VERTICAL DISTRIRBUTION, NITROGEN CONTENT, AND NATURAL 15N AND 13C ABUNDANCE OF EPIPHYTIC MACROLICHEN FUNCTIONAL GROUPS AND SOIL IN SUB-BOREAL SPRUCE FORESTS OF CENTRAL BRITISH COLUMBIA

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Anna Kobylinski

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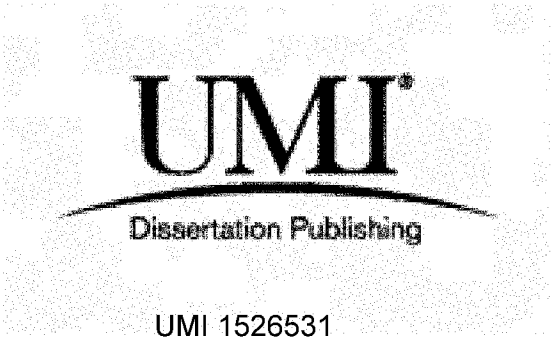

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Vertical Distribution, Nitrogen Content, and Natural ^{15}N and ^{13}C Abundance of Epiphytic Macrolichen Functional Groups and Soil in Sub-Boreal Spruce Forests of Central British Columbia

Abstract for LAC Submission

The importance of epiphytic cyanolichens to N inputs was evaluated in sub-boreal forest ecosystems of central interior BC. Four old growth sub-boreal forest sites were chosen based on cyanolichen abundance and diversity – two High Cyano sites and two Low Cyano sites. Interior hybrid spruce (Picea glauca x engelmannii) and subalpine fir (Abies lasiocarpa) trees were randomly selected at these High and Low Cyano sites and access into canopies achieved through a single-rope technique. Vertical distributions of lichen biomasses were quantified and a vertical niche of foliose chlorolichens was observed in the canopy where cyanolichens were deficient. Lichen functional groups, conifer needle, and soil samples were obtained and their 15N:14N, %N, 13C:12C, %C contents measured. Comparisons of stable isotope ratios between High and Low Cyano sites were made. Sub-boreal forest epiphytes and foliage had isotopically lighter N at sites with high cyanolichen abundance, indicative of greater inputs of biologically fixed-N.

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Glossary

Algae: (sing. alga) The photosynthetic partner of most lichens, most are microscopic unicellular or multicellular green algae.

Apothecia: a spore-bearing structure in many lichens and fungi consisting of a discoid or cupped body bearing asci on the exposed flat or concave surface.

Atom Percent (Atom %): The absolute number of atoms of a given isotope in 100 atoms of total element. For example, the ^{15}N content of air N_2 is 0.3663 Atom %. For calculation, At% = $[R_s / (R_s + 1) * 100]$ where R_s is the ratio of the light isotope to the heavy isotope of the sample.

Biodiversity (biological diversity): The diversity of plants, animals, and other living organisms in all their forms and levels of organization, including genes, species, ecosystems, and the evolutionary and functional processes that link them.

Biogeoclimatic zone: A geographic area having similar patterns of energy flow, vegetation and soils as a result of a broadly homogenous macroclimate.

Biomass: The total mass of living organisms in a given area or volume. Forest autotrophic biomass consists primarily of above-ground and below-ground tree components (stems, branches, leaves, and roots); other woody vegetation; and mosses, lichens, and herbs.

Canopy: The uppermost layer of vegetation formed by the crowns of trees in a forest, often forms a distinct habitat.

Cortex: A structurally distinct outermost layer of a lichen thallus, usually with no algal cells, present in many but not all lichens

Crustose: Crust-forming lichen with only the upper surface visible, growing on or in the substratum.

Cyanobacteria: Prokaryotic organisms without organized chloroplasts but having chlorophyll *a* and oxygen-evolving photosynthesis; capable of fixing nitrogen in heterocysts; occurring in lichens both as primary photobionts and as internal or external cephalodia; still commonly called blue-green algae.

Cyanobiont: The cyanobacterial photosynthetic partner in a lichen symbiosis.

Decomposition: The process by which complex organic compounds are broken down into simpler ones which are then available for use by organisms.

Delta Units (δ): Are expressed in molecules per thousand, or "per mil". For example, $\delta^{15}N_{Air}$ = 12 per mil means that the sample was analyzed against a reference material and found to be 12 molecules per thousand more than air - the accepted zero point for expression of ^{15}N in per mil notation. For calculation, $\delta = [(R_s / R_r) - 1] * 1000$ where R_s is the ratio of the heavy isotope to

the light isotope of the sample and R_r is the ratio of the heavy isotope to the light isotope of the reference.

Ecosystem: A functional unit consisting of all the living organisms (plants, animals, and microbes) in a given area, and all the non-living physical and chemical factors of their environment, linked together through nutrient cycling and energy flow. An ecosystem can be of any size – a log, pond, field, forest, or the earth's biosphere – but it always functions as a whole unit. Ecosystems are commonly described according to the major type of vegetation, for example, forest ecosystem, old-growth ecosystem, or range ecosystem.

Elemental Analyzer (EA): an automated sample preparation instrument in which samples are automatically converted into pure gases for isotope ratio analysis. An elemental analyzer contains the following elements: (i) furnace for combustion, reduction or pyrolysis of sample material; (ii) chemical traps for analyte gas purification; (iii) gas chromatography for time separation of these analyte gases.

Foliose: Leafy or flattened lichen, with upper and lower cortical layers.

Fractionation: The enrichment or depletion of a stable isotope caused by natural or artificial processes.

Fruticose: shrubby or pendant lichen, radially symmetrical.

Isotopes: Atoms whose nuclei contain the same number of protons but a differing number of neutrons. Two or more nuclides having the same atomic number, thus constituting the same element, but differing in the mass number. Isotopes of a given element have the same number of nuclear protons but differing numbers of neutrons. Naturally occurring chemical elements are usually mixtures of isotopes so that observed (non-integer) atomic weights are average values for the mixture.

Isotope Ratio Mass Spectrometry (IRMS): A mass spectrometer is an instrument for separation of molecules based upon their mass-to-charge ratio. In IRMS the mass spectrometer used separates isotopes of different mass within a magnetic field and precisely measures the ratio of two, or more, isotopes.

Isotope Ratio: The ratio of the minor isotope over the major isotope. For example, nitrogen in air contains 0.3663 Atom % nitrogen-15 and 99.6337 Atom % nitrogen-14, giving an isotope ratio of 0.3663 / 99.6337 = 0.003676466.

Macrolichen: Larger lichens of squamulose, foliose or fruticose habit.

Mycobiