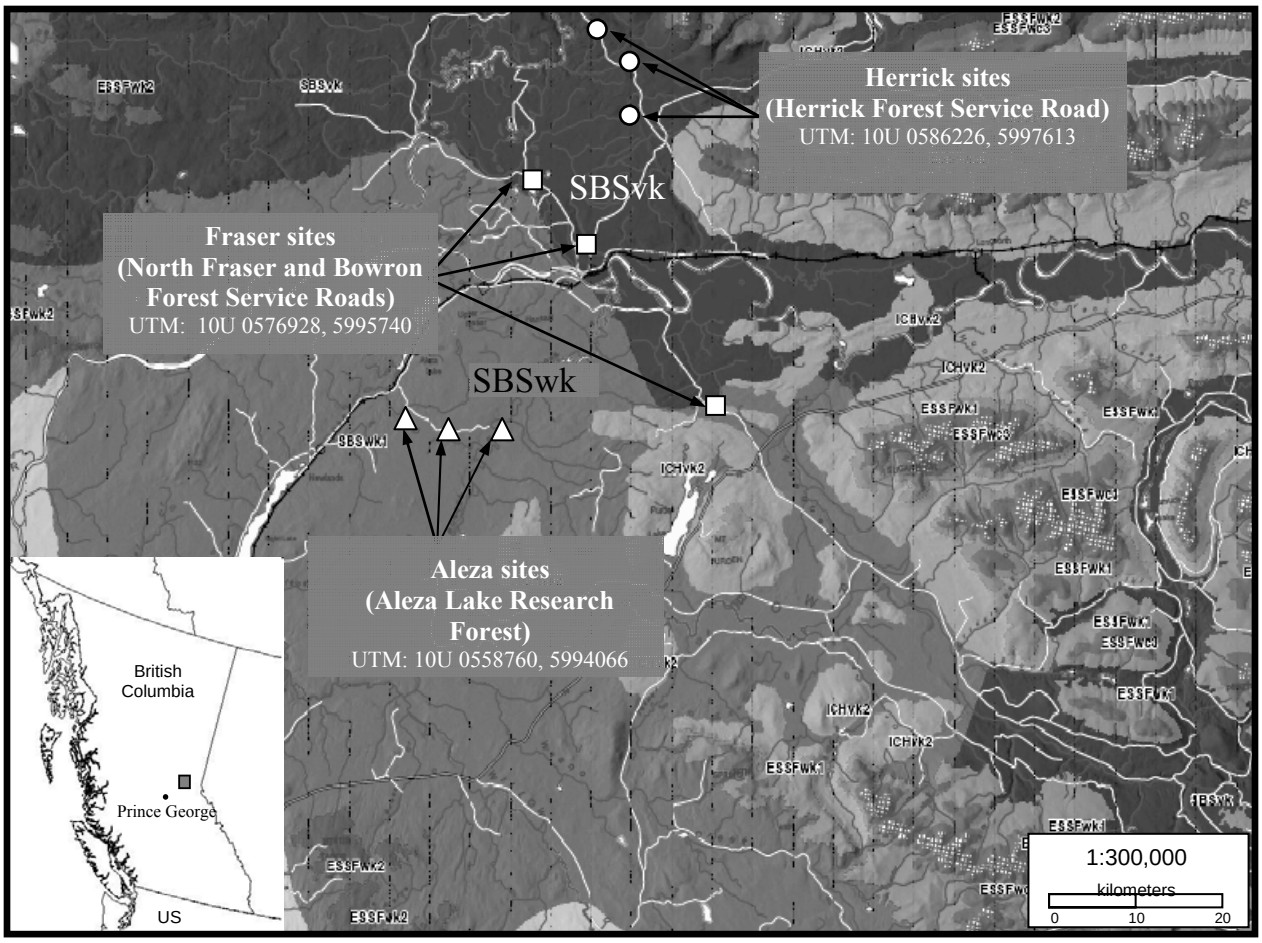

Figure 2.1 Location of the study sites near Prince George, British Columbia, Canada.

Picea glauca (Moench) Voss x *engelmannii* Parry ex Engelmann (interior hybrid spruce) and *Abies lasiocarpa* (Hook.)Nutt. (subalpine fir) were the dominant conifer species at all three site-types, and *Betula papyrifera* Marsh. (paper birch) made up most of the deciduous canopy. *Populus balsamifera* L. ssp. *trichocarpa* Brayshaw (black cottonwood) was a minor component at the Fraser and Herrick sites while *Populus tremuloides* Michx. (trembling aspen) was present at the Aleza sites. *Pinus contorta* Dougl. Ex Loud. var. *latifolia* Engelm. (lodgepole pine), *Pseudotsuga menziesii* (Mirbel.) Franco var. *glauca* (Beissn.) Franco. (Douglas-fir) and *Tsuga heterophylla* (Raf.) Sarg. (western hemlock) were minor components at the Fraser, Aleza and

Herrick sites respectively. For simplicity, tree species are hereafter referred to by genus name only, with *P. tremuloides* and *P. trichocarpa* collectively referred to as *Populus*.

2.2.2 Sampling method

At each study site, three individual trees of each overstorey species were randomly selected from a pool of candidate trees satisfying the following criteria: 1) >20 cm diameter at breast height (DBH), 2) at least 5 m from another canopy tree of a different species and at least 10 m from a mature *Populus*, and 3) surrounded (within 3 m from the bole) by three conifer saplings (<10 cm DBH). Overstorey tree species included *Populus*, *Abies*, *Picea* and *Betula* at all sites, and *Pseudotsuga* at the Aleza sites. At each study tree, the three closest live conifer saplings within a 3-m-radius plot (centered on the study tree) were selected for epiphyte community sampling. Live candidate saplings that had more than 10 branches below 3.5 m were preferentially selected to provide a range of potential habitat for epiphytic lichens. The predominant conifer saplings (*Abies* and *Picea*) were used as a standardized sampling unit to assess the influence of overstorey tree species, rather than the influence of the phorophyte, on lichen community structure. In plots where *Populus* was the central tree, lichens were also sampled on the *Populus* trunk and lower branches (if present) to compare canopy influence versus direct phorophyte contact on the lichen communities.

As the abundance and composition of epiphytic lichen communities are known to vary between the trunks and branches of trees (McCune 1993), lichens were assessed within two sampling zones on the conifer saplings: on trunks up to 2 m and on branches up to 3.5 m in height. Similar sampling zones were also assessed on *Populus* where it was the central study tree. Following Campbell and Fredeen (2007), abundance was recorded for each species as the total area (cm^2) of thallus cover for each species within each sampling zone. Species richness was recorded as the number of epiphytic lichen species observed per plot. Hair lichens (e.g.

Alectoria sarmentosa) and *Cladonia* spp. were excluded due to the ubiquitous nature of the genera, and *Parmelia* spp. were only identified to genus. To assess recruitment success, an estimate (nearest 10) of the number of small (<0.5 cm^2) thalli within each sampling zone was also recorded for each foliose lichen species (or genera in cases where small thallus size prevented in situ identification to species).

2.2.3 Environmental conditions

Air temperature and relative humidity data were recorded at the nine study sites using HOBO Pro RH and Temperature Data-loggers (Onset Computer Corporation, Bourne, MA). At each site, data loggers were installed 1.8 m above ground-level on the north side of the trunk of one *Populus*, *Abies* and *Picea* overstorey tree; additional units were installed in a similar fashion on *Pseudotsuga* trunks at the three Aleza sites. Data were collected continuously from July 2006 to June 2008.

Light availability was assessed using digital hemispherical photographs taken beneath the canopy of the overstorey tree and under each of the three saplings in each plot. Photographs were taken on the north side of each tree both before (summer) and after (winter) leaf abscission to quantify phenological differences in light availability. Photos were taken using a Nikon Coolpix 8700 camera with a Nikon Fisheye converter (UR-E12) and lens (FC-E9). Images were analysed for light availability using Gap Light Analyzer Version 2.0 (Simon Fraser University, Burnaby, Canada). Differences in the light environment between tree species and site-types were evaluated using total light availability (direct plus diffuse light) as both constituents have been demonstrated to affect cyanolichen growth (Gauslaa et al. 2006). Total tree (trunks >20cm DBH) and sapling (trunks <10cm DBH) density, species composition, and overstorey tree ages (using an increment borer) were also determined at each study site.

2.2.4 Precipitation, bark and soil chemistry

Throughfall precipitation collectors were established beneath 5 overstory *Populus*, *Betula*, *Abies* and *Picea* at each site. An additional 3 collectors were placed in the open to collect precipitation unaffected by the foliage of any overstorey tree species, for a total of 23 collectors per site. Collectors consisted of a high-density polypropylene funnel (with a 15-cm diameter opening) secured to a 125-ml high-density polyethylene Nalgene^{TM} bottle (Fisher Scientific, Ottawa, Canada). Glass wool was placed at the funnel mouth to filter debris and a 2-cm diameter polyester capsule containing 10 ml of mixed bed ionic resins (PST-2; Unibest, Bozeman, Montana, USA) was placed in each bottle. Resin capsules were surrounded in a bed of glass wool to maximize water-holding capacity as a 3-mm hole was drilled in the bottom of each bottle to allow water to pass freely throughout the collection period. Supports constructed from PVC piping were installed on each tree trunk at approximately 3 m in height to minimize disturbance by wildlife. Collectors were held in supports approximately 1 m from the trunk and placed to intercept throughfall precipitation from a single tree species and to avoid branches with abundant epiphytes. Winter precipitation collectors were placed in the field following the first snowfall in November 2007 and removed prior to leaf flush in May 2008. Summer collectors were placed at this time and were removed in September 2008 prior to leaf abscission. Similar precipitation collectors were used in July 2007 to collect water for pH analysis. The pH of duplicate water samples was recorded using an Oakton pHTestr 3 (Oakton Instruments, Vernon Hills Il. USA) calibrated with pH 4 and 7 buffers.

Nutrients were extracted from the resin capsules using 2M HCl according the protocol described by Skogley and Dobberman (1996). Element concentrations were analysed at the University of Northern British Columbia Central Equipment Laboratory using inductively

coupled plasma-mass spectrometry (ICP-MS, 7500 Series, Agilent Technologies, Santa Clara, CA) as described by Dolan and Capar (2002).

Samples of conifer sapling branches were collected from the field in July 2007. Branches (approximately 1 cm in diameter) were stripped of foliage and cut to four 15-cm lengths. The branch ends and other bark wounds were coated with paraffin wax to ensure that only the outer bark would contact the water. Samples were immersed in deionized water in HDPE Nalgene^{TM} bottles and stored at 8^{o}C. After 1 week, samples were removed and the pH was recorded for duplicate water samples using an Oakton pHTestr 3.

Organic matter and mineral soil were collected within the drip-zone of three individuals of each tree species at each site in July 2007. Duplicate 25 ml samples were air dried, sifted through a 5 mm sieve, diluted to 10% and 50% (organic matter and mineral soil respectively) and agitated periodically for 1 hour. The pH was recorded using an Oakton pHTestr 3.

2.2.5 Data analysis

Differences in stand age across site-types were tested with a non-parametric Kruskal-Wallis ANOVA and variation in monthly temperature and relative humidity between site-types and tree species were evaluated with a repeated-measures ANOVA and a Bonferonni post-hoc comparison. Differences in light availability among site-types and tree species were assessed with a factorial ANOVA and a Bonferonni post-hoc test. Lichen species were divided into four functional groups (chlorolichens, non-stratified cyanolichens, bipartite stratified cyanolichens, and tripartite stratified cyanolichens sensu McCune 1993) according to the type and distribution of the primary photosynthetic symbiont (Brodo et al. 2001). The total abundance and total number of species observed on *Abies* and *Picea* saplings were compared with one-way ANOVAs following log transformation to achieve a normal distribution (Statistica v. 6.1, StatSoft Inc. Tulsa, OK, USA).

Two multivariate methods, non-metric multidimensional scaling (NMS) and multi-response permutation procedures (MRPP), were used to evaluate overall patterns of variation among the epiphytic lichen communities (PC-ORD v.5.0, McCune and Grace 2002). NMS generates a reduced number of ordination axes ("dimensions") that are well suited for visual display of relationships among plots according to similarities in lichen assemblages. The NMS used a random starting configuration and the Sørensen distance measure calculated on a matrix of 117 plots and 46 species. For consistency, only those lichen species that were readily identifiable in the field were included in the analysis. Rare species (<2 thalli in the entire study area) were either removed for ordination analysis or grouped and analyzed as genera (e.g. *Peltigera* spp.). MRPP, a non-parametric method for testing group differences, was used to test for quantitative differences in lichen community composition among plots grouped by site-type and overstorey tree species (e.g. Fraser - *Populus*) yielding a total of 13 groups of 9 plots each.

Differences in precipitation chemistry, soil and bark pH between site-types and tree species were evaluated following log normal transformation (where necessary) with a full-factorial ANOVA and a Bonferonni post hoc test. The relationship between Ca, P, Mn, and Mo concentrations and the total abundance of bipartite stratified cyanolichens, non-stratified cyanolichens, tripartite cyanolichens and chlorolichens were evaluated using Spearman rank order correlations. Relationships between soil pH, precipitation pH and sapling bark pH were tested using Pearson's correlation (Statistica, StatSoft Inc. Tulsa, OK, USA). Values reported in text and tables are given as the mean ± standard deviation unless otherwise noted.

2.3 Results

2.3.1 Environmental variation

The three site-types had similar mean overstorey tree ages (134±72 y, N=27; H(2,44)=2.0, p=0.34) and had 461±32 mature trees (DBH >20cm) and 1843±147 saplings (DBH <10cm) ha^{-1}. The Herrick sites had the lowest average summer light availability (F(2, 72)= 5.5, p=0.005; Table 2.1). Winter light availability did not differ among sites. Average daily temperatures were higher at the Aleza sites than at the Fraser sites (F(46,552)=50.8, p=0.000). Monthly relative humidity was consistently and significantly lower at the Aleza sites compared to the Herrick and Fraser sites (F(46,552)=7.1, p=0.000; data not shown). There were no climatic differences in temperature or relative humidity among tree species at any site-type.

Table 2.1 Total light availability (direct plus diffuse light expressed as percent of maximum ±SD) recorded under conifer saplings beneath *Populus tremuloides* or *P. trichocarpa, Betula papyrifera, Abies lasiocarpa*, and *Picea glauca* x *engelmannii* at Fraser, Aleza and Herrick sites.

	Site-type	***Populus***	***Betula***	***Abies***	***Picea***	**Site mean**
% winter light-availability	Fraser	19.1±3.3	20.5±2.8	18.9±3.2	18.1±1.2	19.2±2.8
	Aleza	19.3±4.4	17.2±1.5	18.9±1.7	17.1±1.7	18.5±3.8
	Herrick	18.7±4.4	20.2±4.1	18.7±2.9	20.3±3.8	19.4±2.7
% summer light-availability	Fraser	20.7±3.0[a]	17.5±2.6[b]	19.8±3.6[ab]	20.7±2.7[a]	20.3±3.5[a]
	Aleza	21.6±5.1[a]	21.4±5.7[a]	21.6±2.2[a]	21.2±3.3[a]	21.6±3.4[a]
	Herrick	16.9±3.5[b]	17.7±2.2[b]	19.2±3.5[b]	19.6±3.9[b]	18.4±3.6[b]

Note: Winter light availability refers to photographs taken after leaf abscission and summer light availability refers to those taken while deciduous leaves were still on the trees.

2.3.2 Lichen community patterns

Sixty-one taxa of epiphytic foliose macrolichens were recorded across the study area (Table 2.2). These included 26 chlorolichens, 19 bipartite stratified cyanolichens, 1 tripartite cyanolichen and 15 bipartite non-stratified cyanolichens, including 4 species that are not yet described (T. Goward, personal communication 2009). As there were no differences in lichen abundance or species richness observed on *Abies* and *Picea* saplings ($F(1, 66) = 0.5$, $p = 0.49$) the two sapling species were grouped for all subsequent analyses.

Site effects

More epiphytic foliose lichen species were observed at the Fraser sites (total 50, mean 41±5) than at the Aleza (total 41, mean 32±4) and Herrick (total 47, mean 39±3) sites. Community composition differed between site-types with cyanolichens comprising two-thirds (32 species) of the total macrolichen flora at Fraser sites and approximately half of the species at Aleza and Herrick sites (19 and 24 species, respectively; Table 2.3). Moreover, the species richness of non-stratified cyanolichens at Fraser sites (15 species) was double that at the Aleza and Herrick sites (8 and 7 species, respectively).

Overstorey tree effects

Lichen communities beneath overstorey *Populus* trees were the most species-rich with 90% (55 species) of the total observed foliose lichen flora occurring on conifer saplings under *Populus*. Saplings beneath *Abies*, *Betula* and *Picea* trees supported 77% (47 species), 74% (45 species) and 69% (42 species) respectively, of all lichen species observed. Saplings beneath *Pseudotsuga* had only 24% (15 species) of the total foliose lichen flora. This low diversity was partly related to the occurrence of *Pseudotsuga* at only one site-type; however, saplings beneath *Pseudotsuga* also supported lower lichen richness compared to other tree species at the Aleza sites (Table 2.3).

Table 2.2 The mean thallus area (average of total cm^2 $plot^{-1}$, N=9) of epiphytic foliose lichen species observed on saplings (branches up to 3.5 m and trunks up to 2 m) under *Populus tremuloides* or *P. trichocarpa*, *Betula papyrifera*, *Abies lasiocarpa*, *Picea glauca* x *engelmannii* and *Pseudotsuga menziesii* at Fraser, Aleza and Herrick sites. The lichen communities beneath *Populus tremuloides* and *P. trichocarpa* were found to be statistically similar (MRPP, T=-0.61 A=0.01 P=0.19).

Site-type	**Fraser sites**				**Aleza sites**					**Herrick sites**			
Tree species	***Populus***	***Betula***	***Abies***	***Picea***	***Populus***	***Betula***	***Abies***	***Picea***	***Pseudo-tsuga***	***Populus***	***Betula***	***Abies***	***Picea***
Foliose chlorolichens													
Cavernularia hultenii Degel.						0.1				0.3	1	4	3
Hypogymnia austerodes (Nyl.)Rae.	1	2		1	3	2	4	0.4	0.4	0.4	3	1	3
H. canadensis Goward					0.3	0.1	0.8	0.2		0.1	1	1	2
H. metaphysodes (Asah.) Rass.	0.2	2	0.2	0.1	1	1	2	2	1	0.3	4	2	3
H. occidentalis L. Pike	1	21	24	43	45	76	104	112	77	7	26	107	118
H. physodes (L.) Nyl.	3	4	10	36	42	45	55	38	34	27	36	40	50
H. rugosa† (G. Merr.) L. Pike						1						1	
H. tubulosa (Shaerer) Hav.	0.1	2	0.3	2	9	16	14	6	6	10	42	13	12
H. vitatta (Ach.) Parrique		23	2	19		4	3	1	1	2	16	4	4
Melanelia elegantula (Zahlbr.) Essl.					1							3	
M. exasperatula (Nyl.) Essl.		0.2			0.4	1	1	0.1		1	5	1	4
M. fuliginosa (Fr. ex Duby) Essl.		1	2	0.2	0.4		0.1			2	0.1	10	4
M. subaurifera (Nyl.) Essl.		0.1				0.1					4		
M. subelegantula (Essl.) Essl.		0.4		0.1		0.3				0.1	5	2	0.1
M. *trabeculata* (Ahti) Essl.			0.2								1	1	
Parmelia spp.	41	179	132	205	154	384	365	168	223	123	433	278	346
Parmeliopsis ambigua (Wulfen) Ach.	0.4	7	6	35	18	39	80	68	138	3	10	72	47
P. hyperopta (Ach.) Arnold	0.3	26	2	9	9	39	44	54	64	1	23	24	12
Phycsia aipolia† (Ehrn.) Hampe.										0.1			
Platismatia glauca (L.)Culb.&C.F.Culb	6	176	181	347	101	1402	1367	848	1383	17	278	287	542
Ramalina dilacerata (Hoffm.) Hoffm.†	0.1												

Site-type	**Fraser sites**				**Aleza sites**					**Herrick sites**			
Tree species	***Populus***	***Betula***	***Abies***	***Picea***	***Populus***	***Betula***	***Abies***	***Picea***	***Pseudo-tsuga***	***Populus***	***Betula***	***Abies***	***Picea***
Ramalina intermedia (Delise ex Nyl.) Nyl.†					0.2								
Tuckermanopsis chlorophylla (Willd.) Hale	0.4	10	20	16	18	66	38	33	59	6	84	108	60
T. ciliaris (Ach.) Gyelnik											1		0.1
T. orbata (Nyl.) M. J. Lai					1	2	1	0.2	0.1	0.3	1	0.4	3
Vulpicida pinastri (Scop.) Gray	0.3	8	0.1	1	1	4	2	0.4	5		6	0.3	1
Stratified bipartite cyanolichens													
Fuscopannaria spp.† P.M. Jorg.					0.1								
Lempholemma spp.† Körb.	0.1												
Lobaria hallii (Tuck.) Zahlbr.	445	114	105	118	11	2	2			45	18	1	0.1
L. scrobiculata (Scop.) DC.	39	2	3	25	1			1		5	3	5	7
Nephroma bellum (Sprengel) Tuck.	81	53	7	27	4	1	0.3	0.1		254	44	2	2
N. helveticum Ach.	202	708	212	487	41	14	8	3		8	52	19	3
N. isidiosum (Nyl.) Gyeln.	12	8	0.3	6	9	1	1	1		18	23	4	8
N. parile (Ach.) Ach.	265	529	207	210	105	0.1	3	2	2	278	121	24	34
N. resupinatum (L.) Ach.	109	9	15	9	9					3			
Peltigera canina‡ (L.) Willd		5									3		
P. collina (Ach.) Schrader	130	17	17	20						27	0.3		
P. membranacea‡ (Ach.) Nyl.	11			12							1		
P. neckeri Hepp‡ ex Mull. Arg.										0.4			
P. polydactylon‡ (Necker) Hoffm.		3								12			
P. praetextata‡ (Sommerf.) Zopf										8			
Pseudocyphellaria anomala Brodo& Ahti.	5	39	32	82	6	1	1	1		17	24	16	16
Sticta fuliginosa (Hoffm.) Ach.	12	25	10	31	4	0.2				7	8	11	13
S. limbata (Sm.) Ach.	1	1	0.1	0.3									
S. oroborealis Goward & Tønsberg	0.1	0.2	0.2	1							1		

Site-type	**Fraser sites**				**Aleza sites**					**Herrick sites**			
Tree species	***Populus***	***Betula***	***Abies***	***Picea***	***Populus***	***Betula***	***Abies***	***Picea***	***Pseudo-tsuga***	***Populus***	***Betula***	***Abies***	***Picea***
Bipartite non-stratified cyanolichens													
Collema auriforme (With.) Coppins & J.R. Laundon	2		0.1										
C. coniophilum Goward	16	5	4	4	1					2			
C. curtisporum Degel.	0.4	0.1	1		2								
C. flaccidum† (Ach.) Ach.	0.2												
C. furfuraceum (Arnold) Du Rietz	11	2	1	1	0.4					1			
C. spp. nova 1 Goward ined.† ¶	4	0.1	2	1									
C. subflaccidum Degel.	10	1	2	1.2	0.1					0.3			
C. spp. nova 2 Goward ined. ¶	0.4	0.4	0.1	0.1	0.1								
Leptogium burnetii C.W. Dodge	40	6	2	3	7					13			
L. intermedium† (Arnold) Arnold	0.2												
L. spp nova 1Bjork & Goward ined.† ¶	0.1												
L. occultatum† Bagl.	0.2												
L. saturninum† (Dickson) Nyl.	116	45	27	13	68		0.1			49	1		
L. teretiusculum† (Wallr.) Arnold	0.1		0.1	0.1	0.1					0.1			
L. spp. nova 2 Bjork & Goward ined.† ¶	0.1		0.1							0.1			
Tripartite cyanolichen													
L. pulmonaria (L.). Hoffm	4118	4518	3434	5678	2578	1705	952	493	78	880	216	114	144

† - not included in the NMS or MRPP analysis due to rarity in the data set
‡- included in the NMS and MRPP analysis as Peltigera spp.
¶ - not yet described

Table 2.3 Total number of foliose macrolichen species (and mean species per plot ± SD) observed within four functional groups on conifer saplings beneath five overstorey tree species at Fraser, Aleza and Herrick sites.

Site-type	Tree species	Bipartite stratified cyanolichens	Bipartite non-stratified cyanolichens	Tripartite stratified cyanolichens	Chlorolichens	Total
Fraser	*Populus*	14 (8±1)	15 (7±3)	1	12 (3±3)	42 (18±4)
	Betula	14 (8±2)	8 (3±2)	1	16 (6±4)	39 (18±5)
	Abies	12 (6±3)	11 (3±3)	1	13 (6±3)	37 (16±4)
	Picea	13 (8±3)	8 (2±3)	1	14 (7±4)	36 (18±3)
Aleza	*Populus*	10 (5±2)	8 (3±2)	1	17 (9±3)	36 (16±7)
	Betula	7 (2±1)	0	1	19 (11±2)	27 (13±2)
	Abies	6 (2±2)	1 (0.1±0.3)	1	16 (11±2)	24 (13±2)
	Picea	6 (2±2)	0	1	15 (10±1)	22 (12±3)
	Pseudotsuga	1 (0.4±0.5)	0	1	13 (9±1)	15 (10±2)
Herrick	*Populus*	13 (7±2)	7 (3±2)	1	18 (8±4)	39 (19±5)
	Betula	12 (6±2)	1 (0.4±0.5)	1	21 (13±3)	35 (20±4)
	Abies	8 (5±3)	0	1	21 (12±2)	30 (18±3)
	Picea	8 (4±2)	0	1	19 (13±2)	28 (17±4)

Differences in cyanolichen communities on conifer saplings beneath the different overstorey tree species varied with site-type (Table 2.3). At the Fraser sites, cyanolichen communities were similar regardless of the overstorey tree species. By contrast, the cyanolichen communities on saplings beneath overstorey *Populus* at the Aleza and Herrick sites were

significantly more abundant and species-rich compared to those on saplings beneath other tree species (MRPP, T=-20.04, A=0.46, p=0.0001). Furthermore, 24% and 27% of all cyanolichen species observed at the Aleza and Herrick sites, respectively, were observed exclusively on saplings beneath *Populus*. Epiphyte communities on saplings beneath *Betula*, *Abies* and *Picea* at the Aleza and Herrick sites were mainly composed of chlorolichens and were largely devoid of non-stratified cyanolichen species; only two small *Leptogium saturninum* thalli (<1 cm^2) were recorded beneath these trees at the Aleza and Herrick sites.

Cyanolichen abundance, like species-richness, was greater on saplings beneath *Populus* at the Aleza and Herrick sites, and at Fraser sites regardless of tree species (Fig. 2.2). The degree to which lichen abundance on conifer saplings differed by overstorey tree species varied with site-types; cyanolichens were 44%, 96% and 89% more abundant on saplings beneath *Populus* than beneath conifers or *Betula* at the Fraser, Aleza and Herrick sites respectively. Chlorolichens, in contrast, were most abundant on saplings beneath *Betula* or conifer canopies at the Aleza and Herrick sites (Fig. 2.2).

Small thalli of all cyanolichen functional groups were highly abundant on saplings beneath all overstorey tree species at the Fraser sites (Table 2.4). In contrast, small cyanolichen thalli (especially the non-stratified cyanolichens) were limited to saplings beneath *Populus* trees at the Aleza and Herrick sites.

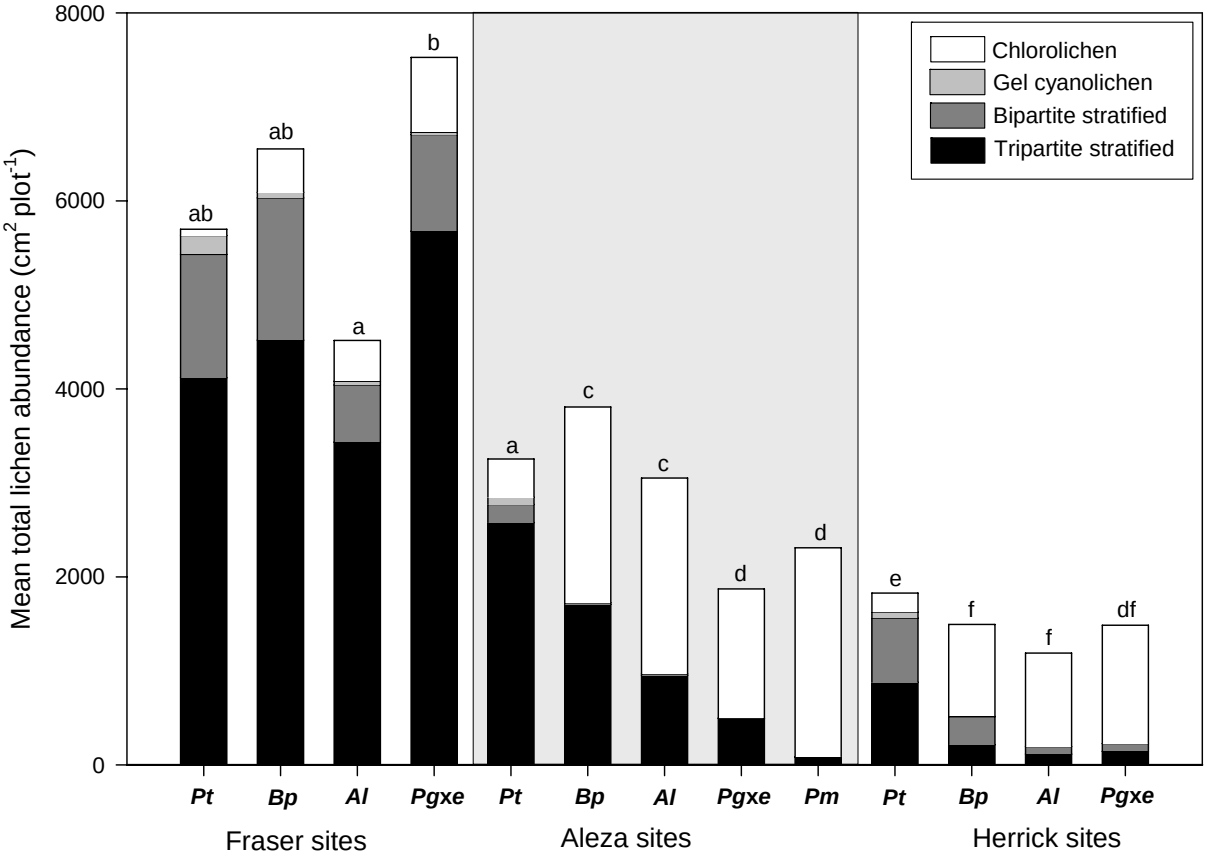

Figure 2.2 Total abundance of four functional groups of epiphytic foliose macrolichens observed on sapling branches beneath five tree species at the three site-types. Tree species codes: Pt (*Populus tremuloides* or *P. trichocarpa*), Bp (*Betula papyrifera*), Al (*Abies lasiocarpa*), Pgxe (*Picea glauca* x *engelmannii*), Pm (*Pseudotsuga menziesii*). Letters above the bars denote significant multivariate differences in lichen diversity and abundance across plot types (overall MRPP; T= -18.11, p<0.0000, A= 0.39).

Table 2.4 Total numbers of small cyanolichen thalli (< 0.5 cm^2) observed within three cyanolichen functional groups on conifer saplings beneath five overstorey tree species at Fraser, Aleza and Herrick sites (N=9).

Site-type	Tree species	Bipartite stratified cyanolichens	Bipartite non-stratified cyanolichens	Tripartite stratified cyanolichens
Fraser	*Populus*	5270	640	4720
	Betula	2500	70	2780
	Abies	2770	220	5170
	Picea	7970	260	6870
Aleza	*Populus*	220	620	2940
	Betula	0	0	70
	Abies	0	0	50
	Picea	40	0	10
	Pseudotsuga	0	0	5
Herrick	*Populus*	1080	540	190
	Betula	340	0	170
	Abies	140	0	240
	Picea	80	0	30

The NMS ordination provided a graphical summary of the overall variation in lichen community composition and abundance (Fig. 2.3). Fraser plots largely grouped with the cyanolichens while Aleza and Herrick sites were more widely spread out, covering a range of species groups. Plots representing saplings beneath overstorey *Populus* from Aleza and Herrick sites clustered with all plots at the Fraser sites due to their association with cyanolichens. In contrast, plots representing saplings beneath overstorey conifer and *Betula* trees at the Aleza and Herrick sites grouped with the chlorolichens and were clearly separated from those representing saplings beneath *Populus* and all the Fraser-site plots. The MRPP comparisons confirmed the quantitative differences in lichen communities across site-types and between overstorey tree species (Fig. 2.2). Whereas differences between the plots representing saplings beneath *Populus* and those representing saplings beneath other overstorey tree species were non-significant at the Fraser sites, this was not the case at Aleza and Herrick sites, where *Populus* plots differed significantly from the other overstorey trees.

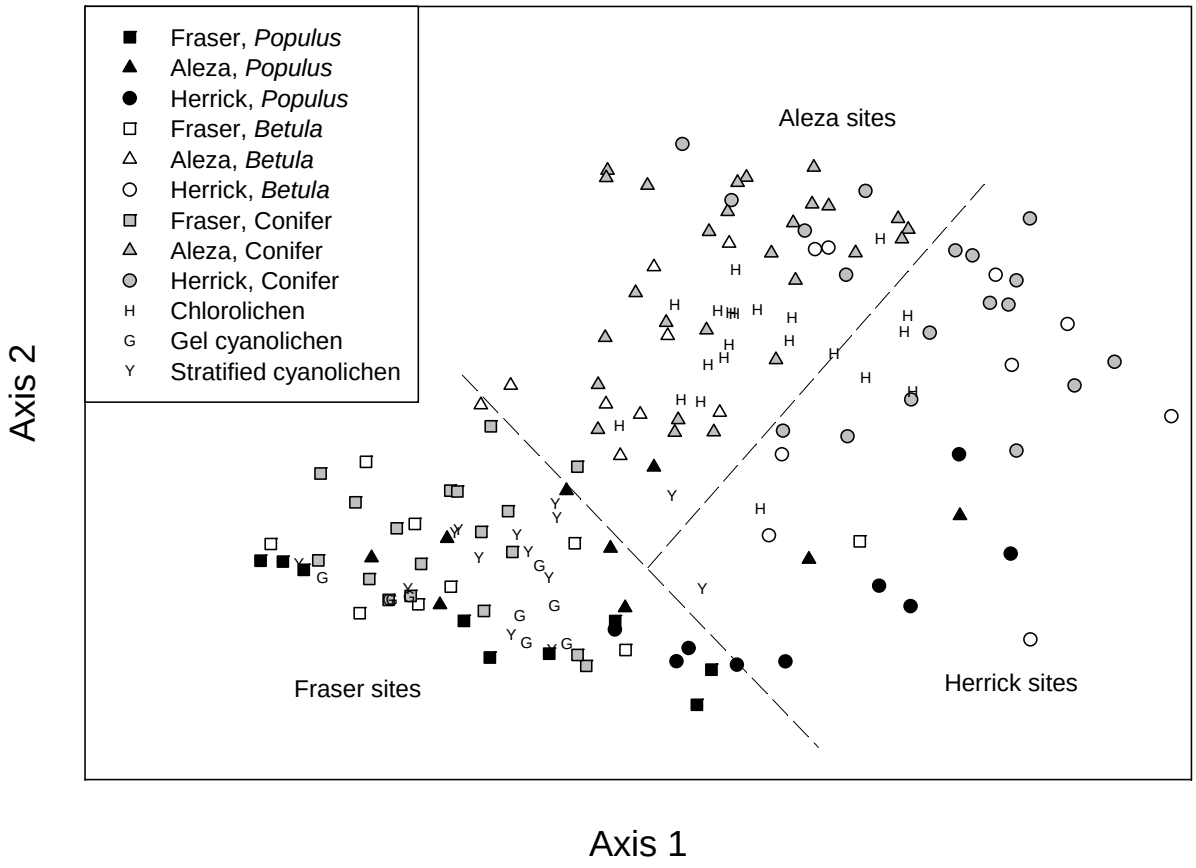

Figure 2.3 NMS ordination of 46 species observed in 117 plots at three site-types. The ordination resulted in a 2-dimensional solution with a final instability criterion <0.0001 after 250 runs with real data. NMS axes 1 and 2 accounted for 47% and 42% of the variation in the distance matrix respectively. Letters (H- chlorolichen, Y – stratified cyanolichen, G – non-stratified cyanolichens) denote individual lichen species and symbols represent plots shown by site-type and overstorey tree species combinations. Dashed lines indicate approximate separation of the three site-types.

Conifer saplings versus overstorey Populus

Cyanolichen abundance and species-richness were higher on conifer saplings beneath the overstorey *Populus* canopy than on the trunks of the *Populus* trees themselves. *Populus* trunks (and branches where present) supported 11, 11 and 14 cyanolichen species at Fraser, Aleza and Herrick site-types, respectively. In contrast, conifer saplings beneath *Populus* canopies supported 31, 19 and 21 cyanolichen species for the same site-types (Fig.2.4).

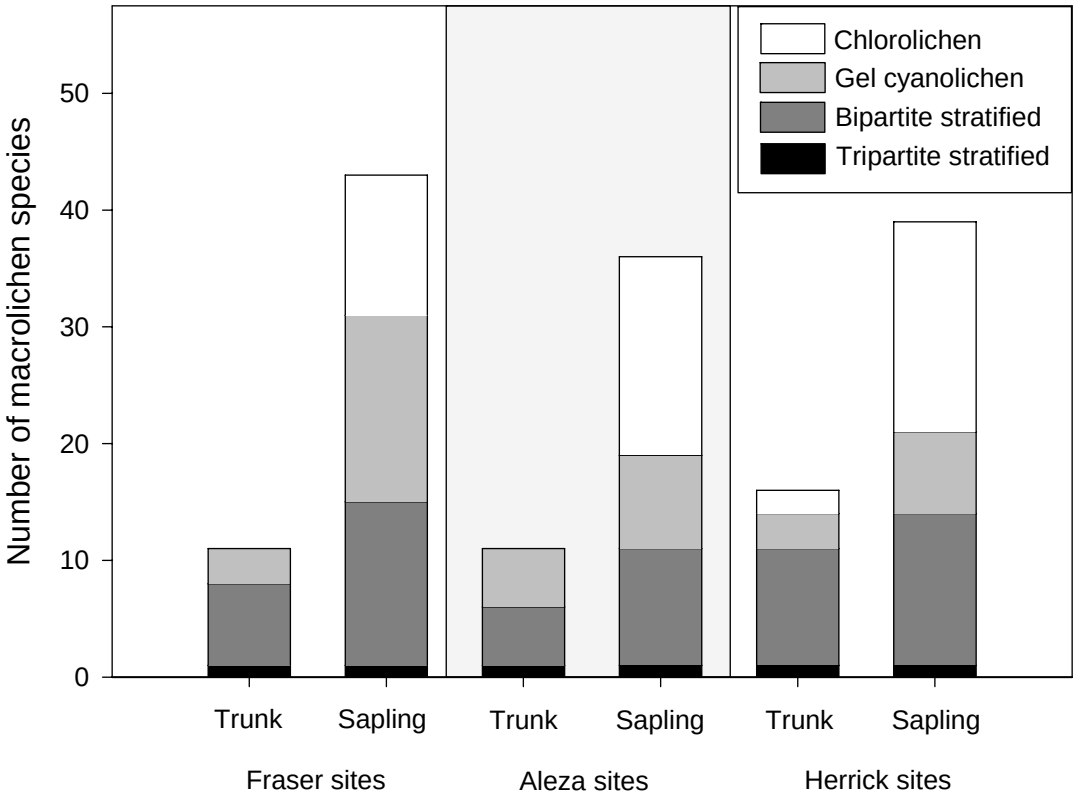

Figure 2.4 Diversity of foliose chlorolichens, non-stratified cyanolichens, stratified bipartite cyanolichens and tripartite cyanolichens observed on *Populus* trunks versus on the conifer saplings beneath *Populus* canopies at Fraser, Aleza and Herrick sites.

2.3.3 Precipitation, bark and soil chemistry

In general, no clear relationships were evident between throughfall precipitation chemistry and foliose macrolichen community patterns. Calcium concentrations beneath *Populus* were similar to all other overstorey tree species at the Fraser sites, *Picea* at the Aleza sites, and *Abies* and *Picea* at the Herrick sites ($F(8,122)=3.36$, $p=0.002$; Fig. 2.5a & b). Phosphorus and manganese concentrations were both significantly lower in precipitation throughfall from beneath *Populus* and *Betula* compared to beneath *Abies* and *Picea* (P: $F(4,122)=94.71$, $p=0.000$; Fig. 2.5c; Mn: $F(4,122)=58.12$, $p=0.000$; Fig. 2.5e). The highest overall phosphorus and manganese concentrations were observed in throughfall precipitation at the Fraser sites ($F(2,122)=6.51$, $p=0.002$; Fig. 2.5c) and at the Herrick sites ($F(2,122)=9.1$, $p=0.000$; Fig. 2.5e), respectively. No differences in throughfall concentrations of molybdenum were detected across site-types or tree species (Fig. 2.5g). In contrast to the other elements studied, throughfall molybdenum levels were significantly lower than in the non-throughfall (open) precipitation across all three site-types ($F(4,122)=11.14$, $p=0.000$), indicating some degree of uptake by trees from the ambient precipitation. Also unlike the other four elements, molybdenum concentrations were higher in the winter precipitation (Fig. 2.5h).

Precipitation chemistry was poorly correlated with lichen abundance; manganese concentrations were negatively correlated with non-stratified cyanolichen abundance ($r= -0.29$, $p=0.003$), and phosphorus was negatively correlated with bipartite stratified cyanolichen abundance ($r= -0.27$; $p=0.004$). All other correlations were non-significant.

The pH of ambient precipitation was 6.5±0.2 and did not differ between site-types. Throughfall precipitation beneath *Abies* and *Picea* was more acidic than that beneath *Populus* and *Betula* at all site-types ($F(4,48)=6.73$, $p=0.0001$; Fig. 2.6a). Conifer sapling bark pH was higher at the Fraser sites regardless of over overstorey tree species. At the Aleza and Herrick

sites, bark pH of saplings beneath *Populus* was similar to that at the Fraser sites, and was significantly higher than that beneath all other tree species ($F(6,271)=7.84$, $p<0.0001$; Fig. 2.6b). Sapling bark pH was positively correlated with cyanolichen abundance ($r=0.62$, $p=0.0001$) but only weakly correlated with precipitation pH ($r= 0.29$; $p=0.04$), and not significantly correlated with soil pH. Soil organic matter was more acidic at the Fraser sites (pH 4.8±0.1) than at the Aleza (5.0±0.1) or Herrick (5.2±0.1) sites ($F(2, 114)=7.52$, $p=0.0009$), with no difference between tree species. Conversely, mineral soil pH was significantly higher beneath *Populus* at Fraser sites (5.2±0.1) than beneath *Pseudotsuga* at Aleza sites (4.6±0.1) or *Picea* at Herrick sites (4.6±0.1; $F(3,104)=2.91$, $p=0.04$).

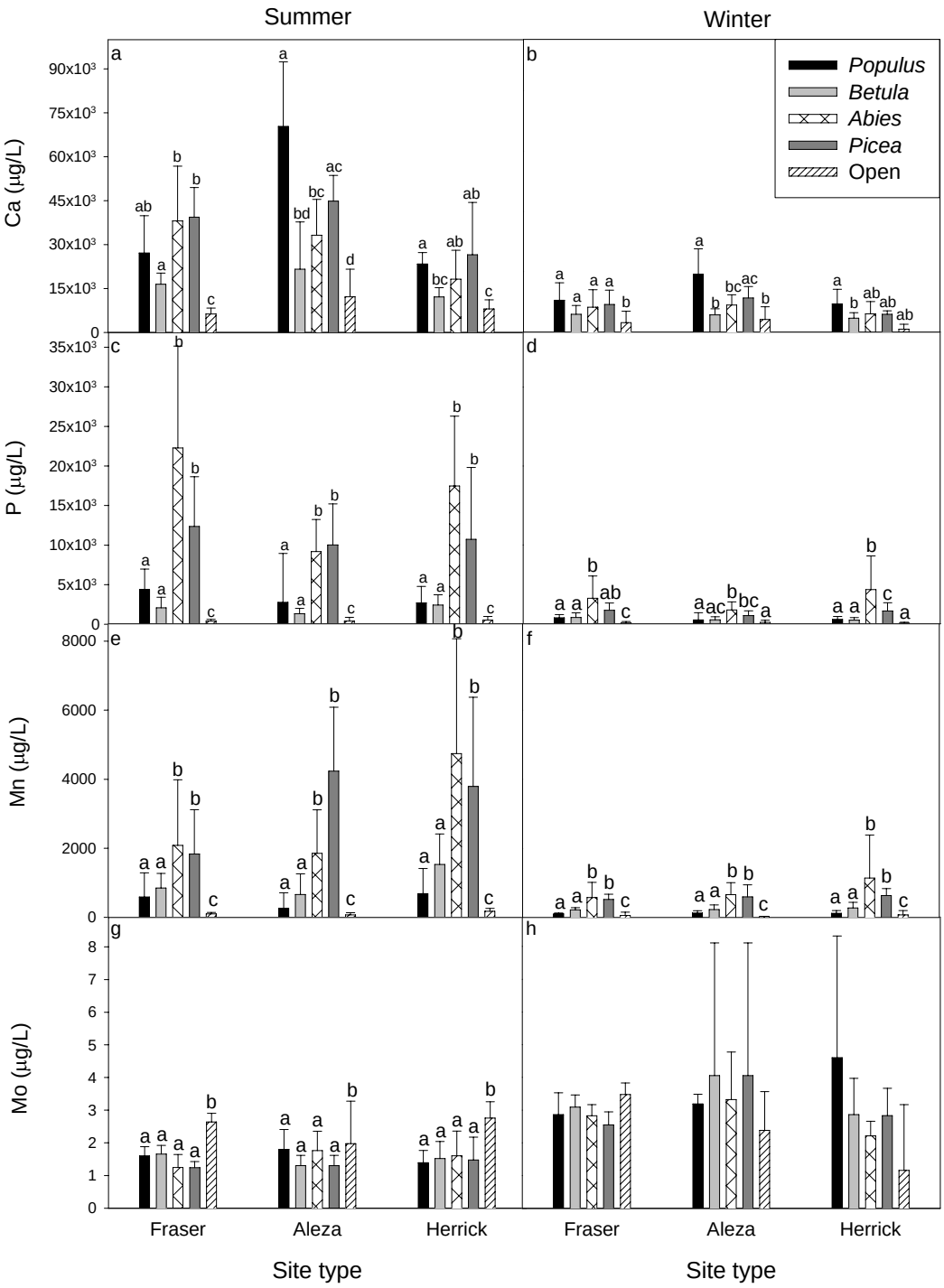

Figure 2.5 Concentration (± SD) of Ca, P, Mn, and Mo in precipitation captured in the open and beneath four tree species at three site-types. Elements were extracted from resin capsules installed in the field from November 2007 to May 2008 (Winter) and from May to September 2008 (Summer). Dissimilar letters represent significant differences ($p<0.05$) between tree species within site-types.

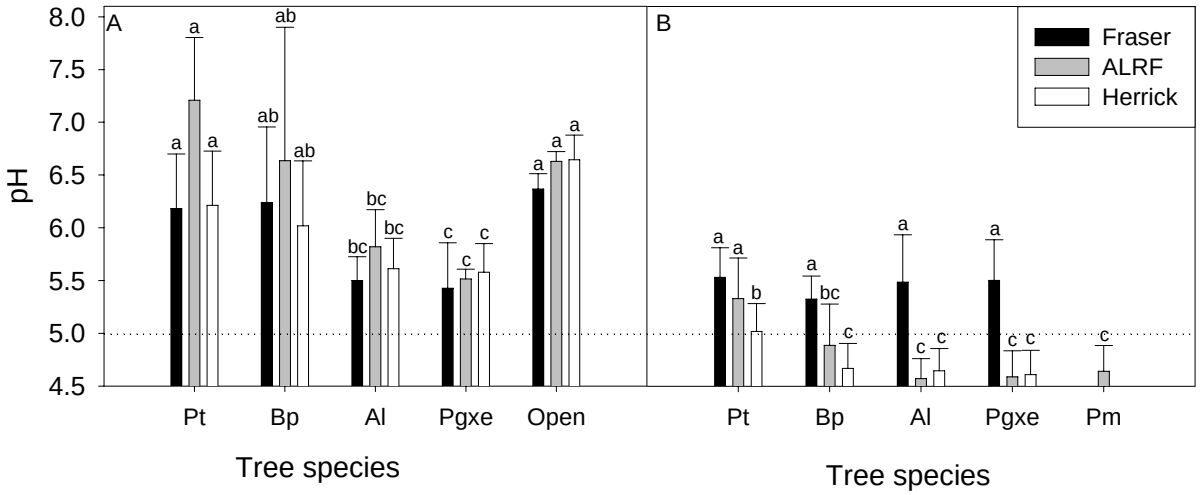

Figure 2.6 Comparisons of (a) throughfall precipitation pH (±SD) and (b) conifer sapling bark pH (±SD) beneath five tree species at three site-types. Tree species codes: Pt (*Populus tremuloides* or *P. trichocarpa*), Bp (*Betula papyrifera*), Al (*Abies lasiocarpa*), Pgxe (*Picea glauca* x *engelmannii*), Pm (*Pseudotsuga menziesii*). Dashed lines represent the lower pH limit for cyanolichen habitat (Gauslaa 1985). Dissimilar letters represent significant differences ($p<0.05$) between tree species and site-types.

2.4 Discussion

2.4.1 Lichen abundance patterns

Abundant and species-rich cyanolichen communities were observed on conifer saplings beneath overstorey *Populus* trees across the study area. This spatial association intensified in areas where sub-optimal environmental conditions (i.e. moisture or light deficiencies) appeared to preclude cyanolichens in the surrounding conifer forests. This suggests that *Populus* may facilitate the development of cyanolichen thalli beneath its canopy. This may be particularly the case with the non-stratified (homoiomerous) lichen species, more than 85% of which were observed exclusively under *Populus* in these sub-boreal forests. The distribution of non-stratified lichens is known to be limited in inland temperate forests (for example, see Spribille et al. 2009 for ecology and distribution of *Collema coniophilum*), however, the nearly exclusive existence beneath *Populus* at sites with moisture or light deficiencies merits further investigation.

The importance of moisture availability in determining the composition of lichen communities is borne out in our results. Lichens are less physiologically active when thallus moisture falls below a certain threshold (Kappen 1988). This threshold varies considerably with species and is generally higher for cyanolichens (>150% dry weight, Lange et al. 2004) than for chlorolichens with green-algal partners (50-70% dry weight, Lange et al. 1986). Accordingly, the epiphyte communities beneath a coniferous overstorey at the Fraser sites were composed largely of cyanolichens while those at the drier, Aleza sites were primarily chlorolichens. However, that overstorey conifers at the Herrick sites, with similar rainfall and relative humidity as the Fraser sites, also supported predominantly chlorolichen communities indicates that other environmental factors are involved. The removal of individual trees from the Herrick sites in the early 1900's effected stand structure such that light was 9% lower than at the other sites. For cyanolichens

that almost invariably occupy the lower canopy where direct sunlight is infrequent, this reduced light availability could result in reduced establishment, growth (Gauslaa et al. 2006) and photosynthesis (Gaio-Oliveira et al. 2004). While climate is a key determinant of regional distribution patterns, moisture and light availability cannot account for the enriched cyanolichen communities observed beneath *Populus* as there were no differences in these factors between overstorey tree species.

Differences in lichen distribution patterns have previously been related to variation in the physical characteristics of the phorophyte itself (Gauslaa 1995; Hauck and Spribille 2005). However, the rich cyanolichen community observed beneath *Populus* cannot be solely attributed to substrate characteristics as all host saplings here were either *Abies* or *Picea*, and no significant floristic differences were noted between them. Furthermore, the greater diversity of cyanolichens observed on saplings beneath the *Populus* canopy, rather than on the *Populus* trunks, indicates that the facilitative role involves more than the physical attributes of the host tree species.

The abundant and species-rich cyanolichen communities observed on conifer saplings beneath overstorey *Populus* trees suggest that *Populus* facilitates one or more of lichen dispersal, establishment or growth. The close proximity between the cyanolichen-rich communities beneath *Populus* and the cyanolichen-poor communities beneath conifers indicates that dispersal may not be a limiting factor. However, the probability of dispersed diaspores becoming established may be low (Scheidegger 1995) and successful recruitment of cyanolichens on conifer branches in a moisture- or light-deficient conifer forest may depend on the possible amelioration of habitat conditions by overstorey *Populus*. This possibility is supported by the greater number of small (and presumably newly established) cyanolichen thalli observed beneath *Populus* compared to all other overstorey trees.

2.4.2 Relationship to precipitation chemistry

These results support the concept of a drip-zone of nutrient-enriched precipitation surrounding *Populus* (Goward and Arsenault 2000). Several chemical factors have been highlighted in the literature as potentially important to cyanolichens. Cyanolichens are sensitive to acidic conditions below pH 5.0 (Gauslaa 1985; Farmer et al. 1991). Goward and Arsenault (2000) attributed substrate pH differences to an increase in available calcium in throughfall precipitation. Our data also show a strong correlation between cyanolichen abundance and sapling bark pH. However, a similar relationship with the pH or calcium concentration of throughfall precipitation was not observed. Overall, the weak correlation between precipitation pH, soil pH, and sapling bark pH suggests that factors other than precipitation or soil chemistry may be involved. It is plausible that the lichens themselves are altering the pH of sapling bark, rather than responding to it. Gauslaa and Holien (1998) reported that cyanolichens can raise the pH of their bark substrate by releasing more cations than protons at ion-exchange sites, whereas chlorolichens have the opposite effect. Thus, the abundant cyanolichens at Fraser sites and beneath *Populus* at Aleza and Herrick sites might have increased the sapling bark pH, while the abundant chlorolichens beneath conifers and *Betula* at the Aleza and Herrick sites might have decreased the substrate pH.

Cyanolichens are capable of N_2-fixation and so may be limited by the availability of phosphorus or molybdenum (Kurina and Vitousek 1999). Indeed, cyanolichen diversity and abundance increased in direct response to long-term phosphorus fertilization in late-seral Hawaiian forests (Benner and Vitousek 2007). In this study, however, the weak correlation between phosphorus concentrations and cyanolichen abundance suggests that phosphorus was not a limiting factor (see also Arocena and Sanborn 1999). Molybdenum additions have likewise been shown to increase the nitrogen-fixing activity of asymbiotic N_2-fixing bacteria (Silvester

1989) and lichens (Horstmann et al. 1982). However, the similar molybdenum concentrations across tree species and study sites here suggest that it is unlikely to be a limiting factor for cyanolichen growth. Finally, the weak inverse relationship between manganese concentrations and non-stratified cyanolichen abundance provides tentative agreement with previous records of lichen-thallus inhibition in response to high manganese concentration (reviewed in Hauck 2003). Nonetheless, significantly different lichen communities were noted beneath *Betula* and *Populus* canopies despite similar manganese concentrations.

Cyanolichen communities often occur only on the trunks of *Populus* trees and are otherwise absent on the surrounding coniferous tree species. Cyanolichens are not similarly restricted to *Populus* trunks in sub-boreal British Columbia and, indeed, are more species-rich on the branches of conifer saplings beneath *Populus* than on the *Populus* trunks themselves. There are several plausible explanations. Sapling conifer branches provide a larger, horizontal surface area which may allow for higher cyanolichen colonization compared to *Populus* trunks. In a similar vein, the varied orientation and exposure of conifer branches beneath *Populus* trees may provide a more heterogeneous habitat which, as ecological theory predicts, can result in greater diversity (Ricklefs 1977). Alternatively, the unexpectedly low diversity of lichens observed on *Populus* trunks may be in response to the ameliorated environment created by frequent interception of stemflow and throughfall precipitation through the *Populus* canopy. In such environments, stress is likely to be minimal and as predicted by Grime (1973), inter-specific competition will strongly define community structure. One or a few strongly competitive species would thus be expected to become dominant to the exclusion of other, less competitive, species (Paine 1966).

While competition may be a dominant factor determining lichen species-richness on *Populus* trunks, the lichens observed on conifer saplings beneath *Populus* suggest that facilitation is equally important in defining lichen community structure. The near-exclusive occurrence of cyanolichens beneath overstorey *Populus* at sites with moisture (Aleza) or light (Herrick) deficiencies suggests that *Populus* supplies some as yet unknown, resource that is critical to cyanolichen establishment or growth. As such, overstorey *Populus* trees appear to be crucial for providing cyanolichen habitat in regions where environmental conditions otherwise limits their establishment in the surrounding conifer forest.

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CHAPTER 3 Growth and mortality of cyanolichens under *Populus*[2]

3.1 Introduction

Populus has been shown to support unique and species-rich lichen communities. In many parts of the world, *Populus* trunks provide habitat for cyanobacterial lichens that are otherwise absent in the predominantly conifer forests (Kuusinen 1996; Hedenås and Ericson 2000). Although many cyanolichen species are also frequently observed on conifer branches in wet, interior forest ecosystems of British Columbia, cyanolichen communities are disproportionately species-rich and abundant on conifers growing beneath an overstorey *Populus* tree compared to those beneath a conifer canopy (Goward and Arsenault 2000; Campbell et al. 2010).

The factors underlying the strong association between cyanolichens and *Populus* are not understood, but it is probable that *Populus* supports the cyanolichen symbiosis at one or more developmental stage. Sillett et al. (2000) describe the development of epiphytic cyanolichens in three stages; propagule dispersal, establishment, and thallus growth. Inadequate propagule dispersal has been shown to contribute to differences in epiphyte community structure (Sillett et al. 2000). However, dispersal limitations may not be involved in defining the observed differences between overstorey tree species; *Populus* trees, heavily loaded with *L. pulmonaria*, are often located within 0.1 ha of other tree species that support cyanolichen-poor epiphyte communities (e.g. Kuusinen 1996; Goward and Arsenault 2000; Campbell et al. 2010). The proximity of these disparate communities is well within the dispersal range of many cyanolichens (Ockinger et al. 2005). Any potential positive influence of *Populus* on cyanolichen communities is thus more likely to occur at a later phase of thallus development. Indeed, Schiedegger (1995) demonstrated that very few dispersed propagules successfully develop into a

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mature lichen thallus, and that this success depends on substrate and microclimatic conditions. The strong spatial association between abundant cyanolichen communities and *Populus* suggest that colonization success is greater near *Populus* than near other trees. However it is unknown whether this success is due to greater rates of establishment or growth.

Previous studies have suggested that lichen growth rates are dependent on the conditions of the study area. Transplant experiments have demonstrated the relative influence of forest age (Sillett et al. 2000), light availability (Coxson and Stevenson 2007a), moisture availability (Gauslaa et al. 2007), seasonality (Muir et al. 1997) and nutrient availability (McCune and Caldwell 2009) on lichen growth. However, despite repeated accounts of highly disparate lichen communities on broadleaf versus conifer trees, few studies have controlled for the influence of different host or overstorey tree species on lichen growth. This study examines the effect of overstorey *Populus* by comparing growth rates of cyanolichens transplanted beneath the canopies of mature *Populus tremuloides* or *P. balsamifera* ssp. *trichocarpa*, *Picea glauca* x *engelmannii* and *Pseudotsuga menziesii* var. *glauca*. The influence of overstorey tree species is evaluated, relative to regional climate, by assessing growth rates in three sub-boreal spruce forests with varying climatic conditions. Furthermore, the influence of overstorey tree species on growth rates of tripartite (both a cyanobacterial and green-algal partner) and bipartite (only a cyanbacterial partner) cyanolichen species are compared.

3.2 Methods and materials

3.2.1 Study area

The study area was located north-east of Prince George British Columbia in old growth (mean tree age >240 years) forests of the Sub-Boreal Spruce (SBS) biogeoclimatic zone (Meidinger and Pojar 1991). Three replicate stands were selected in each of three site-types. The

'Aleza' sites were located in the Aleza Lake Research Forest at 680 m in elevation. Relatively humidity was consistently lower at these three sites and so they were considered to be the most moisture-deficient of the three site-types (Campbell et al. 2010). 'Herrick' sites were located along the Herrick Forest Service Road approximately 28 km north-east of the Aleza sites at an elevation of 850 m. Lower light levels were recorded at these sites compared to the other site types and so they were considered to be light-deficient (Campbell et al. 2010). Finally, three sites where neither moisture nor light were limiting (the Fraser sites) were located in an ecotonal region between the two aforementioned subzones at 680 m in elevation (see Campbell et al. 2010 for detailed site information).

3.2.2 Cyanolichen growth

Healthy, intact thalli of *Lobaria pulmonaria* (L.) Hoffm. and *Lobaria hallii* (Tuck.) Zahlbr. were collected from a single spruce-subalpine fir dominated forest stand in early June 2006. *Lobaria pulmonaria* is a tripartite cyanolichen species and is among the more highly studied of all lichen species and is abundant beneath all tree species in many sub-boreal spruce forests (Campbell et al. 2010). *Lobaria hallii* is a bipartite cyanolichen with more limited distribution; it is highly abundant beneath *Populus* but is otherwise infrequent in sub-boreal British Columbia (Campbell et al. 2010).

Forty large (initial mass: *L. pulmonaria* 0.42±0.07 g, *L. hallii* 0.23±0.07 g) and 120 small (initial mass: *L. pulmonaria* 0.003±0.001 g, *L. hallii* 0.004±0.001 g) thalli of each lichen species were collected to represent mature and establishing lichens, respectively. Samples were cleaned, carefully removed from the substrate, and placed in a climate-controlled environment (25^{o}C and 40% relative humidity) where thallus-water content was allowed to stabilize for at least 72 h prior to weighing as per Coxson and Stevenson (2007b). Selected thalli were secured to strips of silicone-coated (TSNet-Tech Spray, Amarillo TX) aluminium mesh using clear silicone sealant

(GE Sealants and Adhesives, Huntesville, NC). Strips were attached to a 28 cm x 18 cm plastic tray (1 cm x1 cm grid) using binder clips and enclosed by UV-resistant, translucent mesh. Light transmission in these growth enclosures was >95% of ambient light (Coxson and Stevenson 2007b).

Growth enclosures contained one large and three small thalli of either *L. pulmonaria* or *L. hallii* (see Coxson and Stevenson 2007b for photograph of growth enclosure). Two enclosures (one of each lichen species) were hung from wooden supports attached at approximately 3 m in height on the north side of each of two mature trees of each tree species at each site (N=6 large thalli, 18 small thalli per lichen species x tree species x site-type). Tree species included *Picea glauca* (Moench) Voss x *engelmannii* Parry ex Engelmann (interior hybrid spruce) and *Populus* spp. at each site (*Populus tremuloides* Michx. (trembling aspen) at Aleza sites and P. *balsamifera* L. ssp. *trichocarpa* Brayshaw (black cottonwood) at Fraser and Herrick sites). Enclosures were also hung beneath *Pseudotsuga menziesii* (Mirbel.) Franco var. glauca (Beissn.) Franco. (Douglas-fir) at the Aleza sites only. For simplicity, supporting tree species are hereafter referred to by genus name only, with *P. tremuloides* and *P. trichocarpa* collectively referred to as *Populus*.

Transplants were initially placed in the field in late June 2006 and brought back into the lab for remeasurement in early October 2006, late June 2007, early October 2007 and late June 2008. Each time they were cleaned of debris and pollen and placed in the climate-controlled environment for 72 hours prior to remeasurement. Transplants were replaced in the field after no more than 96 hours in the lab. Large lichen thalli were weighed at each time interval to calculate growth rate. Growth of small thalli was represented by changes in surface area over time as recorded in digital photographs. Areas rather than masses were thus used for small thalli to

prevent growth being obscured by small variations in measuring conditions. Briefly, thallus size was standardized by resizing a digital photograph (CorelDRAW™ 12; Corel Corporation Inc., Mountain View, CA) so that 50 mm on a plastic ruler included in the photograph equaled a 50 mm digital standard. Lichen thalli were then digitally traced and exported to ArcView (3.2; ESRI, Redlands, CA) to quantify the surface area of each thallus at each time period. The initial surface areas of small *L. pulmonaria* and *L. hallii* were 31.8±10.2 mm^2 and 27.1±13.0 mm^2, respectively. Lichen thalli were removed from the experiment if they were disturbed by wildlife or falling trees, visibly lost large fragments, or were completely discolored (either entirely bleached or entirely black) and visibly unresponsive to rewetting.

3.2.3 Data analysis

Lichen transplant growth was analyzed at each site-type using a repeated-measures ANOVA with a Fishers LSD post hoc test. The main effects and interactions between lichen species (*Lobaria pulmonaria* and *L. hallii*) and tree species (*Populus* and *Picea*) were tested across the 27 month transplant period. A Chi-square was used to test group differences in mortality frequency between tree species and site types. Values are given as mean± standard deviation unless otherwise stated.

3.3 Results

3.3.1 Large thalli

Growth rates of both species varied considerably, ranging from 29 to 238% and 5 to 225% in *Lobaria pulmonaria* and *L. hallii*, respectively, over 27 months. However, faster growth rates were observed with *L. pulmonaria* transplants than *L. hallii* at all three sites (Fig. 3.1a). Accordingly, the mass of *L. pulmonaria* transplants was significantly larger than that of *L. hallii* transplants throughout the entire transplant period at all three site types (Fig. 3.1b)

The growth rate of large lichens was also consistently faster beneath *Populus* than beneath *Picea* for both lichen species (Fig. 3.1a; see Table 3.1 for ANOVA statistics). Transplant mass of *L. pulmonaria* increased by 117±40% and 80±38% beneath *Populus* and *Picea*, respectively, over 27 months, while *L. hallii* mass increased by 112±46% and 45±36% for the same comparison. The slowest growth for both lichen species was observed beneath *Pseudotsuga* with cumulative growth rates of 36±2% and 3±2% for *L. pulmonaria* and *L. hallii*, respectively. The final transplant mass of both lichen species was greater beneath *Populus* than *Picea* at all three site types (Fig. 3.1b). There were no significant differences in cyanolichen growth between site types.

3.3.2 Small thalli

Growth of small *Lobaria pulmonaria* and *L. hallii* thalli was significantly faster beneath *Populus* than beneath *Picea* at all site types (Fig. 3.2a; see Table 3.1 for ANOVA statistics). While most small thalli beneath *Populus* had modest area gains over 27 months, the majority of small thalli beneath conifers exhibited slow or negative-growth resulting in final thallus areas that were as small, or smaller, than the initial thallus size (Fig. 3.2b).

After 27 months, 2% of small *L. pulmonaria* and 27% of small *L. hallii* showed a net decrease in total area. Under the *Pseudotsuga* overstorey, small *L. pulmonaria* thalli had modest area-gains (16±17%) over the first 12 months and lost area thereafter. *Lobaria hallii* thallus-areas consistently decreased over 15 months after which point all small *L. hallii* were removed from the experiment due to visible necrosis or death.

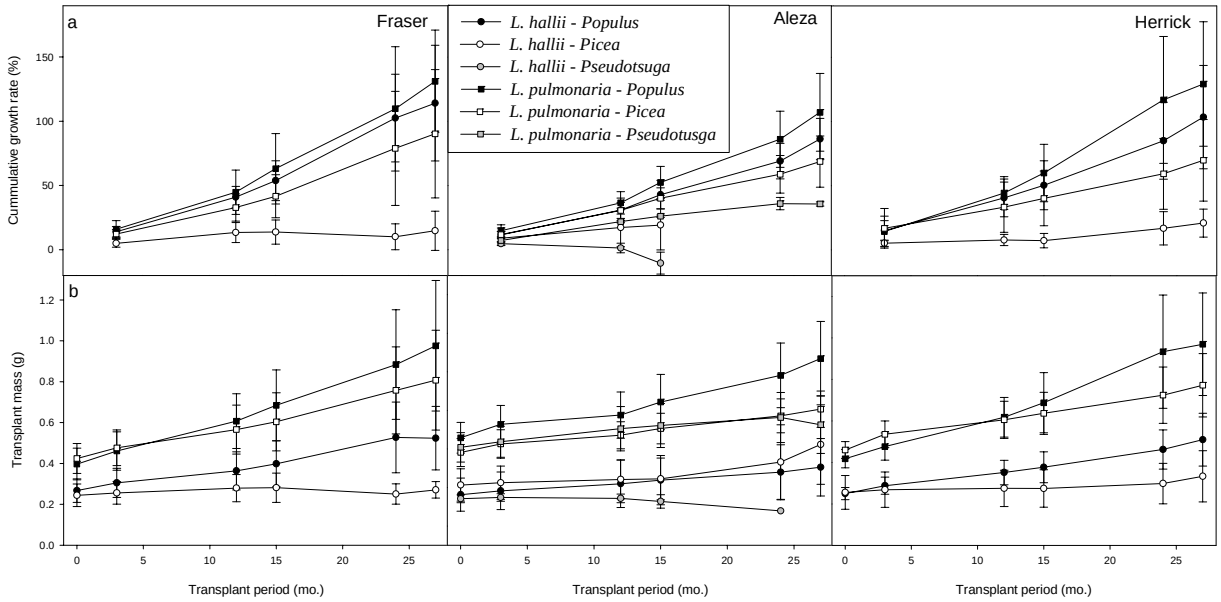

Figure 3.1 Cumulative percent growth rate of large thalli (a) and transplant mass (b) over 27 months beneath mature *Populus*, *Picea*, and *Pseudotsuga* trees at Fraser (left panel), Aleza (middle panel), and Herrick (right panel) sites.

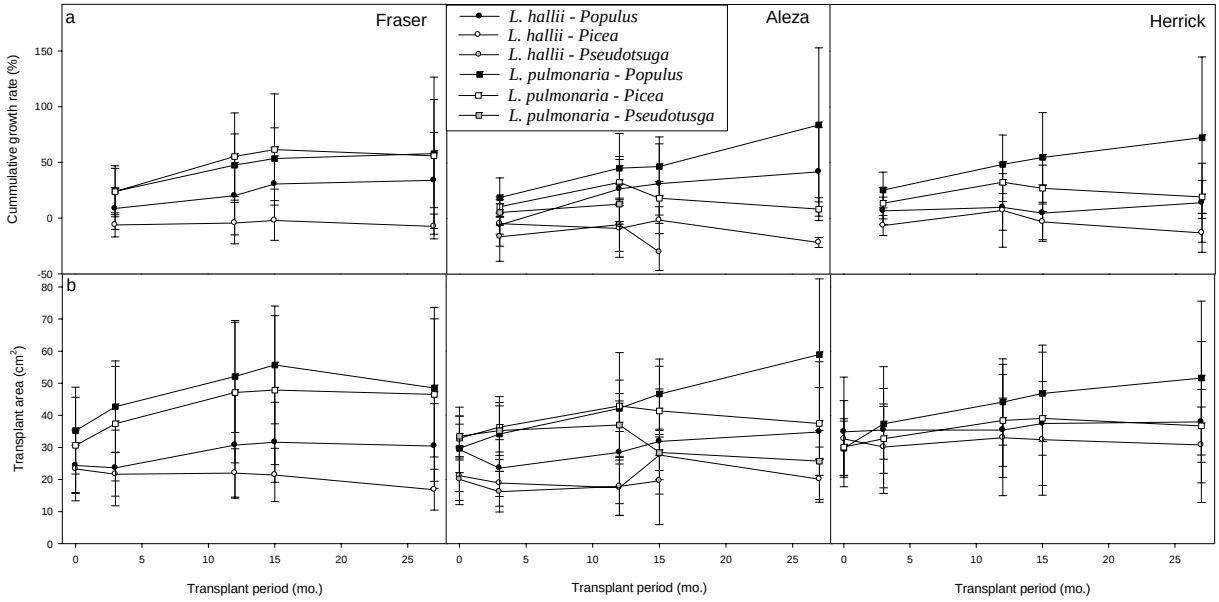

Figure 3.2 Cumulative percent growth rate of small thalli (a) and transplant mass (b) over 27 months beneath mature *Populus*, *Picea*, and *Pseudotsuga* trees at Fraser (left panel), Aleza (middle panel), and Herrick (right panel) sites.

Table 3.1 Repeated Measures ANOVA statistics at Fraser, Aleza and Herrick sites. Models tested significant differences in the change in transplant mass (large thalli) or transplant area (small thalli) over 3, 12, 15, 24 and 27 months (time as the repeated measure) between lichen species (*Lobaria pulmonaria* and *Lobaria hallii*) and tree species (*Populus* and *Picea*). Differences associated with *Pseudotsuga* were not tested due to low sample size.

	Large thalli					**Small thalli**				
	SS	**DF**	**MS**	**F**	**p**	**SS**	**DF**	**MS**	**F**	**p**
Fraser sites										
time	1.534	5	0.307	44.750	**0.0000**	3093.7	4	773.4	12.398	**0.0000**
Time*lichen	0.393	5	0.079	11.450	**0.0000**	77.3	4	19.3	0.310	0.8710
time*tree	0.181	5	0.036	5.279	**0.0002**	1522.9	4	380.7	6.103	**0.0001**
time*lichen* tree	0.008	5	0.002	0.226	0.9502	381.4	4	95.3	1.528	0.1965
Error	0.651	95	0.007			9731.9	156	62.4		
Aleza sites										
time	0.626	5	0.125	70.417	**0.0000**	1563.9	4	391.0	6.720	**0.0001**
Time*lichen	0.102	5	0.020	11.515	**0.0000**	1398.4	4	349.6	6.008	**0.0002**
time*tree	0.022	5	0.004	2.483	**0.0414**	898.5	4	224.6	3.861	**0.0056**
time*lichen* tree	0.035	5	0.007	3.920	**0.0038**	150.3	4	37.6	0.646	0.6311
Error	0.107	60	0.002			6516.7	112	58.2		
Herrick sites										
time	1.170	5	0.234	39.110	**0.0000**	1183.0	4	295.8	5.679	**0.0003**
Time*lichen	0.254	5	0.051	8.499	**0.0000**	924.8	4	231.2	4.439	**0.0020**
time*tree	0.186	5	0.037	6.223	**0.0001**	2105.3	4	526.3	10.106	**0.0000**
time*lichen* tree	0.011	5	0.002	0.369	0.8683	88.3	4	22.1	0.424	0.7913
Error	0.419	70	0.006			8541.0	164	52.1		

3.3.3 Mortality

Cumulative small-thallus mortality of both lichen species tended to be higher beneath *Picea* than *Populus* at all site-types and was significantly greater beneath conifers than *Populus* at the Aleza sites (χ^2 (6) = 26.35, p=0.0002). All small lichen thalli transplanted to beneath *Pseudotsuga* were either dead or highly necrotic following the second summer (Fig. 3.3). Indeed, with few exceptions, the highest mortality rates were observed between June and October 2007 (12 – 15months; Fig. 3.3). The highest mortality rates were consistently observed beneath conifers at the Aleza sites, where 90-100% of both species were dead after 27 months. Although several large lichen thalli appeared at least partially necrotic after 27 months, there was no large-thallus mortality in either lichen species. Unfortunately all large *L. hallii* thalli transplanted to beneath *Pseudotsuga* were disturbed by wildlife over the second winter and were excluded from the experiment.

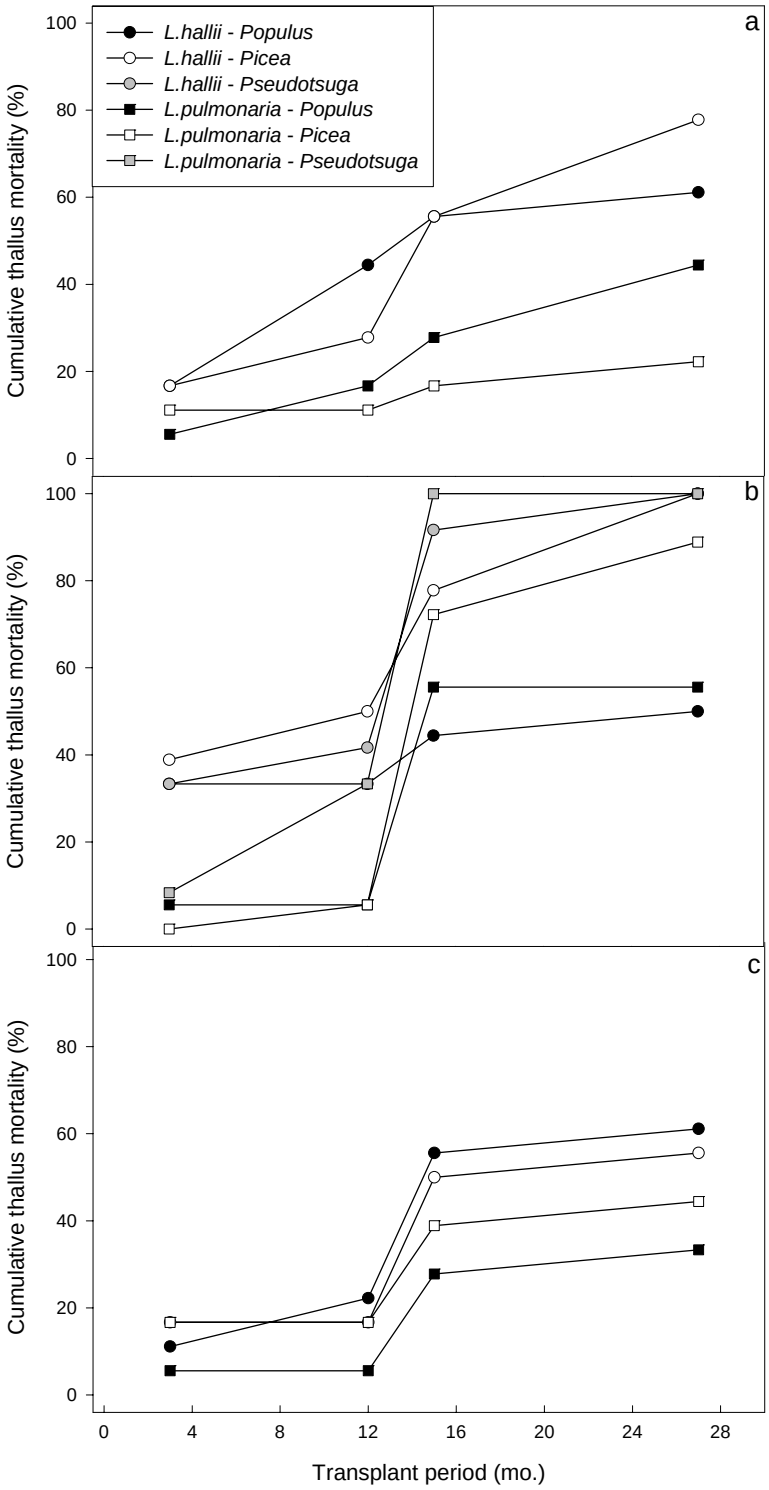

Figure 3.3 Cumulative mortality of small *Lobaria hallii* and *L. pulmonaria* thalli beneath *Populus*, *Picea* and *Pseudotsuga* trees at a) Fraser, b) Aleza and c) Herrick sites over 27 months.

3.4 Discussion

3.4.1 Effect of overstorey tree species

Growth of both *Lobaria pulmonaria* and *Lobaria hallii* was faster beneath *Populus* than beneath *Picea* at all three site types (Fig. 3.1a and Fig. 3.2a). In addition, growth rates of large thalli for both lichen species beneath *Populus* were faster than most other published rates (Table 3.2). The sole exception was 68% growth of *Lobaria pulmonaria* over 15 months in Western Portugal (Gaio-Oliveira et al. 2004) where calcareous soils and high pH may have promoted cyanolichen growth (Gauslaa 1995). The mean annual growth of *L. hallii* under *Populus* was almost three times the mean of published annual bipartite cyanolichen growth rates (14%; Table 3.2).

Lobaria pulmonaria is among the more studied of all forest lichens and growth rates have been documented across a wide range of forest types under different moisture regimes (Table 3.2). The most comparable are from other wet interior forests, where mean growth rates range from 16 to 19% over 12 months in even-aged, and old-growth Thuja-Tsuga forests, respectively (Coxson and Stevenson 2007b). The mean first-year *L. pulmonaria* growth recorded here under *Picea* was faster than that of Coxson and Stevenson, but is similar to other studies under similar moisture regimes (Table 3.2).

Annual growth of *Lobaria hallii* beneath *Picea* varied with site-type but, like *L. pulmonaria*, remained within the range of reported growth rates for other bipartite cyanolichen species (Table 3.2). Specifically, growth rates under *Picea* were similar to those recorded for two closely related species, *Lobaria retigera* and *L. scrobiculata*, which had annual growth rates of 4.5-10.5% (Coxson and Stevenson 2007a) and 19% (Hilmo 2002) respectively.

The slowest growth rates for both cyanolichen species were recorded beneath overstorey *Pseudotsuga*. The response of *L. hallii* was particularly striking, with growth rates that were as low as the lowest published rate of annual growth for a bipartite cyanolichen species (Table 3.2). These observations, in combination with high juvenile thallus mortality, suggest that cyanolichens are inhibited by *Pseudotsuga* at the establishment and growth life stages (Sillett et al. 2000), but the causal factor remains unknown.

Table 3.2 Comparative studies on growth rates of cyanolichens in old forest environments. Precipitation is given as mm year^{-1} unless (except in cases where the number of measurement days is included in parentheses). Approximate annual growth rates are based on calculations from seasonal or multi-year growth rates. Annual growth rates in parentheses indicate those that are extrapolated from shorter-term studies and may therefore overestimate annual growth. See Coxson and Stevenson 2007a for other records of *L. pulmonaria* growth.

Citation	**Location**	**Precipitation (mm)**	**Growth (%)**	**Time period (mo.)**	**Annual growth**
Lobaria pulmonaria					
Antoine & McCune (2004)	*Pseudotsuga-tsuga* forest, northern Oregon	2500	4 -13	12	4-13
Asplund & Gauslaa (2008)	Broadleaf forest, south & south-east Norway	346 (104d.)	5-8.2	3.5	(23)
Coxson & Stevenson (2007a)	*Thuja-Tsuga* forest, British Columbia	840	16.1-19	24	9
Denison (1988)	western Oregon	1300	8	12	8
Gaio-Oliviero et al. (2004)	*Quercus faginea* forest, western Portugal	927	68	15	54
	Picea abies forest, northern Sweden	1204	1.9-9.5	10.5	6
Gauslaa (2006)	*Picea abies* forest, south-east Norway	487 (100d.)	11.6-18.5	3.5	(52)
Gauslaa et al. (2006)	Old *Picea abies* forest, south-east Norway	487 (100d.)	16	3.5	(55)
Gauslaa et al. (2007)	*Picea abies* forests, northern Sweden	276 (110d.)	16.5	3.5	(57)
	Picea abies forests, western Norway	440 (110d.)	34.6	3.5	(119)

Citation	Location	Precipitation (mm)	Growth (%)	Time period (mo.)	Annual growth
Gauslaa et al. (2009)	*Picea abies* forests, northern Sweden & western & southern Norway	276-440 (110d.)	21.2	3.5	(73)
McCune & Caldwell (2009)	*Fraxinus latifolia* forest, western Oregon	1080	14.8	12	14.8
McCune et al. (1996)	*Pseudotsuga-Tsuga* forest, western Oregon	1800	13-41	9-12	13-41
Muir et al. (1997)	*Fraxinus latifolia*, western Oregon	1000	28	12	28
Palmqvist & Sundberg (2000)	*Picea abies* forest, northern & southern Sweden	650	-1.2 & -0.4	12	-1.2 &-0.4
Renhorn et al. (1997)	*Picea abies* forest, northern Sweden	600	28	16	21
Shirazi et al. (1996)	*Fraxinus latifolia*, western Oregon	1080	24.5	4	(74)
Sillett et al. (2000)	*Pseudotsuga-Tsuga* forest, western Oregon	1000	15.2	12	15.2
Sundberg et al. (1997)	*Picea abies* forest, northern Sweden	600	2.9	16	2
This study	*Populus*, British Columbia	897-964	42.5	12	42.5
	Picea, British Columbia	897-964	32.3	12	32.3
	Pseudotsuga, British Columbia	897-964	21.9	12	21.9
Lobaria hallii					
This study	*Populus*, British Columbia	897-964	39.4	12	39.4
	Picea, British Columbia	897-964	17.3	12	17.3
	Pseudotsuga, British Columbia	897-964	4.3	12	4.3
L. retigera					
Stevenson & Coxson (2008)	*Thuja-Tsuga* forest, British Columbia	840	4.5-10.5	12	4.5-10.5
L. scrobiculata					
Hilmo (2002)	*Picea abies* forest, western Norway	788	19	12	19
Pseudocyphellaria berberina					
Caldiz (2004)	*Nothofagus* forest, Pategonia, Argentina	3000	12.6	12	12.6
P. crocata					
Gauslaa et al. (2007)	*Picea abies* forest, western Norway	440 (110d.)	35.7	3.5	(122)
P. rainierensis					
McCune et al. (1996)	*Pseudotsuga-Tsuga* forest, western Oregon	1800	4-8	12	4-8
Sillett & McCune (1998)	*Pseudotsuga-Tsuga* forest, western Oregon	1000	14	12	14
Sillett (1994)	*Pseudotsuga-Tsuga* forest, western Oregon	2000	5.7	12	5.7

Lobaria pulmonaria generally grew faster than *L. hallii* beneath *Picea*. Stevenson and Coxson (2008) similarly reported slower growth rates in the bipartite cyanolichen species *L. retigera* compared to *L. pulmonaria* in the same environment. The faster growth of the tripartite cyanolichen may be attributed to the supplemental photosynthetic activity of the green-algal biont.

That the growth rate of both *L. pulmonaria* and *L. hallii* beneath *Populus* was considerably higher than most published accounts may account for the disproportionately high cyanolichen abundance observed under *Populus* at each of the three site-types (Campbell et al. 2010). The decrease in size, and high mortality rates, of small cyanolichen thalli beneath conifers may also provide insight into cyanolichen abundance patterns in these sub-boreal forests. Denison (1988) attributed a decrease in the mass of lichen transplants to early stages of thallus necrosis and death and indeed, a large proportion of small thalli under *Picea* and *Pseudotsuga* died during the 27-month experiment. The mortality rates beneath conifers here substantially exceed mortality in other transplant experiments involving large thalli; Sillett et al. (2000) recorded 26% and 42% mortality of *Lobaria oregana* and *Psuedocyphellaria rainierensis*, respectively. This suggests that small thalli are disproportionately sensitive. Indeed, Scheidegger (1995) observed that 90% of diaspores died within the first 20 months and concluded that *L. pulmonaria* distribution was limited by establishment and early thallus growth. The small thalli in this experiment were initially >5 mm in diameter and therefore likely to be at least 12 months old at the time of transplant (Scheidegger 1995). Nevertheless, the high mortality rate of these thalli supports Scheidegger's conclusions, and may explain the near absence of small cyanolichen thalli beneath conifers at the Aleza sites (Campbell et al. 2010). More generally,

high mortality of lichen thalli during the establishment and juvenile growth phases may be a key factor limiting cyanolichen populations under conifers in sub-boreal forests.

Sillett et al. (2000) showed that cyanolichens will survive and grow in unsuitable climates but it is important to note that the *L. pulmonaria* transplant samples in that study were 'adult' thalli, having mean dry masses of 0.123g. As shown here, large thalli will survive and grow when transplanted, even to unfavourable environmental conditions. In contrast, as demonstrated here, and by Scheidegger (1995), thallus mortality is frequent in small thalli. The greater surface area decreases the water-holding capacity of small thalli which may shorten the duration of physiological activity following each wetting event in young thalli compared to older thalli (Gauslaa and Solhaug 1998). Such shorter hydration events may prevent photosynthetic carbon gains from overcoming respiratory carbon losses. Young thalli may therefore be more susceptible to sub-optimal moisture (Gauslaa and Solhaug 1998) and light (Coxson and Stevenson 2007b) conditions. In this study, this is most evident at the drier Aleza sites where large thalli beneath conifers survived the experiment while 80-100% of small thalli died. Moisture-related thallus mortality may be an important reason why *L. pulmonaria* and bipartite cyanolichens were 12 and 171 times less abundant, respectively, beneath *Picea* at Aleza than at Fraser sites (Campbell et al. 2010). Bipartite cyanolichens may be particularly susceptible to moisture-related mortality as net photosynthesis is only achieved in these species at high thallus hydration. In contrast, tripartite species such as *Lobaria pulmonaria* can depend on photosynthetic output from the green-algal biont which can rehydrate from water vapour alone and remain active at lower thallus moisture (Lange et al. 1986). The higher rates of thallus mortality in *L. hallii* compared to *L. pulmonaria*, and the more limited distribution of bipartite cyanolichens across the sub-boreal spruce landscape (Radies et al. 2009; Campbell et al. 2010)

suggests that these species are less able to survive the phases of establishment and juvenile growth.

3.4.2 Facilitation

The rapid cyanolichen growth rates beneath *Populus* suggest that *Populus* may have a facilitative influence on cyanolichen communities. Indeed, *Populus* may act as a nurse-species, promoting cyanolichen establishment and growth by either directly or indirectly controlling the availability of some unknown resource. Disparate cyanolichen growth rates have been attributed to the availability of exogenous nutrients. McCune and Caldwell (2009) demonstrated faster cyanolichen growth rates with a treatment of phosphorus and Gauslaa et al. (2006) saw increased growth rates with nitrogen additions. However, Campbell et al. (2010) examined the chemical composition of precipitation throughfall between *Populus* and *Picea* at these sites and concluded that mineral nutrient availability explained little of the variation in lichen community structure. Although the specific resource remains unknown, some insight may be gleaned from a qualitative comparison between treatments here. Small cyanolichen transplants beneath conifers died or decreased in size by month 12 at the moisture-deficient (Aleza) and light-deficient (Herrick) sites. Both moisture- and light-limitations have previously been demonstrated to limit photosynthetic activity in cyanolichens (Lange et al. 1986; Palmqvist and Sundberg 2000). That transplants beneath *Populus* at these sites continued to increase in size throughout the experiment, despite such limitations, suggests that some factor within the drip-zone of a *Populus* tree compensates for the more limited photosynthesis in these transplanted thalli.

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CHAPTER 4 The influence of exogenous glucose on epiphytic cyanolichens[3]

4.1 Introduction

Lichens are formed by a relationship between a fungus and either a cyanobacterium (cyanolichens), green alga (chlorolichens), or both (tripartite cyanolichens). The prevailing moisture conditions under which certain lichen species are found is determined, in part, by the photosynthetic partner. Chlorolichens require only 50-70% thallus hydration (Lange et al. 1986; Lange et al. 2001) while cyanolichens require up to 150% thallus hydration and contact with liquid water for physiological activity (Lange et al. 1986). Drier forest ecosystems in sub-boreal British Columbia are characterized by chlorolichen communities (Lehmkulh 2004) while cyanolichens are most abundant in wet forest ecosystems (Sillett and Neitlich 1996; Goward and Spribille 2005). A comparison of epiphytic lichens on conifer saplings beneath five mature tree species in sub-boreal spruce forests revealed that cyanolichens are uniformly abundant throughout some wet mixed-conifer forests but are restricted to saplings beneath the drip-zone of *Populus* trees in drier forest types (Campbell et al. 2010). Such patterns suggest that *Populus* trees provide some factor that compensates for inadequate moisture. Abundant cyanolichen communities have previously been attributed to available calcium (Goward and Arsenault 2000), manganese (Hauck 2003), molybdenum (Horstmann et al. 1982) and phosphorus (Benner and Vitousek 2007). However, analysis of throughfall precipitation beneath different tree species suggests that mineral nutrients are not responsible for the patterns observed in the sub-boreal forests (Campbell et al. 2010).

A plausible alternative is found by examination of the chemical secretions of *Populus* trees themselves. Leaves of many *Populus* species have large extrafloral nectaries (EFN) at the

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junction of the petiole and leaf blade (Trelease 1881; Curtis and Lersten 1974). Nectar is secreted from *Populus* EFNs regardless of tree size, including from leaves in the crowns of mature trees (Curtis and Lersten 1978). Of the 93 families that develop foliar EFNs (Pemberton 1998), only *Salicaceae,* containing the genera *Salix* and *Populus,* is present in sub-boreal forests of British Columbia (Elias 1983). Both *Salix* and *Populus* are observed to support abundant cyanolichen communities despite the rarity or absence of cyanolichens from surrounding conifer forests (Kuusinen 1994; Hedenås and Ericson 2000).

Although there are no published accounts of the carbohydrate concentrations of *Populus* extrafloral nectar, extrafloral nectar from a phylogenetically related species, *Prockia crucis,* contains up to 49.6% sugar, 16.2% of which is glucose (Thadeo et al. 2008). This concentrated nectar is secreted onto the leaf surface where it dries to an extremely viscous film during dry atmospheric conditions. EFNs and the associated glucose-rich secretions are theorized to be a reward for insects and thus provide a protective function to the plant (Thadeo et al. 2008). However, the accumulation of nectar on the leaf surfaces will also wash off during subsequent rain events and thus may drip onto epiphytic cyanolichens on lower branches.

Whether one ascribes to a mutualistic or a more parasitic paradigm (see Richardson 1999), lichens are a symbiotic relationship. Under appropriate climatic conditions, this relationship provides a somewhat reliable source of fixed C for fungal metabolism in return for a conduit for mineral nutrients to, and a protective shell of hyphae for, the photosynthetic partner (Honegger 1985). The widespread success of this arrangement implies that these are reasonable trade-offs for the fungus, particularly when no other photosynthate source is readily available. However, lichenized fungi may retain the opportunistic approach to C acquisition characteristic of their free-living counterparts. Indeed lichen-forming fungi have been shown to preferentially

metabolize exogenous glucose over glucose from the photobiont under laboratory conditions (Drew and Smith 1967). Exogenous glucose has also been shown to promote nitrogen-fixation by epiphyllic cyanobacteria (Bentley 1987). Cyanolichens on the *Populus* trunks and on conifer saplings beneath mature *Populus* canopies may intercept and metabolize the glucose-rich extrafloral nectar, thereby acquiring an exogenous source of reduced C despite long periods of drought-induced inactivity of photobiont photosynthesis.

To our knowledge, the only record of C concentration in throughfall precipitation beneath a *Populus* canopy showed saccharide concentrations of 9-24 mg L^{-1} beneath mature *Populus balsamifera* (Sanborn and Pawluk 1983). However, these values represent an average concentration across multiple rain events over two growing seasons and are not necessarily representative of the glucose concentrations on which cyanolichen communities might depend. Several authors have demonstrated that C concentration in throughfall precipitation beneath broadleaf trees fluctuates dramatically throughout the growing season (Carlisle et al. 1966; DeBoois and Jansen 1976). Furthermore, rainfall following a dry period of some duration will both wash the existing accumulation of nectar from the leaf surface, as well as stimulate further release from EFN (Trelease 1881). Both factors may result in glucose concentrations that are orders of magnitude higher than reported averages.

Relatively dry conditions will both reduce photosynthetic activity of cyanobacteria (Lange et al. 1986) and apparently increase the availability of glucose from EFN (Trelease 1881). Taken together, these factors may favour alternative nutritional strategies to meet the metabolic requirements of cyanolichens under less suitable environmental conditions. In this paper, we explore whether the proliferation of cyanolichens beneath *Populus* is a consequence of facilitation by the exogenous source of labile C provided by poplar EFNs. We experimentally

test three hypotheses which, if true, provide supporting evidence for the hypothesized maintenance of cyanolichen communities by exogenous-glucose. These include: a) net photosynthesis will decrease and nitrogen fixation will increase in cyanolichen thalli with the addition of exogenous glucose, b) exogenous glucose will be readily taken up and assimilated into lichen fatty-acids, and c) the rate of cyanolichen establishment will be significantly enhanced with the provision of exogenous glucose.

4.2 Methods and materials

4.2.1 Physiological response to $^{13}C_6$-glucose

Healthy and intact specimens of four cyanolichens species were collected from *Picea glauca* (Moench) Voss x *engelmannii* Parry ex Engelmann and *Abies lasiocarpa* (Hook.) Nutt. branches in a single old (mean tree age >240 years) mixed-conifer forest in September 2008. Lichens growing on branches within 5 m of a *Populus* tree were avoided. Samples included; two stratified bipartite cyanolichen species (*Nephroma helveticum* Ach. and *Lobaria hallii* (Tuck.) Zahlbr.), where the cyanobacteria are located in a discrete layer beneath the thallus surface; one stratified tripartite cyanolichen (*Lobaria pulmonaria* (L.) Hoffm.) where the thallus consists of a fungal and an algal photobiont (photosynthetic partner) and the cyanobacteria are in discrete pockets embedded at the surface of the fungal matrix; and one homoeomerous (gel) cyanolichen species (*Leptogium saturninum* Dickson) Nyl.) where the cyanobacteria are located throughout the lichen thallus. *Nostoc* spp. was the cyanobacterial partner in all lichens species (Brodo et al. 2001). Samples were maintained in an open container on a 12-hour light: 12-hour dark day: night cycle with day temperatures of 12^{o}C (6am to 6pm) at 200 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) and night temperatures of 8^{o}C. Relative humidity remained at approximately 60-70% throughout and thalli were periodically re-wetted (approximately every 3

days) with a de-ionized water spray to prevent desiccation. Lichen thalli were stored under these conditions for a 10-day acclimatization period. Thalli were moved to a sealed container 24 hours prior to the experiment and were pre-treated with either 10 ml of deionized water (control) or 10 ml of 2% glucose ($^{13}C_6$-glucose for C fixation). Thalli were pre-incubated at 19 ^{o}C and 200 µmol m^{-2} s^{-1} of light for the 3 hours immediately preceding experiments.

Net photosynthesis and respiration

Cyanolichen thalli were treated with either water or $^{13}C_6$-glucose to determine the photosynthetic response to exogenous glucose. Glucose was used because it is the principal form in which fixed C moves between symbionts (Drew and Smith 1967) and because it is absorbed preferentially over other saccharides (Harley and Smith 1956). A glucose concentration of 2% was used to ensure carbohydrate-saturation of the lichen thallus (Harley and Smith 1956) and to ensure a measurable physiological response over the measurement period.

A second 10-ml application of either water or 2% $^{13}C_6$-glucose was applied less than a minute prior to CO_2-flux measurements. Thalli were spot-dried and sealed inside a 2 x 3-cm Li-COR 6400 – 02B LED gas-exchange chamber in concert with the Li-6400 gas-exchange system (LiCOR Inc., Lincoln, NE.). Photosynthetically active radiation (PAR) was manipulated through sequential 5 - 10-min equilibration intervals at 400, 200, 100, 50, 25, and 10 µmol PAR m^{-2} s^{-1}. Net photosynthesis was measured at 20 ^{o}C and an external CO_2 concentration of 400 µmol mol^{-1}. Differences in net photosynthesis between control and glucose-treated thalli were evaluated using a one-way t-test at each of the six levels of photosynthetically active radiation (PAR). Net photosynthesis differences between lichen species were evaluated with a one way ANOVA (Statistica v 6.1, StatSoft Inc. Tulsa, OK).

Nitrogen-*fixation*

Nitrogen fixation of lichen thalli was measured using the acetylene reduction assay (ARA) method according to Stewart et al. (1967). Following pre-treatment as above, five additional transplants of each lichen species were treated with water or 2% glucose (n=6 per species per treatment) and placed in 250-ml canning jars modified by inserting rubber septa into the lid and sealing with silicone sealant (GE Sealants and Adhesives, Huntersville, NC). Air in the jar was replaced with acetylene gas (10% v/v). Gas samples (0.7 cc) were taken after a 3-hour incubation at 20^{o}C and 200 µmol PAR $m^{-2}s^{-1}$ and immediately analyzed for ethylene with a gas chromatograph (SRI 8610A Wennick Scientific Corporation, Ottawa, ON.) fitted with a Porapak Column (Alltech Canada, Guelph, ON.) and a flame ionization detector. Hydrogen was used as the carrier gas (pressure 179 kPa) and the column was maintained at 68^{o}C. Mass was recorded for each transplant after drying for 48 hr at 105^{o}C. Differences in ARA rates between lichen species and between control and glucose-treated samples were detected with a factorial ANOVA with a Fishers LSD post hoc test.

4.2.2 Fatty-acid extraction and analysis

Assimilation of ^{13}C into lichen tissue was assessed by extracting fatty acids from the same lichen samples for which the photosynthetic responses to exogenous glucose were recorded. Samples were removed from the Li-6400 chamber, weighed, flash-frozen in liquid nitrogen to prevent further respiration, and freeze dried for 24 hours. Freeze-dried lichen samples were ground to a fine powder with a grinding mill (Retsch MM200 stainless steel mixer mill, Sigma Aldrich, St. Louis, MO). Phospho- and neutral-lipids were extracted from the ^{13}C-glucose-treated lichens using techniques proposed by Bligh and Dyer (1959) and described by Olsson et al. (1995). Briefly, 100-200-mg samples were vortex mixed in a 0.8:1:2 (v/v/v) solution of citrate buffer, chloroform and methanol. Extracted acids were eluted with chloroform

and methanol through pre-packed Accubond II Solid Phase Extraction silica columns (Agilent Technologies Inc., Santa Clara, CA) to fractionate into neutral-lipid fatty acids (NLFA) and phospholipid fatty acids (PLFA), respectively. Following alkaline methanolysis, fatty-acid residues were flash-evaporated under N_2-gas and stored in 200 µl hexane at -20^{o}C until analysis.

The PLFAs and NLFAs were separated with an Agilent 6890 N gas chromatograph and an Agilent 5975 Inert XL Mass Selective Detector (Agilent Technologies Inc., Santa Clara, CA) with a 30-m J&W HP-5 column as described by Bengston et al. (2009). Specific fatty acids were identified using a combination of mass spectra and retention times relative to an internal standard (19:0) and authentic standards. The ^{13}C concentration in the individual fatty acids was determined on an Isoprime^{TM} stable-isotope ratio mass spectrometer (GV instruments) connected to an Agilent 6890A gas chromatograph (Agilent Technologies Inc.) under conditions described by Bengston et al. (2009). The µg ^{13}C g^{-1} of lichen sample and the %^{13}C enrichment were determined for each ^{13}C-labelled fatty acid. PLFA and NLFA nomenclature identifies the number of C atoms in the fatty acid chain (e.g. 18 in 18:1ω7), the number of double bonds in the chain (e.g. 1) and the position of the first double-bonded C from the methyl end of the fatty-acid molecule (e.g. ω7). Fatty-acid common names, where available, follow Robinson (1982).

4.2.3 Establishment response to glucose

To evaluate whether an exogenous source of labile C would enhance establishment of cyanolichen thalli, *Picea* branches were inoculated with propagules of four cyanolichen species; *Lobaria pulmonaria, L. hallii, Nephroma helveticum* and *Leptogium saturninum*. Following Benner and Vitousek (2007), propagules were obtained by grinding air-dried samples of mature thalli to a fine powder with a grinding mill (as above). *Picea* branches were scrubbed with a wire brush and rinsed with 0.05% HCl and deionized water to eliminate any existing lichen propagules. Five sets of two branches were installed in a 100 x 100-m area approximately 2 km

west of the University of Northern British Columbia (UTM: 10U 510330, E5971738) in May 2008. The site neither contained cyanolichens nor was it generally permissive to their occurrence. Mean annual precipitation for the experimental area is 600 mm based on monthly averages for 1961-2000 from Meteorological Services of Canada, Environment Canada. Each set included one control branch and one glucose-treated branch that were sufficiently spaced to prevent glucose-contamination of controls. Branches were hung between mature, live *Pinus contorta* trees in the mixed conifer forest. Deionized water was sprayed along a 100-cm segment of each branch to increase propagule retention and approximately 1.5 g of each lichen species was evenly sprinkled over the upper surface. Branches were sprayed once per week with either 30 ml of deionized water (control) or 30 ml of 2% glucose solution (in de-ionized water) from May 5 to Aug 30, 2008 and removed from the field on May 4, 2009. The total number of small (<1 mm long) cyanolichen thalli were counted and identified to species (when possible) by observing each branch through a dissecting microscope. Differences in the number of established thalli between treatment and control were evaluated with a two-sample t-test assuming unequal variances.

4.3 Results

4.3.1 Physiological response to $^{13}C_6$-glucose

Net photosynthesis and respiration

Lobaria pulmonaria, Leptogium saturninum and *Nephroma helveticum* thalli treated with ^{13}C-glucose had significantly lower rates of net photosynthesis than control thalli (Fig. 4.1; Table 4.1). Furthermore, there was a greater difference in net photosynthesis between control and glucose-treated thalli at 400 μmol PAR m^{-2} s^{-1} than at 10 μmol PAR m^{-2} s^{-1} (Fig. 4.1). Net photosynthesis in the $^{13}C_6$-glucose treated thalli was below the light compensation point at all

light levels in the bipartite stratified cyanolichens (*Nephroma helveticum* and *Lobaria hallii*) and at light levels below 200 µmol PAR m^{-2} s^{-1} in *Leptogium saturninum* and *Lobaria pulmonaria.* Net photosynthetic responses of control thalli were only below the light compensation point when light levels dropped below 50 µmol PAR m^{-2} s^{-1}. There were no statistical differences in photosynthetic light responses between cyanolichen species ($F(3, 40) = 0.84$, $p = 0.48$).

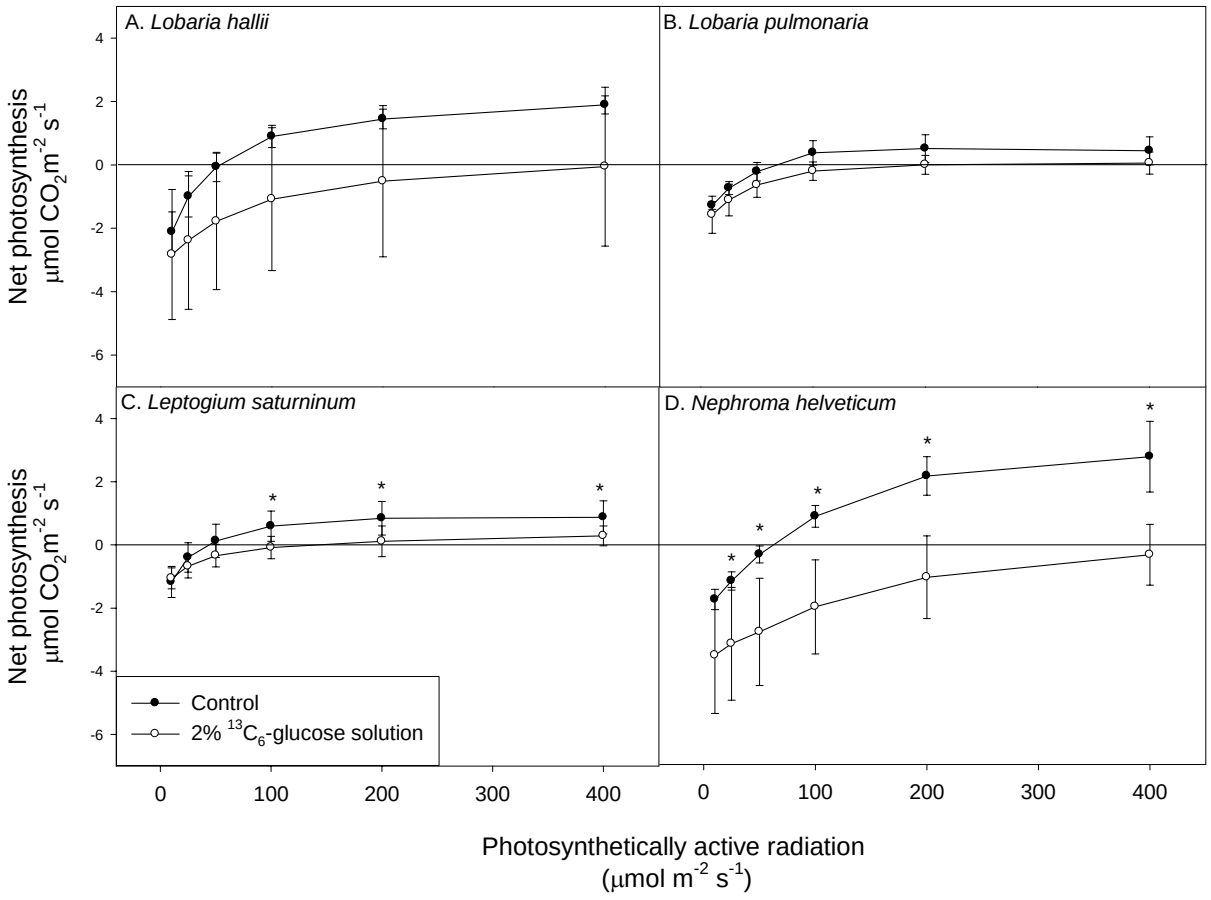

Figure 4.1 Net photosynthesis of water- and 2% $^{13}C_6$-glucose-treated a) *Lobaria hallii* b) *Lobaria pulmonaria* c) *Leptogium saturninum* and d) *Nephroma helveticum*. Error bars represent standard deviation and stars represent significant differences between treatments at individual light levels for individual species (N=6).

Table 4.1 One-tailed t-test statistics between water- and $^{13}C_6$-glucose-treated cyanolichens at six levels of photosynthetically active radiation (PAR).

Species	PAR	Mean		Variance		df	t	p
		Control	Glucose	Control	Glucose			
Lobaria hallii	400	1.87	-0.32	0.09	8.79	4	1.65	0.09
	200	1.47	-0.77	0.10	7.96	4	1.76	0.08
	100	0.90	-1.40	0.14	7.44	4	1.86	0.07
	50	-0.05	-2.15	0.22	6.98	4	1.75	0.08
	25	-0.96	-2.62	0.39	6.67	4	1.40	0.12
	10	-2.10	-3.11	0.45	6.17	4	0.88	0.21
Lobaria pulmonaria	400	0.42	0.06	0.26	0.14	5	1.42	0.09
	200	0.50	-0.01	0.24	0.10	5	2.10	0.03
	100	0.34	-0.21	0.18	0.10	5	2.55	0.02
	50	-0.23	-0.66	0.10	0.19	5	2.01	0.04
	25	-0.73	-1.15	0.03	0.31	5	1.73	0.07
	10	-1.28	-1.62	0.02	0.44	5	1.23	0.14
Leptogium saturninum	400	0.90	0.29	0.28	0.11	5	2.40	0.02
	200	0.88	0.11	0.32	0.11	5	2.85	0.01
	100	0.57	-0.08	0.25	0.14	5	2.58	0.01
	50	0.13	-0.35	0.31	0.13	5	1.76	0.06
	25	-0.39	-0.67	0.22	0.13	5	1.13	0.14
	10	-1.17	-1.06	0.25	0.12	5	-0.43	0.34
Nephroma helveticum	400	2.85	-0.34	1.30	1.11	5	4.61	<0.0001
	200	2.16	-1.06	0.46	1.89	5	4.69	0.002
	100	0.87	-2.00	0.14	2.65	5	3.84	0.01
	50	-0.33	-2.79	0.07	3.44	5	2.94	0.02
	25	-1.18	-3.14	0.09	3.59	5	2.29	0.04
	10	-1.77	-3.54	0.11	4.03	5	1.94	0.06

Nitrogen-fixation

Nitrogen-fixation differed between lichen species. The mean (±SD) ARA rate in *Nephroma helveticum* (2.10±0.90 μmol C_2H_4 g^{-1} hr^{-1}) was significantly higher than in *Lobaria hallii* (1.57±0.44 μmol C_2H_4 g^{-1} hr^{-1}) and *Leptogium saturninum* (1.14±0.66 μmol C_2H_4 g^{-1} hr^{-1}; $F(3, 32 = 3.61, p = 0.02)$. Acetylene reduction by the tripartite species *Lobaria pulmonaria* (1.67±0.44 μmol C_2H_4 g^{-1} hr^{-1}) did not differ from any other cyanolichen species and there was no difference in ARA between the control and glucose-treated thalli for any cyanolichen species ($F(1,32) = 1.85$, $p=0.7$; data not shown).

4.3.2 Fatty-acid analysis

The composition of phospholipids (PLFA) and neutral lipids (NLFA) varied among cyanolichen species, but the dominant fatty acids in all cyanolichen species were 16:0, 18:1ω9 and 18:2ω6,9 (Table 4.2). Each of the fatty acids detected showed ^{13}C-enrichment with the exception of 18:3ω3 which was only ^{13}C-enriched in *Nephroma helveticum* and *Leptogium saturninum* PLFAs (Fig. 4.2a). This fatty acid was also a minor constituent of the NLFAs in *Lobaria hallii, L. pulmonaria* and *Nephroma helveticum* (0.03%, 0.1% and 1.58%, respectively; Table 4.2) but was only ^{13}C-enriched in the latter species (Fig. 4.2c). Of the analyzed PLFAs, 20:0 and 20:3 were the most enriched with ^{13}C in all lichen species (Fig. 4.2a). The highly ^{13}C-enriched NLFAs included 26:0 in *Lobaria pulmonaria* and *Lobaria hallii*¸ 16:1ω9, 18:1ω9, 18:0 and 20:0 in *Leptogium saturninum* and 18:0 and 20:0 in *Nephroma helveticum* (Fig. 4.2c). The largest proportion of total ^{13}C-uptake was assimilated into the PLFAs and NLFAs 18:1ω9 and 18:2ω6,9 in all four cyanolichen species (Fig. 4.2b and d).

The amount of ^{13}C that was applied to fatty-acid synthesis by *Lobaria pulmonaria* and *Nephroma helveticum* was nearly double that of *L. hallii* and *Leptogium saturninum* (Table 4.3). The three bipartite cyanolichens invested more of the assimilated ^{13}C into structural

(phospholipids) as opposed to storage (neutral) lipids with 68%, 74% and 56% of total assimilated-^{13}C detected in PLFAs in *Lobaria hallii*, *Leptogium saturninum* and *Nephroma helveticum*, respectively. By contrast, the assimilation of ^{13}C in *Lobaria pulmonaria* was similar between the two lipid types. The ratios of ^{13}C assimilated into PLFA and NLFA and of total PLFA-C to NLFA-C reflect these disparate investments (Table 4.3).

Table 4.2 Relative abundance (%) of phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) in four $^{13}C_6$-glucose treated cyanolichen species. ND- not detected (N=6).

	Lobaria hallii		***Lobaria pulmonaria***		***Leptogium saturninum***		***Nephroma helveticum***	
	PLFA	**NLFA**	**PLFA**	**NLFA**	**PLFA**	**NLFA**	**PLFA**	**NLFA**
14:0	0.05	0.69	0.42	0.44	0.16	0.08	0.15	0.04
16:0	21.51	13.48	18.75	12.73	19.61	10.99	20.26	8.89
16:1ω5	0.18	0	0.12	0	0.75	0	0.30	0
16:1ω7	3.46	0	0.01	0	0	0	0	0
16:1ω9	4.81	0.07	0.82	0.66	8.77	0.47	7.00	1.00
17:0	0.72	0.15	0.60	0.16	0.60	0.08	0.48	0.05
18:0	3.88	5.34	3.41	3.29	2.17	6.90	4.99	3.48
18:1ω7	6.64	0	3.03	0	6.77	0	6.24	0
18:1ω9	20.73	36.05	18.61	33.02	18.61	22.19	11.36	9.21
18:2ω6,9	34.38	41.20	51.17	47.82	31.81	53.44	42.57	70.87
18:3ω3	0.01	0.10	0.02	0.03	0.03	0.18	1.78	1.58
20:0	0.08	0.10	0.18	0.12	0.21	0.13	0.29	0.11
20:2	0.26	0	0.19	0	0.79	0	0.24	0
20:3	0.50	0	0.24	0	0.52	0	0.15	0
21:0	0.52	0.47	0.43	0.09	2.02	2.57	0.30	0.08
22:0	1.19	1.14	0.95	0.37	2.40	1.31	1.55	0.83
23:0	0.58	0.17	0.54	0.10	2.42	0.12	0.98	0.09
24:0	0.50	0.43	0.47	0.14	2.37	0.77	1.36	0.78
26:0		0.59		1.05		0.77		2.99

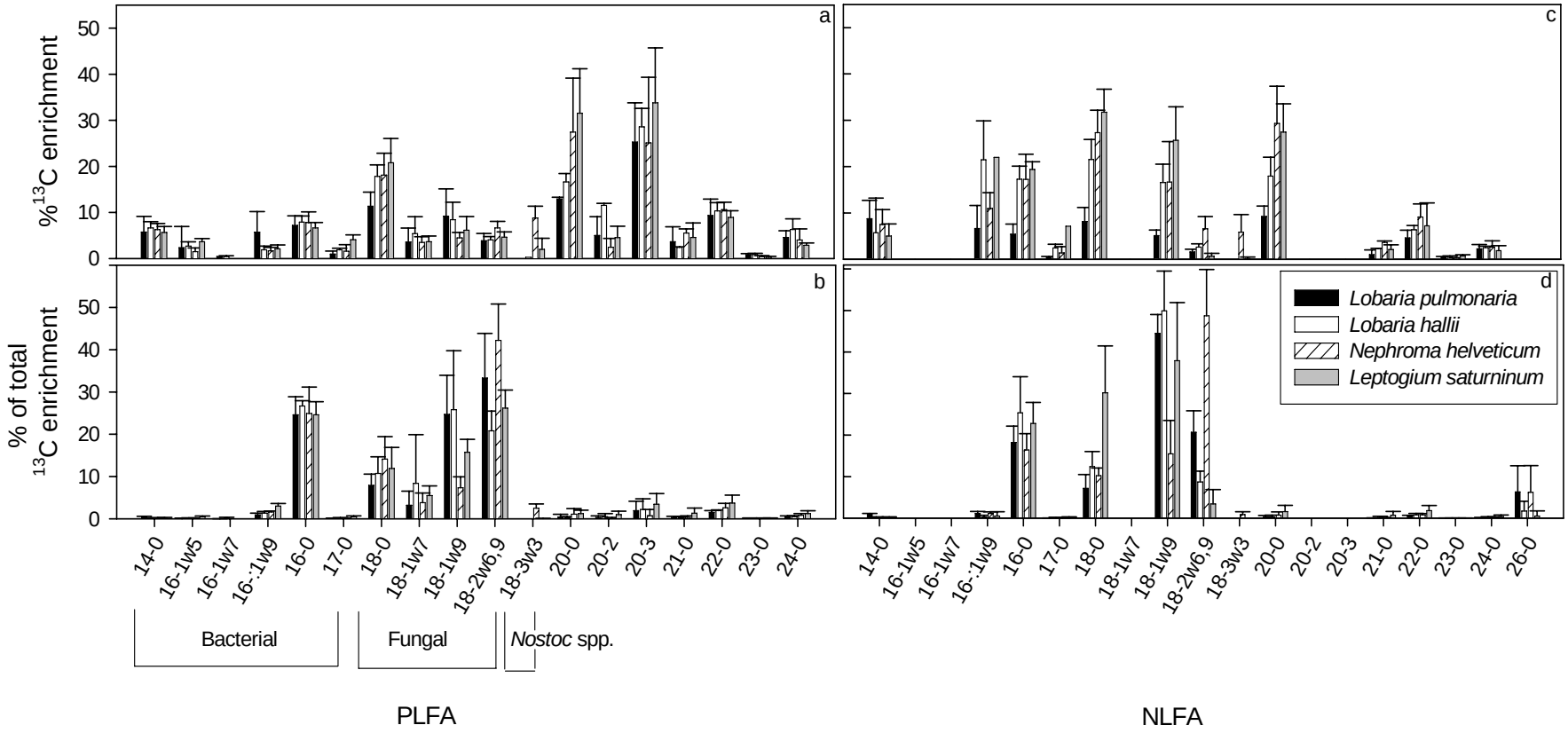

Figure 4.2 The % ^{13}C enrichment in extracted (a) phospho-lipid fatty acids (PLFA) and (c) neutral-lipid fatty acids (NLFA) and the % of total ^{13}C enrichment in each (b) PLFA and (d) NLFA from $^{13}C_6$-glucose treated samples of *Lobaria pulmonaria, Lobaria hallii, Nephroma helveticum* and *Leptogium saturninum.* Associations of specific fatty acids with specific lichen bionts are indicated. Error bars represent standard deviation. Natural abundance of ^{13}C (1.15%) was subtracted from values prior to analysis (N=6).

Table 4.3 Mean quantity (±SD) of ^{13}C assimilated into PLFAs, NLFAs and total lichen fatty acids, the ratio of ^{13}C assimilated into PLFA (μg ^{13}C g sample $^{-1}$) to NLFA (μg ^{13}C g sample $^{-1}$), and the ratio of total PLFA-C g sample^{-1} to total NLFA-C g sample^{-1} in four cyanolichen species (N=6).

	μg ^{13}C in PLFA g sample $^{-1}$	**μg ^{13}C in NLFA g sample $^{-1}$**	**μg ^{13}C total g sample $^{-1}$**	**^{13}C in PLFA: ^{13}C in NLFA**	**TOTAL PLFA:NLFA**
Lobaria hallii	102.5±34.2	48.6±22.4	151.1±56.0	2.2	6.0
Lobaria pulmonaria	108.6±59.4	103.2±51.9	211.9±94.8	1.1	1.4
Leptogium saturninum	86.3±.31.3	30.9±21.0	117.2±44.3	4.0	8.1
Nephroma helveticum	133.7±49.0	104.0±76.9	237.7±118.5	1.6	5.3

4.3.3 Glucose fertilization experiment

Significantly more cyanolichen thalli became established on the glucose-treated branches than on the control branches ($t(4)=-2.5$, $p=0.019$). A total of 242 cyanolichen thalli were observed on the glucose-treated branches, while only 35 cyanolichen thalli became established on the control branches (Fig. 4.3). *Nephroma helveticum* was the most abundant species, making up 66% and 69% of all cyanolichens on control and glucose-treated branches, respectively. *Leptogium saturninum* thalli were observed only on the glucose-treated branches.

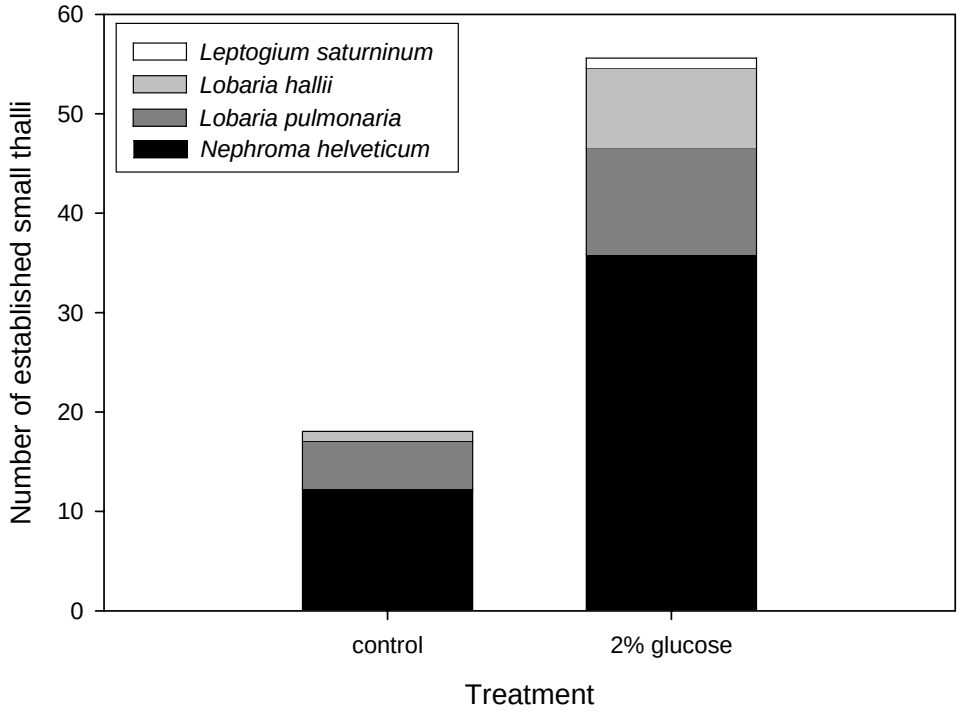

Figure 4.3 The number of small (<1mm long) thalli observed on control and 2%-glucose treated *Picea* branches. Establishment on glucose-treated branches was significantly higher than control ($p=0.019$).

4.4 Discussion

Success of cyanolichen establishment was substantially enhanced by the provision of exogenous glucose. Although sugars may improve the adherence of lichen diaspores to their substrate (Y. Gauslaa, Pers.comm. March 2010), the uptake and physiological response to exogenous glucose observed here suggest that the benefit of glucose is related to the ecophysiological limitations of cyanolichens. Lichens can completely desiccate during dry periods and reactivate once thallus moisture increases, but the thallus is subject to respiratory C-loss during rewetting (Brown et al. 1983). The degree to which photosynthetic C-gains compensate for rewetting respiration depends on the duration of hydration; long hydration events may allow for significant gain of C while shorter events truncate gains and may even result in a net loss of C. Although the proportion of fixed C that is lost during respiration varies with lichen species and physiology (Palmqvist 2000), cyanolichens may be more susceptible to C loss because significant fungal respiration can occur under humid conditions (Lange et al. 1986) but upregulation of the photosynthetic pathways in *Nostoc* spp. requires contact with liquid water (Lange et al. 2004). In contrast, chlorolichens may rehydrate with water vapour alone and require only 50-70% thallus moisture for photosynthesis (Lange et al. 1986). Photosynthesis in these green-algal species may therefore quickly compensate for respiratory C loss while cyanolichens require longer wetting periods to achieve positive C gain.

Establishing thalli may be particularly vulnerable to rewetting C-loss; the large surface area to volume ratio of small thalli limits the duration of thallus hydration following wetting events (Gauslaa and Solhaug 1998). The exogenous glucose provided in this experiment may provide a source of reduced C that begins to compensate for rewetting respiration in establishing cyanolichen thalli before up-regulation of photosynthesis occurs and before subsequent thallus

dehydration. Establishing cyanolichens on glucose-treated branches may thus overcome C loss more rapidly than control thalli, thereby achieving a positive C balance. Thalli on control branches, by contrast, would experience a consistent loss of C.

Cyanolichen thalli treated with 2% $^{13}C_6$-glucose had lower or more negative net photosynthesis than those treated with deionized water. While the decreased net photosynthesis in glucose-treated thalli may be interpreted as an increase in respiration at low levels (no photosynthesis occurred in either treatment at 10 umol PAR $m^{-2}s^{-1}$), a proportion of the response to exogenous glucose must also be attributed to a reduction in photosynthesis. A larger difference between control and glucose-treated responses was observed at high compared to low light intensities, indicating that glucose also decreased photosynthesis in cyanolichens. This is consistent with Nátr et al. (1974) who showed a considerable reduction in photosynthesis rate in barley leaves following glucose uptake, however the specific mechanism remains unknown.

The ability of the studied cyanolichen species to use exogenous glucose as a C source was confirmed by the ^{13}C experiment. Although the total ^{13}C incorporated into lipids varied between samples and species, almost all lichen fatty-acids were highly ^{13}C enriched. The three bipartite cyanolichens invested more ^{13}C into structural (PLFA) lipids compared to storage (NLFA) lipids despite the fact that neutral lipids make up the highest percentage of total lipids in lichens (Dembitsky 1992). The ratio of PLFA to the corresponding NLFA has been reported as an indicator of physiological condition of the fungus (Olsson et al. 1998) because it indicates an investment into structure rather than storage. The PLFA:NLFA ratio was relatively high for each of the bipartite cyanolichen species signifying a substantial investment into structural lipids. By contrast, *Lobaria pulmonaria* appeared to invest equally between storage and structural compounds. Taken together, the lower PLFA:NLFA ratio and the weaker respiratory response to

exogenous glucose in *Lobaria pulmonaria* compared to other stratified species, might reflect a lesser dependence of the former on exogenous C at sub-optimal moisture conditions. Indeed, this may explain why *Lobaria pulmonaria* is commonly observed throughout sub-boreal spruce forests while bipartite cyanolichen species are largely restricted to regions beneath a *Populus* drip-zone in moisture-deficient forests (Campbell et al. 2010).

The composition of fatty acids varied across the four cyanolichen species, but the primary fatty acids in each case were 16:0, 18:1ω9 and 18:2ω6,9. These results are consistent with Dembitsky (1992) who observed that 16:0 was the predominant fatty acid in many lichen species and reflects the presence of 16:0 in both fungal (Riley et al. 2000) and cyanobacterial (Gugger et al. 2002) fatty-acid extractions. That the fungal fatty acids 18:1ω9 and 18:2ω6,9 made up a large proportion of the fatty acid composition in this study is also consistent with previous work on cyanolichens (Rezanka and Dembitsky 1999) and likely reflects the greater fungal biomass in the lichen thallus (Honegger 1985).

The largest proportion of ^{13}C taken up by the cyanolichen thalli was assimilated into 18:1ω9 (oleic acid) and 18:2ω6,9 (linoleic acid). These lipids are commonly used to identify fungi in soil microbial communities (Frostegård and Bååth 1996) and have been shown to be relatively abundant in stratified lichens (Bowker et al. 2008) and in the homiomerous lichen *Leptogium saturninum* (Rezanka and Dembitsky 1999). The disproportionate ^{13}C enrichment of these fatty acids suggests that the mycobiont absorbed and assimilated the majority of exogenous $^{13}C_6$-glucose.

The neutral lipid fatty acids 20:0 (icosanoic acid) and 20:3 (homo-γ-linolenic acid) were also highly enriched in most lichen species. These results are consistent with the fatty-acid composition of *Peltigera* spp. which had abundant long-chain neutral lipids in comparison with

the chlorolichens investigated, many of which had no fatty acids longer than 18-carbons (Dembitsky 1992).

Some enrichment in the fatty-acid 18:3ω3 (linolenic acid) was observed. Though absent from fungi, 18:3ω3 is the major fatty acid in thylakoid membranes and present in green algae and many filamentous cyanobacteria, including *Nostoc* spp. (Potts et al. 1987). The absence of ^{13}C enrichment NLFA 18:3ω3 is expected as lipids are not a primary C-storage compound in cyanobacteria (Neidhardt et al. 1990). Enrichment of PLFA 18:3ω3 may indicate some degree of glucose uptake by the cyanobacteria. Alternatively, ^{13}C enrichment of 18:3ω3 may have resulted from photosynthetic fixation of the $^{13}CO_2$ that was respired by the mycobiont following uptake of $^{13}C_6$-glucose.

Exogenous glucose did not affect the rate of nitrogen-fixation in any of the four lichen species investigated. Kershaw et al. (1977) similarly showed that 2%-glucose failed to elevate N_2-fixation in a bipartite cyanolichen following a drop in nitrogenase activity in the dark. The authors concluded that exogenous glucose could not replace the C contribution from light energy. These results are inconsistent with those of Bentley (1987) who demonstrated that exogenous glucose supports cyanobacterial nitrogen-fixation despite a darkness-induced reduction in photosynthate production. These disparate results may be explained by the fact that the cyanobacteria in Bentley's study were epiphyllic and not surrounded by absorptive fungal-hyphae. As demonstrated by the fatty-acid extractions here, most of the exogenous-glucose is taken up by the fungus in lichen studies, leaving comparatively little to potentially affect nitrogen-fixation in the cyanobacterial partner.

4.4.1 Conclusion

Cyanolichens are strongly associated with moist environmental conditions in interior British Columbia (Goward and Spribille 2005) where drying events are infrequent and the

duration of thallus hydration may be adequate to maintain positive C balance. Cyanolichens are comparatively rare under less suitable climatic conditions where cyanobacterial photosynthesis may be insufficient for metabolism in both symbionts. Other nutritional strategies may be necessary to maintain the symbiosis in such cases. The observation of abundant and species-rich cyanolichen communities beneath *Populus* in otherwise unsuitable moisture conditions (Campbell et al. 2010) suggests that *Populus* compensates for a drought-induced reduction in photosynthetic activity. Furthermore, experimental evidence presented here supports the hypothesis that glucose-rich nectar produced by *Populus* EFNs facilitates cyanolichen establishment and survival beneath *Populus* canopies. Our results demonstrate that fungal respiration and fatty-acid metabolism are enhanced by exogenous glucose, and suggest that lichenized fungal partners may not always be 'faithful' to the symbiosis. Under suboptimal moisture conditions, nutritional philandering may be necessary to maintain the relationship.

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CHAPTER 5 Decomposition and nutrient release from four lichen litters[4]

5.1 Introduction

Litter decomposition provides an important source of nutrients for plant growth in forest ecosystems, e.g. 69-87% of the annual nutritional requirements of forest plants come from nutrients mineralized from decomposing litter (Waring and Schlesinger 1985). The rate at which litter is broken down and nutrients are released by microbial communities is regulated by climatic factors (i.e. Aerts 1997; Berg et al. 2000) and by the initial chemical composition of the litter (i.e. Swift et al. 1979; Taylor et al. 1989). In general, litter decomposition rates are positively correlated with increasing moisture and increasing temperature (Waksman and Gerretsen 1931; Zhang et al. 2008). In a meta-analysis of litter decomposition from 70 studies, Zhang et al. (2008) confirmed that mass loss at specific sites was best explained by a combination of climatic factors (latitude, mean annual temperature) and chemical factors. The initial chemical make-up of the litter is often well correlated with the rate of initial decomposition; litter types with high initial N concentrations tend to have higher rates of initial decomposition than those with lower initial N (Taylor et al. 1989).

The rate at which nutrients are released from decomposing litter also varies among nutrients and litter types. Generally, relatively mobile nutrients such as Mg, Ca and K are rapidly released during early decay, often at a rate faster than mass loss, while other elements (notably N and P) are retained or even immobilized in litter with high initial C:N or C:P ratios (Manzoni et al. 2008). If, however, the ratio of C to N or P is low, these elements may be released from litters during early decay (Prescott 2005). The release or retention of mineral nutrients during early

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decay results in a convergence of litter chemistry on common C:P and C:N ratios (Vesterdal 1999). Convergence values vary substantially in forest litter and tend to increase with the proportion of recalcitrant material in the litter. For example, broadleaf litters tend to converge at C:N and C:P ratios of 20-25 and 400, respectively, while needle litters converge at C:N and C:P of 28-35 and 500, respectively (Prescott 2005).

Epiphytic lichens generally decay rapidly (McCune and Daly 1994; Esseen and Renhorn 1998; Holub and Lajtha 2003; Caldiz et al. 2007). This may be related to the chemical make-up of the lichen thallus. Cyanolichens (species with cyanobacterial symbionts), in particular, are able to fix atmospheric nitrogen and consequently have a very high N content (up to 3.5%, Campbell and Fredeen 2007). More than 80% of this lichen-N is in highly labile forms such as amino acids and proteins (Dahlman et al. 2003). Given that initial N-concentration and C:N ratios are a strong predictor of initial decay rates, particularly in litters with low lignin content (Taylor et al. 1989), cyanolichen litters are expected to decompose and release nutrients faster than other lichen and vascular plant litters.

In many late-seral temperate forest ecosystems, N is thought generally to be a growth-limiting element. While reported rates of symbiotic-N_2-fixation in young forest ecosystems range from 0.8-2 kg N ha^{-1} yr^{-1} in *Lupinus arcticus* and *Sheperdia canadensis* (Hendrickson and Burgess 1989) to 10-15 kg N ha^{-1} yr^{-1} in *Alnus viridis* ssp. *sinuate* (Sanborn et al. 2002), these species are not abundant following crown closure (Sanborn et al. 2002). In late-seral forests, N is tightly cycled (Davidson et al. 1992) and largely supplied by decomposing forest litter (Sollins et al. 1980). There are therefore limited new N inputs into older sub-boreal forest ecosystems of central British Columbia where atmospheric N inputs are as low as 0.9 kg N ha^{-1} yr^{-1} (Hope 2001). The abundance of N_2-fixing cyanolichens increases with forest age (Sillett and Neitlich

1996; Campbell and Fredeen 2004) and may therefore be a substantial source of new N to these forests. For example, Denison (1979) estimated the annual N-inputs from epiphytic cyanolichens in mature conifer forests of the Pacific Northwest to be 3-4 kg N ha^{-1} yr^{-1}. The quantity of *in situ* cyanolichen-N has been calculated from biomass estimates in wet-temperate interior forests of B.C. (Campbell and Fredeen 2007), but this provides only limited insight into the role of these lichens in N-cycling. There are no estimates of lichen-litter contributions to forest nutrient cycling in sub-boreal spruce ecosystems.

In this study we compare mass loss rates and nutrient release dynamics of four lichen species (two cyanolichens and two chlorolichens) with different N concentrations in three sub-boreal spruce forests. We hypothesize that cyanolichens will decompose faster and lose N and P faster than chlorolichens, resulting in a convergence of N and P concentrations in the four litters as decomposition proceeds.

5.2 Methods and materials

5.2.1 Study area

The study was located north-east of Prince George British Columbia in old-growth forests (mean tree age >240 years) of the Sub-Boreal Spruce (SBS) biogeoclimatic zone (Meidinger and Pojar 1991). The sub-boreal forests are characterized by cool, moist summers and cold, snowy winters. Annual precipitation in the study area ranges from 897 mm in the western, wet-cool subzone (SBS wk), to 964 mm in the eastern, very wet-cool subzone (SBS vk, Murphy 1996).

Three site-types were established in the SBS to evaluate the relative rates of litterfall, decomposition and nutrient release across varying climatic conditions. “Herrick” sites were located at the north-eastern end of the study area in the SBS vk at an elevation of 850 m. Mean

summer temperatures and relative humidity levels were 10.8±5.3°C and 77.6%, respectively (Fig. 5.1). "Aleza" sites were located approximately 40km south-west in the Aleza Lake Research Forest (SBS wk) at 680 m elevation. Mean summer conditions were slightly warmer (12.4±5.5°C) and drier (relative humidity 69.1%) than at the Herrick sites (Fig. 5.1). The "Fraser" sites were located at 680 m elevation in the ecotonal region between the SBS wk and the SBS vk. Summer temperatures were 11.8±5.3°C and relative humidity was 77.6% (Fig. 5.1). Relative humidity was consistently lower at the Aleza Lake sites than at the Fraser or Herrick sites. Records of light availability also show that total (direct and indirect) light levels were 9% less beneath the forest canopy at the Herrick sites compared to the Fraser and Aleza sites (Campbell et al. 2010).

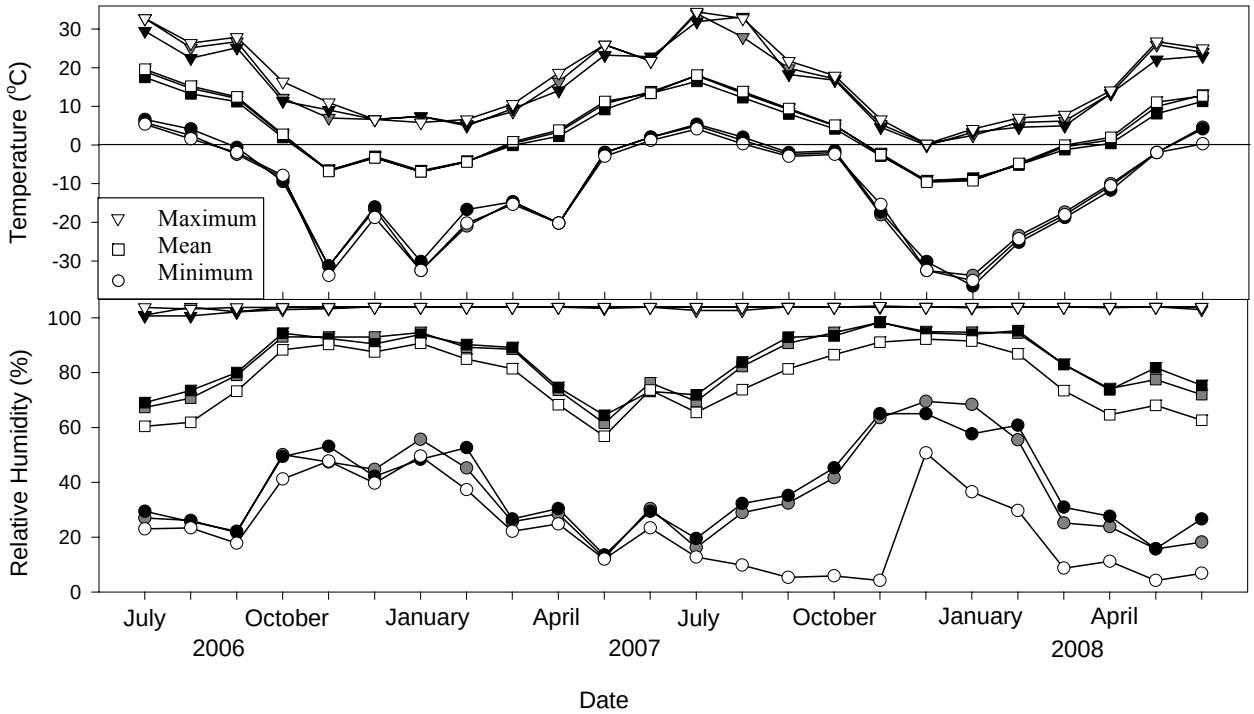

Figure 5.1 Monthly mean air temperature (top) and relative humidity (bottom) at the Fraser (dark symbols), Aleza (open symbols) and Herrick (shaded symbols) sites.

Picea glauca Parry x *engelmannii* (Moench) Voss. (interior hybrid spruce) and *Abies lasiocarpa* Hook.(Nutt.; subalpine fir) are the dominant conifer species at all sites. *Betula papyrifera* Marsh (paper birch) made up most of the deciduous canopy and *Populus balsamifera* L. ssp. *trichocarpa* Brayshaw (black cottonwood) was a minor component at the Fraser and Herrick sites while *Populus tremuloides* Michx. (trembling aspen) was infrequently present at the Aleza sites. Three stands (hereafter referred to as study sites) were chosen at each site-type, yielding a total of nine study sites. Fraser site soils varied from fine-loamy glaciolacustrine materials at two sites to an orthic humo-ferric podzol formed from sandy-skeletal glaciofluvial materials at the third. Herrick site soils were orthic humo-ferric podzols formed from sandy-colluvial materials and Aleza site soils were a fine-textured orthic luvic gleysols formed from glaciolacustrine parent materials. For more detailed site information and locations see Campbell et al. (2010).

5.2.2 Litterfall

To capture canopy (tree and lichen) litter, 1-m x 1-m litterfall traps were constructed from medium-duty landscape fabric stapled to PVC piping frames. Three parallel 40-m transects were established 10 m apart at each of the nine study sites. Five litterfall traps were randomly placed along each of the three transects for a total of 15 traps per site. Placements were cleared of vegetation, plant litter and coarse woody debris prior to trap placement. Traps were installed in June 2006 and all lichen and vascular plant litter falling into the trap was collected in October 2006 and June 2007. Litter was separated into seven categories: leaves, conifer needles, twigs and other small woody debris, ‘other materials’ (consisting mainly of cones and buds), bipartite cyanolichens (with cyanobacterial symbionts), tripartite cyanolichens (with both cyanobacterial

and green-algal symbionts) and chlorolichens (with green-algal symbionts). Terrestrial litter (shrubs and herbs) was discarded. Sorted samples were dried at 65ºC for 72 hours and weighed.

5.2.3 Litter decomposition and nutrient release

Samples of four lichen species were collected from *Abies lasiocarpa* branches from a single site in the SBS vk. A hair chlorolichen (*Alectoria sarmentosa* (Ach.) Ach.), a foliose chlorolichen (*Platismatia glauca* (L.) Culb.&C.F.Culb), a tripartite cyanolichen (*Lobaria pulmonaria* (L.) Hoffm.), and a bipartite cyanolichen (*Nephroma helveticum* Ach.) were chosen to represent four epiphytic macrolichen functional groups. A 1.4-1.6 g sample of each lichen was placed into a 10-cm x 10-cm woven fiberglass-mesh bag with 160±40 μm opening size. Lichens were dried at 65ºC for 72 hours, weighed, and placed in litterbags which were sewn closed with polyester thread. Litterbags were tied to a length of polyester thread (spaced approximately 30 cm apart) to increase the success of litterbag retrieval. The thread was staked to the forest floor adjacent to 14 of the 15 litterfall traps at each site. Each position initially contained one bag each of *A. sarmentosa*, *L. pulmonaria* and *P. glauca* and a litterbag containing *N. helveticum* was also sewn onto the polyester thread at 6 of the 14 positions. Fewer bags of *N. helveticum* were prepared because of the lower abundance of the species and the conservation concern over removing substantial quantities from the forest canopy.

Litterbags were retrieved from the field in October 2006, June 2007, October 2007 and June 2008. Bags were re-dried and weighed to determine mass loss during each interval. One litterbag of each lichen species from each of the nine sites was destructively sampled at each time interval for nutrient analysis. These litter samples were oven-dried at 65^{o}C for 72 hours and ground to a fine powder with a grinding mill (Retsch MM200 agate mixer mill, Sigma Aldrich, St. Louis, MO). Nitrogen and C content were determined by a flash combustion of duplicate 6-8 mg subsamples (NA 1500 NC elemental analyzer, Fisons Instruments, Italy). Samples were

prepared by microwave digestion and analysed for Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn content using inductively coupled plasma-mass spectrometry (ICP-MS, 7500 Series, Agilent Technologies, Santa Clara, CA) as described by Dolan and Capar (2002). Samples were analyzed at the University of Northern British Columbia Central Equipment Laboratory.

5.2.4 Soil nitrogen

The availability of soil nitrogen was measured as the quantity of NO_3^- and NH_4^+ adsorbed to PRS^{TM} ion exchange probes (Western Ag Innovations Inc., Saskatoon, Canada) over a 10-week period from June 16 - August 25, 2006. Probes were inserted into the mineral soil inside root-exclusion tubes (constructed from 10-cm diameter and 20-cm long PVC piping) to a depth of approximately 10 cm. Three sets of probes were buried near each of three randomly selected trees of each dominant species (*Picea glauca* x *engelmannii*, *Abies lasiocarpa*, and *Populus tremuloides* or *trichocarpa*). Three root-exclusion tubes and probe sets were also buried outside of the influence of all canopy trees. Probes were extracted, washed with deionized water, and shipped to Western Ag. Innovations for elemental analysis. Compounds are represented as the µg of N adsorbed to the resin probes over the surface area of resin ($10cm^2$) over the burial period (10 weeks).

5.2.5 Data analysis

Differences in decay rates between litter species and across site-types were analysed using two, one-way repeated measures ANOVAs with four repeated-measure decay periods and a Bonferroni post-hoc test in each analysis. One-way ANOVAs with Bonferroni post-hoc tests were used to evaluate differences in total litter, vascular litter and lichen litter biomass (analysed as pooled totals by litter category at each site; n = 3 per site-type) and soil nitrogen (log transformed for normality; n = 12 per site-type) across site-types. The relationship between mean cyanolichen litter biomass and soil N was evaluated for each site using a Product-Moment

correlation. Cyanolichen litter biomass, soil NO_3^- and NH_4^+ were averaged for each site and log transformed for normality prior to correlation analysis. All results are presented as means ± standard deviations.

Decomposition rate constants (k) were calculated according to Olson (1963) from the difference between initial and measured litter mass at each stage. The quantity of N-input was calculated by multiplying litter-N content by annual litterfall biomass. Nitrogen release over two years was calculated by subtracting the final mass of N in cyanolichen litter [mg-N ha^{-1} yr^{-1}] (calculated from cyanolichen litter-mass [kg-cyanolichen litter ha^{-1} yr^{-1}] x % mass remaining x final N concentration [mg-N kg-cyanolichen $litter^{-1}$]) from the initial mass of N in the cyanolichen litter (initial N concentration x initial lichen litterfall mass). N-input and potential N-release was corrected by increasing the mass of cyanolichen litterfall by 20% to account for the expected litterfall mass-loss between collection periods (based on median mass-loss over the initial four months).

5.3 Results

5.3.1 Decomposition rates

More lichen litter mass remained after four months of decay at the Aleza sites than at the other two sites. Mass loss at the three sites was similar after 1 year, but after 2 years less mass remained at the Fraser sites than at the other sites (Fig. 5.2a). Decomposition at all three sites followed a two-stage pattern with an initial rapid mass loss that slowed such that only an additional 11-18% (of the original mass) was lost in year 2 (Fig. 5.2a).

During the first four months, decomposition of the two cyanolichens, *N. helveticum* and *L. pulmonaria* was faster than that of the two chlorolichens, *A. sarmentosa* and *P. glauca*. This is reflected in the higher decay constants for the cyanolichen species (Table 5.1). Decay of *A.*

sarmentosa was more rapid over the first winter (months 4-12) than for the other three species. Consequently, after 24 months only *P. glauca* differed significantly with more mass remaining ($F(3,326)=57.52$, $p<0.0001$; Fig. 5.2b), and a consistently lower cumulative decay constant (Table 5.1) than the other three litter types. After two years in the field, mass remaining ranged from 42.0% (*P. glauca*) to 26.6% (*N. helveticum*; Fig. 5.2b).

Initial rates of decay (during the first 4 months; Fig. 5.2b) and decay constants (Table 5.1) were highly correlated with initial %N (R2=0.75; see Table 5.2). Low correlations thereafter are attributed to the relatively fast decay of *A. sarmentosa* during the first winter (Fig. 5.2b). Mass loss of lichens was faster than those measured in past studies of lodgepole-pine needle litters at Aleza Lake (Prescott et al. 2004). Decomposition rates of *A. sarmentosa* and the two cyanolichens were also more rapid than those observed for aspen leaf litter, but mass loss of *P. glauca* was similar to that of aspen leaf litter (Fig. 5.2b).

Table 5.1 The initial C:N and decay rate constants (k) for four lichen species (AS = *Alectoria sarmentosa*, PG = *Platismatia glauca*, LP = *Lobaria pulmonaria* and NH = *Nephroma helveticum*). Decay constants are calculated according to Olson (1963) for four lichen litters during four time intervals and cumulatively over the two year incubation.

		k for time intervals				**Cumulative k**		
	Initial C:N	**0-4mo**	**4-12mo**	**12-16mo**	**16-24mo**	**12mo**	**16mo**	**24mo**
AS	129.4	0.59	0.48	0.17	0.17	0.84	0.79	0.63
PG	88.0	0.79	0.22	0.13	0.12	0.56	0.55	0.44
LP	17.3	1.49	0.25	0.17	0.15	0.83	0.77	0.61
NH	12.4	1.77	0.27	0.19	0.15	0.96	0.93	0.69

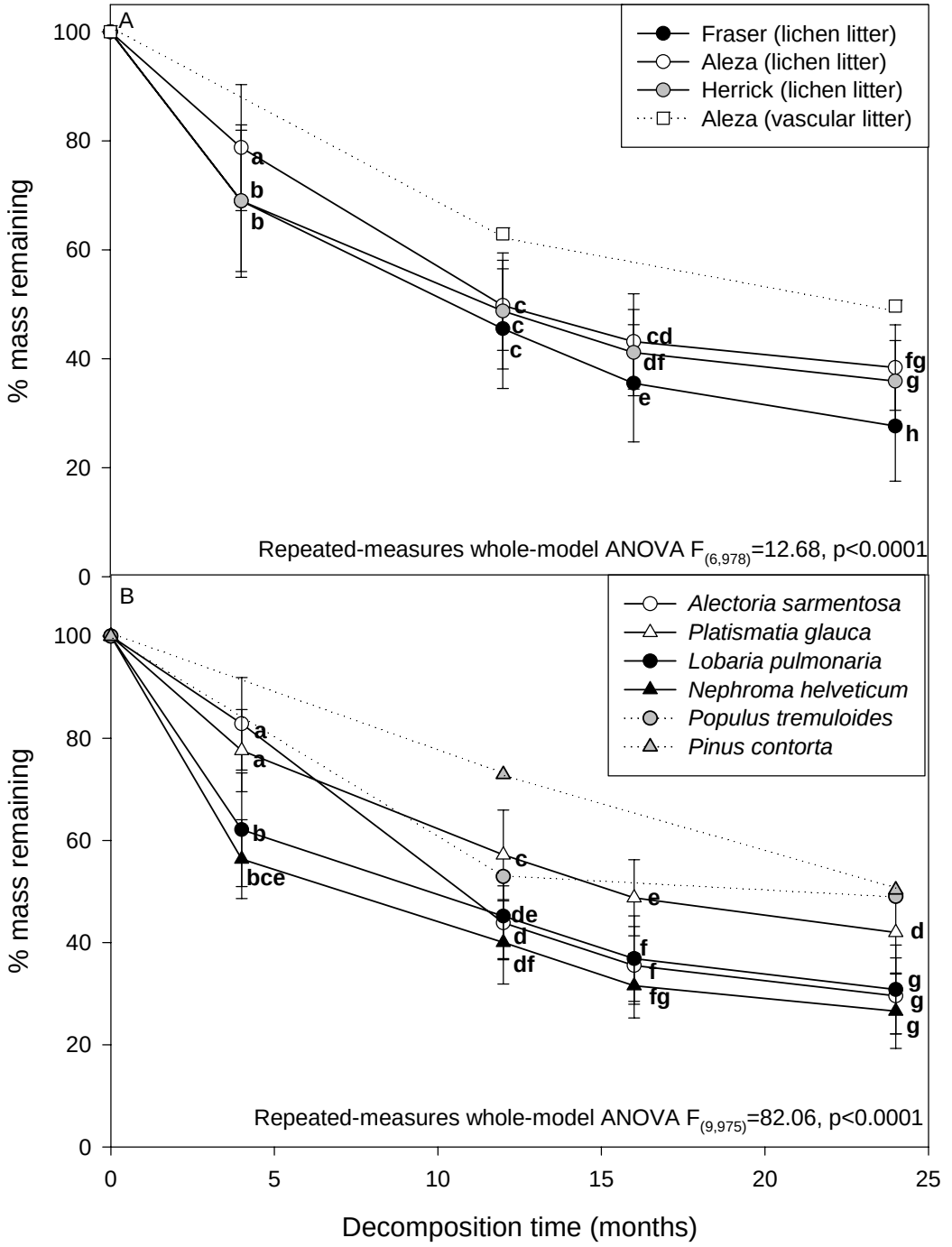

Figure 5.2 Mean (±SD) decomposition rates over two years at (a) three site types for (b) four species of lichen litter. Dotted lines represent decomposition of vascular plant litter incubated from 1993-1997 (from Prescott et al. 2004). Dissimilar letters represent significant differences between site types (a) or species (b).

Table 5.2 Chemistry of litter from four lichen species (AS = *Alectoria sarmentosa*, PG = *Platismatia glauca*, LP = *Lobaria pulmonaria* and NH = *Nephroma helveticum*) at five sequential decay stages.

Lichen species	Decay period (mo)	Element Concentration (mg g^{-1})					(mg kg^{-1})						
		N	**P**	**Ca**	**Mg**	**K**	**Al**	**Fe**	**B**	**Cu**	**Mn**	**Na**	**Zn**
AS	0	3.3	0.8	3.4	0.4	2.0	0.04	0.03	7.7	3.2	204.4	68.1	28.1
	4	-	0.4	5.2	0.6	0.9	0.09	0.7	8.4	2.4	344.1	54.1	28.7
	12	5.9	0.7	10.1	1.7	1.1	1.0	0.3	9.5	9.6	583.0	60.8	72.8
	16	9.5	0.8	9.9	1.9	1.3	1.0	0.4	4.6	10.5	654.1	244.2	85.8
	24	7.9	0.9	8.0	1.5	0.8	1.5	0.7	18.7	22.0	545.8	54.0	89.3
PG	0	4.9	1.0	2.4	0.5	1.9	0.2	0.2	10.0	5.3	271.7	94.5	27.3
	4	-	0.6	3.4	0.7	1.1	0.4	0.5	11.5	4.7	405.8	79.4	41.0
	12	6.0	0.6	6.5	1.3	1.1	1.4	0.9	8.9	10.4	517.1	120.3	64.9
	16	7.7	0.7	6.5	1.4	1.4	1.4	1.1	4.6	10.0	643.5	266.7	67.8
	24	7.1	0.8	7.3	1.6	0.8	1.2	1.2	20.4	19.0	613.6	73.4	121.6
LP	0	25.9	1.5	1.0	0.4	4.5	0.1	0.1	10.1	15.9	93.1	98.7	21.4
	4	-	0.9	2.0	0.6	1.5	0.2	0.2	13.8	20.4	223.3	90.5	26.6
	12	21.3	0.9	3.3	0.8	1.2	0.7	0.5	9.4	25.8	441.1	100.8	44.9
	16	23.7	0.8	3.3	0.9	1.0	0.9	0.6	5.1	24.4	411.5	176.5	45.9
	24	22.1	0.9	3.0	0.8	0.7	0.9	1.1	17.5	42.5	450.4	69.3	69.7
NH	0	35.2	2.1	2.0	1.1	7.0	0.6	1.2	29.9	5.2	296.2	133.8	49.8
	4	-	1.2	3.3	1.2	2.1	1.3	2.3	16.1	5.4	310.7	132.1	56.5
	12	25.2	0.9	4.4	1.5	1.6	3.1	3.1	15.4	9.2	425.3	240.1	79.7
	16	29.3	1.2	6.0	1.9	2.1	3.7	4.3	11.7	12.1	633.9	326.8	113.1
	24	27.3	1.2	4.8	1.7	1.4	3.5	6.1	28.0	27.5	560.8	135.3	111.3

5.3.2 Nutrient release rates

Initial N concentrations of lichen litter varied by an order of magnitude from 3.3 mg g^{-1} in the chlorolichen *A. sarmentosa* to 35.2 mg g^{-1} in the cyanolichen *N. helveticum* (Table 5.2). Lichens with high initial N rapidly lost N during the first year while species with a lower initial N retained N (Fig. 5.3a). After the first year of decay, N concentrations slightly increased indicating that C was lost more rapidly than N during later decay in all lichen litter types (Fig. 5.3b). Nitrogen concentrations after 2 years of decomposition ranged from 7.1 mg g^{-1} in *P. glauca* to 27.3 mg g^{-1} in *N. helveticum*. Cyanolichen litters had initial C:N ratios of 17 and 12 (for *L. pulmonaria* and *N. helveticum*, respectively), which did not change significantly as decomposition proceeded. In contrast, the C:N ratio of chlorolichen litter decreased significantly as a function of decreasing C content (R2=0.4, F(1,28)=20.47, p=0.0001). Neither the N content nor the C:N ratios of chlorolichens and cyanolichens had converged by the end of the second year (Fig. 5.4).

Phosphorus and potassium were also lost from all lichen litters during the first year and the rate of loss was positively related to the initial content. Contents (and concentrations) by the end of the first year had converged at approximately 500mg P (1 mg g^{-1}) and 1000 mg K (1 mg g^{-1}; Fig. 5.3c-f).

The concentrations of other elements including Al, Ca, Cu, Mg, Mn, Zn increased during decay (Table 5.2). However, there was no evidence of net element gain as an associated increase in the elemental mass was not detected. Initial Fe concentration was higher in *N. helveticum* than in other lichen litters, an observation likely related to the role of Fe in N_2-fixation. While the low Fe concentration remained largely stable in most lichen litters with no change relative to C, there was a consistent increase in concentration with decay time observed in *N. helveticum*.

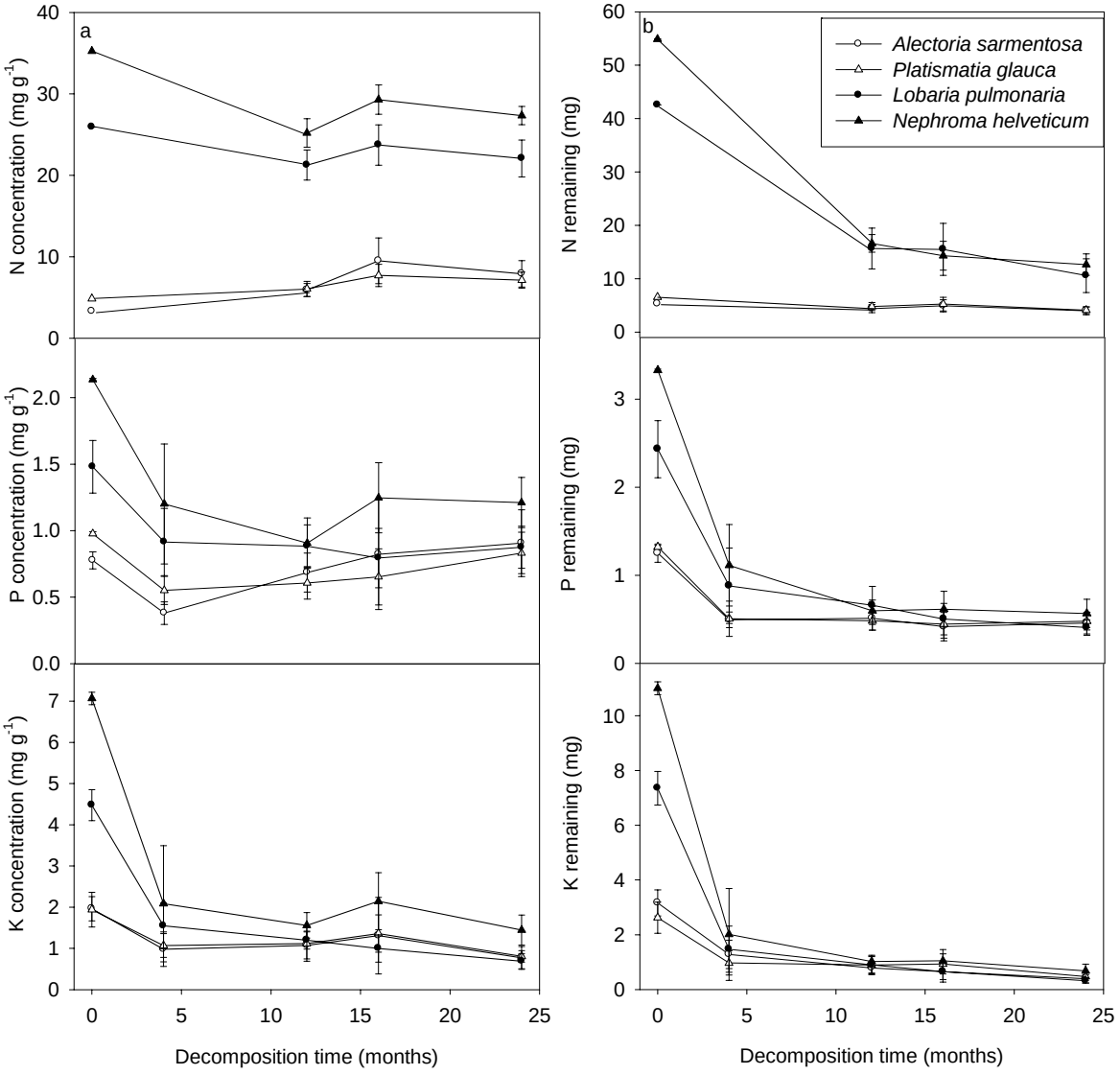

Figure 5.3 Changes in elemental concentrations (mg g^{-1} ±SD; panel a) and elemental mass remaining in the litterbags (mg ±SD; panel b) of N, P and K over two years of lichen litter decomposition.

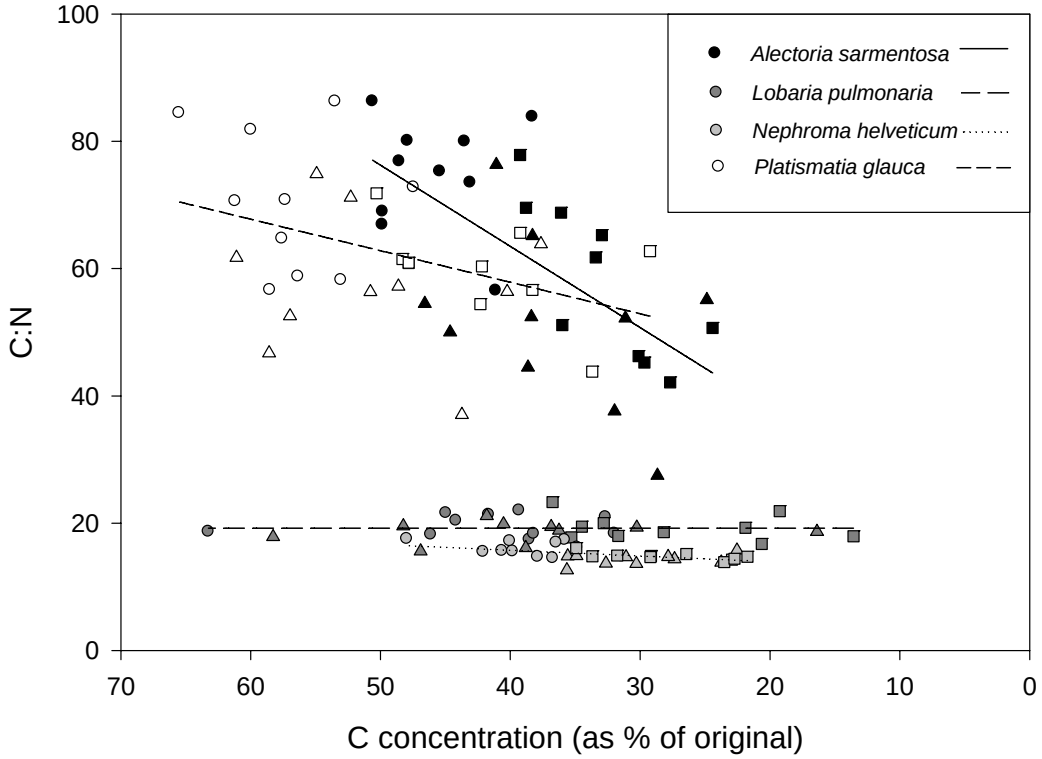

Figure 5.4 The ratio of C:N as a function decreasing C concentration (%) in litter of four epiphytic lichen species. Twelve-, 15-, and 24-month decay periods are denoted by circles, triangles and square symbols respectively.

5.3.3 Litterfall rates

The total mass of aboveground litterfall collected did not differ between site-types. Conifer needle litter made up the majority (58-61%) of litter at all three site types (Table 5.3). However, there was significantly more deciduous leaf litter at the Fraser and Herrick sites than at the Aleza sites ($F(2, 132)=9.86$, $p=0.0001$). Lichen litter made up 2.8% and 2.2% of total aboveground litterfall at Fraser and Aleza sites, respectively. Significantly less lichen litter was collected at Herrick sites where it accounted for only 0.4% of total aboveground litter ($F(2, 132)=19.12$, $p=0.0001$).

Table 5.3 Lichen, leaf, needle and twig litterfall at each of three sites. Litterfall values are mean kg ha^{-1} yr^{-1}± SD. Different superscript letters represent significant differences in litterfall mass between site-types (ANOVA, $p<0.05$).

	Fraser sites		**Aleza sites**		**Herrick sites**	
Litter type	**Litterfall biomass (kg ha^{-1} yr^{-1})**	**% of total litter**	**Litterfall biomass (kg ha^{-1} yr^{-1})**	**% of total litter**	**Litterfall biomass (kg ha^{-1} yr^{-1})**	**% of total litter**
Chlorolichens	$21±6^{a}$	0.6	$59±12^{b}$	1.7	$12±3^{a}$	0.3
Bipartite cyanolichens	$6±3^{a}$	0.2	$0.5±0.1^{b}$	0	$1±1^{b}$	0
L. pulmonaria	$77±41^{a}$	2.1	$19±12^{b}$	0.5	$2±3^{b}$	0.1
Total lichen	$104±38^{a}$	2.8	$78±13^{a}$	2.2	$15±2^{b}$	0.4
Leaves	$548±156^{a}$	15.0	$161±84^{b}$	4.6	$653±475^{a}$	16.3
Conifer needles	2165±142	59.2	2164±235	61.4	2301±231	57.6
Twig	739±211	20.2	932±206	26.4	663±277	16.6
Other	103±50	2.8	192±63	5.4	361±352	9.0
Total	3659±275	100.0	3526±441	100.0	3993±856	100.0

The composition of lichen litter also differed significantly between site-types. Chlorolichens comprised most of the lichen litter at the Aleza sites (75%), whereas cyanolichen litter (particularly *L. pulmonaria*) made up 74% of the lichen litter at the Fraser sites. Although this cyanolichen biomass made up only 2.3% of the total annual aboveground litter mass at the Fraser sites, it represented 11.5% of the total estimated N-input from aboveground litterfall (Table 5.4). The estimated N-input from N_2-fixing lichens (comprised of various bipartite cyanolichen species plus the tripartite lichen, *L. pulmonaria*) was 2.6, 0.6, and 0.1 kg N ha^{-1} at Fraser, Aleza and Herrick sites, respectively (Table 5.4). Cyanolichen litters after two years of mass- and nutrient-loss would be expected to contain 0.5, 0.2 and 0.02 kg N ha^{-1} at Fraser, Aleza and Herrick sites, respectively. The difference between initial and final N-contents suggests that cyanolichen litter releases approximately 2.1, 0.4 and 0.08 kg N ha^{-1} over two years at Fraser, Aleza and Herrick sites, respectively

5.3.4 Soil nitrogen

Nitrate (NO_3^-) was significantly more available in mineral soils at the Fraser sites than at the Aleza and Herrick sites ($F(2, 40) = 5.24$, $p = 0.01$; Fig. 5.5). Ammonium (NH_4^+) was most available at the Fraser and Herrick sites ($F(2, 35) = 11.312$, $p = 0.0001$). Soil NO_3^- was positively correlated ($r=0.7$, $p=0.03$) with total cyanolichen litter biomass.

Table 5.4 Estimated annual nitrogen input and release from lichen, leaf, needle and twig litterfall at each of three sites. Nitrogen release (kg N ha^{-1} yr^{-1}) is calculated from the annual litterfall mass multiplied by the initial N content and the change in N content during the first year of decay. The mass of chlorolichen and cyanolichen (including *Lobaria pulmonaria*) litterfall was increased by 10% and 20% respectively to account for the expected litterfall mass loss between collection periods (based on median mass loss for each species over the initial 4 months).

		Fraser sites			**Aleza sites**			**Herrick sites**		
Litter type	**N conc. (mg g^{-1})**	**N input (kg N ha^{-1} yr^{-1})**	**% of total N input from litter**	**N release (kg N ha^{-1} yr^{-1})**	**N input (kg N ha^{-1} yr^{-1})**	**% of total N input from litter**	**N release (kg N ha^{-1} yr^{-1})**	**N input (kg N ha^{-1} yr^{-1})**	**% of total N input from litter**	**N release (kg N ha^{-1} yr^{-1})**
Chlorolichens	4.9	0.11	0.48	0.05	0.32	1.64	0.13	0.06	0.28	0.03
Bipartite cyanolichens	35.2	0.25	1.08	0.15	0.02	0.10	0.01	0.04	0.19	0.03
L. pulmonaria	25.9	2.39	10.37	1.43	0.59	3.024	0.3	0.06	0.28	0.03
Total lichen	--	2.75	11.93	1.63	0.93	4.76	0.44	0.16	0.74	0.09
Leaves	7.81	4.3	18.66	--	1.3	6.66	--	5.1	23.77	--
Conifer needles	5.12	11.1	48.16	--	11.1	56.84	--	11.8	54.99	--
Twig	6.63	4.9	21.26	--	6.2	31.75	--	4.4	20.50	--
Total	--	23.05	100	--	19.53	100	--	21.46	100	--

1 From Prescott et al. 2004 Can. J. For. Res. 34:1714-1729 – mean of values for Trembling Aspen and Black Cottonwood

2 From Prescott et al. 2004 Can. J. For. Res. 34:1714-1729 – mean of values for Subalpine fir and Engelmann spruce

3 From Laiho and Prescott 1999. Can.J.For.Res. 29:1592-1603 – N concentration in small woody debris from the 'fir' site with an overstorey composed of Subalpine fir and Engelmann spruce.

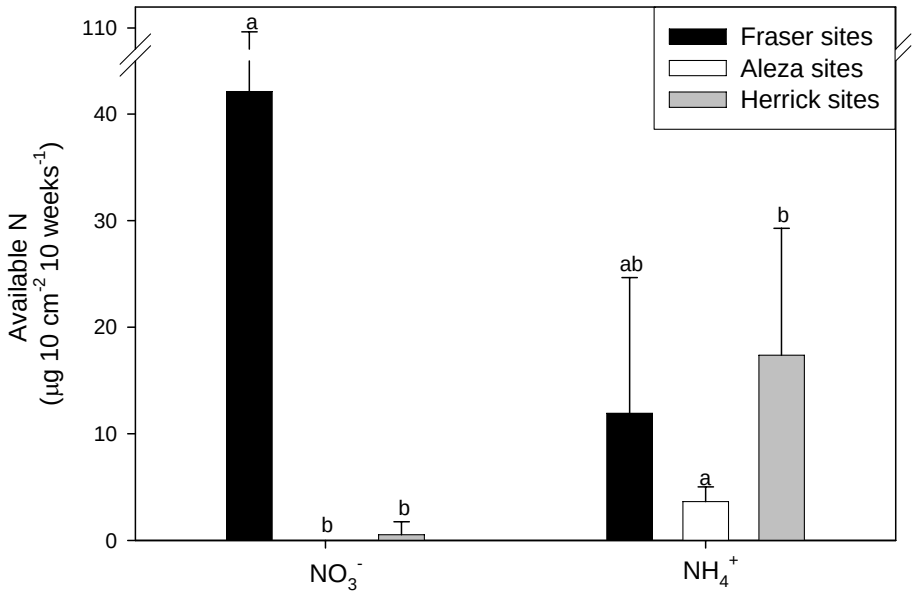

Figure 5.5 Nitrogen availability (±SD) in mineral soil measured as NO_3^- and NH_4^+ supply rates onto PRS™ ion exchange probes incubated in mineral soil within root-exclusion tubes for 10 weeks.

5.4 Discussion

5.4.1 Decomposition of lichen litter

The relative rates of initial lichen litter decay were positively correlated with the initial N content of the litter which varied from very low (0.5% in *P. glauca*) to very high (3.5% in *N. helveticum*). As such, the two lichens with a cyanobacterial component, *L. pulmonaria* and *N. helveticum* had a much faster rate of initial decay, than did the green-algal lichen *P. glauca*. Mass remaining after 12 months of decay ranged from 40-45% of cyanolichen litter to 57% of *P. glauca* litter. These results concurred with those of Esseen and Renhorn (1998) and McCune and Daly (1994) who showed faster rates of cyanolichen decomposition (39% and 60% mass remaining after one year for *L. pulmonaria* and *L. oregana* respectively) than for *P. glauca* (58% and 80% mass remaining for the two studies respectively). Guzman et al. (1990) also reported rapid decay rates for cyanolichen species with more than 70% mass loss over one year in a beech forest in southern Chile. That similar mass of *N. helveticum, L. pulmonaria* and *A. sarmentosa* remained after two years is consistent with the results of Lang et al. (2009) who observed no differences in mass loss over two years between chlorolichens and tripartite cyanolichens.

A meta-analysis of decomposition studies indicate that physical characteristics of plants and bryophytes associated with nutrient-use efficiency, leaf mass per area and phenology strongly influenced decay rates (Cornwell et al. 2008). Lichen litters were not considered in that study but several characteristics of lichens, notably the high N, the relatively labile chemistry of many species and the lack of woody or otherwise recalcitrant tissues suggest that decay of lichen litter would be rapid relative to others evaluated by Cornwell et al. (2008). The rapid decay of *A. sarmentosa* in this study in particular may be attributed to the physical features of this finely-branched hair-chlorolichen. Following 12 months of decay, the percent mass remaining of *A.*

sarmentosa was not different from that of the cyanolichens, despite an N-content that was 87% lower. Similar results observed by Esseen and Renhorn (1998) and McCune and Daly (1994) were also explained by the morphology of the hair lichen thallus. The highly branched and friable thallus structure of hair lichens makes them more susceptible to mechanical breakdown which may speed up decay by softening the litter (Cortez 1988) or increasing the surface area for microbial action (Fyles and McGill 1987).

Other lichens, notably *L. pulmonaria*, possess physical traits such as a thickened thallus structure or chemical composition that may inhibit decay. Initial decomposition of *L. pulmonaria* was slower than that of *N. helveticum* despite both species being N-rich compared to most vascular litters (see Harmon et al. 2009). *Lobaria pulmonaria* litter decay may have been retarded by the relatively thick outer cortex and chemical resistance of the species. A closely related species, *L. oregana*, contains stictic acid, norstictic acid and constictic acid (Culberson 1969) which have all been shown to slow decomposition (Hättenschwiler and Vitousek 2000) and to reduce the rate of N mobilization from chitin (Greenfield 1993). The existence of toxic or unpalatable phenols would also explain why such an N-rich lichen is not commonly foraged upon, while *A. sarmentosa* is readily consumed by caribou (Edwards et al. 1960; Rominger and Oldemeyer 1990), and other mammals (Stevenson and Rochelle 1984).

Chlorolichens and tripartite cyanolichens have been previously shown to decompose at similar rates to vascular litter (Lang et al. 2009). Although no side-by-side comparisons are available here, the lichen litter in this study appears to decompose more rapidly than vascular litters. The mean first-year decay constant of lichen litters in this study ($k= 0.79$ year^{-1}) are larger than those computed for vascular plant litters ($k=0.51$ year^{-1}) in a comprehensive meta-analysis (Harmon et al. 2009). In addition, there was less mass remaining of three lichen litters than of

vascular plant litter after an earlier two-year incubation at the same site (data from Prescott et al. 2004). While inter-annual climatic differences likely influenced the disparate decay rates recorded in this study and that of Prescott et al. (2004), the faster decay of cyanolichen litter may be related to its much lower C:N ratio (12 to 17) compared to that of *Pinus contorta* (104) or *Populus tremuloides* (87, Prescott et al. 2004). Fast decay of lichens may also be related to the relatively labile thallus structure of lichens. The hyphal matrix that makes up 95% of the lichen thallus is composed of N-rich chitin and the algal or cyanobacterial cells enclosed in this fungal matrix are largely comprised of labile cellulose and protein (König and Peveling 1984).

Decomposition of vascular litter is generally described in two phases. Mass loss during the first phase is adequately described by negative exponential decay models, while mass loss during the second phase (late-stage) is imperceptibly slow (Aber et al. 1990). Decay of lichen litter here did not appear to enter late-stage decay as no asymptote was detected in the percent mass remaining. In evaluating rates of decay in 234 cases, Harmon et al. (2009) demonstrated that overall decay rate constants for litters in late-stage decay were approximately 70% of the short-term (first-year) decomposition rates constants. Overall decay constants for the lichen litters ranged from 0.44 $year^{-1}$ for *P. glauca* to 0.69 $year^{-1}$ for *N. helveticum*. These constants are 79% to 71% of the first-year constants for the two species, respectively. This suggests that the two-year incubation was insufficient for lichen litter to enter late-stage decay. Alternatively, it may also be that some high-N lichen litters decompose completely and so do not enter a late-stage of slow decay, as demonstrated by Holub and Lajtha (2003). Indeed, Greenfield (1993) noted that most of cyanolichen mass was lost by the end of 135 days under laboratory conditions. This suggests that some lichen tissues may rapidly and, potentially, completely decay.

The slower lichen decomposition rates observed at the driest sites (Aleza) indicate that moisture is also an important rate-determining factor for lichen decay in these forests. The slowing of decay rates at the Herrick sites may be related to the deeper and more persistent snow pack at these higher-elevation sites.

5.4.2 Element changes

Unlike vascular litters which often have net immobilization of nitrogen and phosphorus during early decay (Lousier and Parkinson 1978), the cyanolichens in this study showed immediate and significant loss of N (up to 28% of total N lost during the first 12 months). This was consistent with what was expected given that approximately 80-90% of the N in the lichen thallus is in readily leached compounds such as labile proteins, chitin and nucleic acids (Dahlman et al. 2003) which can be solubilized and rapidly released during early decay (Greenfield 1989; Rai 1998).

There was no evidence that N was immobilized in the chlorolichen litter over two years. The small increase in N concentration in these species, however, indicates that N was retained during mass loss (relative to C) resulting in a narrowing of the C:N ratios. Both *A. sarmentosa* and *P. glauca* had a high initial C:N (129 and 88 respectively), which declined to 40-50 through the gradual loss of C. The C:N ratios of both chlorolichen litters were above the critical C:N ratio (23-35, Prescott 2005) and so, as expected, there was no net N-release from these litters during 24 months of decay.

Phosphorus was released from all lichen litters during the first four months of decay. Although the mass of P inside litter bags consistently decreased during decomposition thereafter, neither the P concentration nor the C:P ratio changed significantly between 4 and 24 months suggesting that P and C were lost at similar rates.

There are few previous studies of N and P dynamics in decomposing lichens with which to compare these results. Mat-forming lichens in dry spruce forests are extremely nutrient-poor and were found to retain P and accumulate N (up to 200% of initial concentrations) during decomposition (Moore 1984). Net N-uptake was also recorded during the first 100 days of decay of *L. oregana,* despite an initial N content of 2.28% (Holub and Lajtha 2003). Although this N was subsequently released, at a rate 19% faster than mass loss, the initial rapid release of N from high-N lichens in our study is more consistent with elemental changes commonly observed during decomposition of high-N vascular plant litters (Prescott 2005).

The rate and quantity of the accumulation or release of other nutrients during decay has been recorded for vascular plant litter (i.e. Lousier and Parkinson 1978; Berg and Laskowski 1997; Titus and Malcolm 1999). Generally, K, Ca, Mn, and Mg are readily released during decomposition while Fe, Zn, Cu and Na are retained in the litter. In the lichen litters studied here, only K was rapidly released during early decay and all other nutrients were retained in the litter. Caldiz et al. (2007) also reported rapid K loss from all lichen litters during initial decay in wet, *Nothofagus* forests in Patagonia. However, while Ca and Mg were consistently retained in our lichen litters during decomposition, these elements were immobilized in chlorolichen litter and retained in cyanolichen litter in the Pategonian forest study.

5.4.3 Litterfall

Annual biomass of vascular plant litterfall in these wet, sub-boreal forests (3448-3978 kg ha^{-1} yr^{-1}) is within the range of litterfall estimates from other coniferous forests in North America (Prescott et al. 1989; Ferrari 1999). Lichen litterfall is likewise comparable to estimates from coastal, wet temperate forests (McCune 1994). However, the total lichen litterfall (78 kg $ha^{-1}yr^{-1}$) observed at the Aleza sites here represents 13-17% of the 472-603 kg ha^{-1} of *in situ* lichen recorded by Campbell and Fredeen (2007) at the same sites (biomass estimates are unavailable

for the other two site types). This is over an order of magnitude higher than the 1% of total standing epiphytic lichen biomass observed to enter litter pools in other wet, temperate forests (McCune 1994). The biomass of lichen litter made up 0.4 - 2.8% of the total litter collected at the three sites. This is low compared to nearby high-elevation, spruce-fir forests where lichens made up 2.4 - 6.1% of vascular plant litter (Stevenson and Coxson 2003).

5.4.4 Significance of lichen N inputs

The relative contribution of lichen litter to ecosystem N-cycling is perhaps higher than litterfall biomass would suggest. Lichens accounted for up to 12% of litter-N (2.75 kg N $ha^{-1}yr^{-1}$) despite contributing less than 3% of total litterfall (Table 5.4). As discussed earlier, the N in lichens is mostly in labile and leachable forms and so may be readily available. This cyanolichen-N may therefore contribute to the observed positive correlation between available NO_3^--N and total cyanolichen litter biomass. Decomposition of cyanolichen litter at the Fraser sites is estimated to release approximately 2.1 kg N ha^{-1} yr^{-1} to the ecosystem. This is less than the estimated input (3-4 kg N ha^{-1} yr^{-1}) from cyanolichens in coastal forests of the Pacific Northwest (Denison 1979) but is within the range of most symbiotic and free-living N_2-fixation reported for late-seral interior B.C. forests (Fisher and Binkley 2000). Although Sanborn et al. (2002) reported rates of annual N_2-fixation in Sitka alder as high as 10-15 kg N ha^{-1} yr^{-1} in a 9-yr-old *Pinus contorta* plantation, symbiotic N_2-fixation by vascular plants is not likely to be a substantial source of N in late-seral, closed-canopy forests. Therefore, with N release rates as high as 2.1 kg N ha^{-1} yr^{-1}, the deposition of cyanolichen litter onto the forest floor presents a significant source of newly fixed-N that would not otherwise be available to these mature, wet sub-boreal forests.

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CHAPTER 6 CONCLUSIONS

This dissertation evaluates cyanolichen ecology in sub-boreal spruce forests with reference to the role of these nitrogen-fixing communities in nutrient and carbon cycling. This work provides evidence that improves our understanding of two important questions, namely: what is the contribution of cyanolichens to nutrient cycling and what are the environmental factors that promote cyanolichen communities?

Cyanolichens form a distinct assemblage of epiphytes most frequently observed in mature forest ecosystems under humid microclimatic conditions (Goward and Spribille 2005). With biomass loadings as high as 1400 kg ha^{-1} in wet-interior forests of British Columbia (Campbell and Fredeen 2004) these nitrogen-fixing species have the potential to contribute to ecosystem N-cycling. Indeed, the cyanolichens studied here rapidly decomposed and showed immediate and significant N-loss. Analysis of litterfall rates, decomposition rates and N-release rates revealed that up to 2.1 kg N ha^{-1} yr^{-1} may be made available through lichen litter deposition and decay. As demonstrated by Holub and Lajtha (2004) a large proportion of the N released from cyanolichens remains in the litter and organic layers and thus may be a significant source of newly-fixed N that would not otherwise be available.

Cyanolichen communities are most abundant in wet, old forests and as shown here, cyanolichen communities were more abundant at the Fraser sites where relative humidity was higher than at the Aleza sites, and where the more mature stand structure allowed greater penetration of light to the lower canopy than at the Herrick sites. Although such site characteristics have been previously shown to promote cyanolichens (Goward 1994, Campbell and Fredeen 2004), other site factors may also have been involved in defining regional differences in cyanolichen communities. For example, the higher pH of mineral soil at the Fraser

sites may have elevated the pH of bark substrate as has been observed in Norway (Gauslaa 1985).

Regardless of the microclimatic or chemical factors influencing regional cyanolichen distribution patterns, it is clear that cyanolichens are strongly spatially associated with *Populus.* Cyanolichens are restricted to the trunks of *Populus* species in areas where acid deposition is thought to preclude their wide-spread occurrence (Gauslaa 1995). In British Columbia however, cyanolichens are also observed on conifer branches, but are more abundant within a *Populus* drip-zone (Goward and Arsenault 2000). Indeed, a comprehensive survey of cyanolichen communities on conifer saplings beneath overstorey *Populus* compared to overstorey *Betula, Picea, Abies* and *Pseudotsuga* provides evidence for a strong spatial association between cyanolichens and *Populus.* Cyanolichen communities beneath overstorey *Populus* are disproportionately species-richness and abundant. Furthermore, the association between cyanolichens and *Populus* varies with climatic regime. At sites with adequate moisture and light, cyanolichen communities are equally abundant and species-rich beneath all overstorey tree species. By contrast, at sites where either moisture or light were deficient, chlorolichens comprised most of the epiphyte communities and most cyanolichens were abundant only within the *Populus* drip-zone. This suggests that *Populus* provides some factor not otherwise available under unfavourable climatic conditions.

The factors underlying the strong association between cyanolichens and *Populus* are not well understood, but it is probable that *Populus* supports cyanolichens during one or more developmental stages. Sillett et al. (2000) describe the development of epiphytic cyanolichens as going through the stages of dispersal, establishment and thallus growth. Dispersal rates were not evaluated here, but cyanolichens grew faster beneath *Populus* than beneath conifers (*Picea* and

Pseudotsuga) in these sub-boreal forests. More revealing, cyanolichen growth rates beneath *Populus* were considerably higher than most previously published accounts (Table 3.2). In addition, while most small cyanolichen thalli either decreased in size or died when transplanted beneath conifer trees, many small thalli beneath *Populus* survived the experiment and even experienced modest area gains. This indicates that conditions during the most vulnerable life-stages of establishment and early growth (Scheidegger 1995) may be ameliorated by *Populus*. Taken together, the rapid growth rates and lower rates of mortality suggest that both establishment and thallus growth are important factors contributing to the abundance of cyanolichens observed beneath *Populus*.

Differences in lichen distribution patterns have previously been related to the climate (Goward and Spribille 2005; Gauslaa et al. 2006) and chemical characteristics of the host tree (Gauslaa 1995; Hauck and Spribille 2002). However, these results, showing greater cyanolichen abundance beneath, compared to on, the *Populus*, suggest that cyanolichens are not responding exclusively to substrate characteristics. Rather, these results suggest that some factor present in the *Populus* canopy facilitates cyanolichen communities. A higher density of diaspores released from a diverse community of mature lichens beneath the *Populus* canopy may promote cyanolichen species-richness on the saplings below. However, the results presented here provide evidence in support of the drip-zone effect (Goward and Arsenault 2000) in which precipitation that is chemically altered by falling through the *Populus* canopy, is intercepted by the cyanolichen communities. The concentration of calcium (Gauslaa 1995; Goward and Arsenault 2000), molybdenum (Horstmann et al. 1982), manganese (Hauck and Spribille 2002) and phosphorus (Benner and Vitousek 2007) have all been shown to strongly influence cyanolichen community structure. However, differences in mineral nutrient availability in throughfall

precipitation beneath *Populus*, *Betula*, *Picea*, *Abies* and *Pseudotsuga* were poorly correlated with lichen community structure. The extensive sampling regime for each climatic (relative humidity, temperature, light availability) and chemical (soil nutrients, branch and soil pH, throughfall precipitation chemistry) factor suggests that these results accurately reflect the ecological patterns and provide confidence in the conclusion that these factors do not drive the *Populus*-cyanolichen association.

As an alternative, this work presents data in support of a novel hypothesis regarding the factors influencing the association. *Populus* trees belong to the *Salicaceae* family, many members of which possess extrafloral nectaries (EFN) at the junction of the leaf-blade and petiole (Trelease 1881). Glucose-rich nectar secreted from EFNs accumulates on the leaf surface (Curtis and Lersten 1978) and may be subsequently washed off and intercepted by epiphytic cyanolichens on the conifer branches below. Experimental evidence presented here demonstrates that fungal respiration is enhanced by exogenous glucose and that glucose is rapidly assimilated into lichen fatty-acids. Furthermore, the establishment success of cyanolichens was substantially enhanced by the provision of exogenous glucose.

A mechanistic basis for this difference may be found in the significant C-loss that occurs during rehydration (Brown et al. 1983). Although all lichens can withstand desiccation, cyanolichens may be more susceptible to respiratory losses during rewetting than chlorolichens. In the former, contact with liquid water is required to become photosynthetically active, but significant fungal respiration may occur with a lower thallus-water content reached under high atmospheric humidity (Lange et al. 1986). In contrast, chlorolichens may rehydrate with water vapour alone and require only 50-70% thallus-moisture for respiratory and photosynthetic activity (Lange et al. 1986). Thus, cyanolichens are rarely observed in drier ecosystems where

the duration of hydration is insufficient to allow photosynthetic C-gains to compensate for respiratory C-losses. Exogenous glucose may compensate for respiration C-losses, allowing the lichen thallus to achieve a positive C-balance despite the moisture-related inactivity of the cyanobacterial partner.

The experimental and ecological results support the hypothesis that cyanolichen communities are sustained by glucose from *Populus* EFNs. However, no data showing the concentration of carbohydrates in throughfall precipitation beneath the trees studied here are available. Although resin capsules were used to quantify carbohydrates in precipitation, analysis of extracts revealed no carbohydrates above detectable limits. This may actually reflect the chemical composition of the throughfall precipitation, but many other reports of saccharide concentrations in precipitation beneath broadleaf canopies makes this unlikely (Carlisle et al. 1966; DeBoois and Jansen 1976; Sanborn and Pawluk 1983). Rather, this omission is likely related to the timing of collection as the concentration of organic carbon in throughfall precipitation fluctuates seasonally (Carlisle et al. 1966; DeBoois and Jansen 1976). Alternatively, this may have been due to an issue with the materials used. The resin capsules were specifically designed to capture hydrocarbons from solution, but to date, have only been tested on more complex molecules and so may not be effective at adsorbing the simpler sugar molecules. Regardless of causal factors, the lack of detection of glucose-data means that this work must remain hypothetical. Discovering the concentration of glucose in throughfall precipitation and tracing nectar from *Populus* EFNs to understorey cyanolichen communities thus provides an avenue for future work.

Nevertheless, this work constitutes a significant contribution to our understanding of cyanolichens and cyanolichen ecology. The highly abundant and species-rich cyanolichen

communities beneath *Populus*, and the faster cyanolichen growth rates and lower mortality rates beneath *Populus*, strongly suggest that *Populus* facilitate cyanolichen communities. Furthermore, the experimental use and uptake of exogenous glucose, and the increased establishment success of cyanolichens with exogenous glucose, provide support for the hypothesis that it is the glucose-rich nectar produced by *Populus* EFNs that facilitates cyanolichen establishment and survival beneath *Populus* canopies. This may challenge our view of what a lichen is and may substantiate Goward's (2009) evaluation of the lichen symbiosis as shifting according to environmental conditions. The predominant view of the lichen symbiosis is one of mutual and somewhat exclusive benefit. That is, apart from water and exogenous mineral nutrients, all of the requirements of the mycobiont may be obtained from within the symbiosis. However, if under unfavourable environmental conditions, cyanolichen mycobionts are instead reliant on an external source of photosynthate, then the accepted paradigm of the nutritional fidelity in the lichen symbiosis may not always be correct.

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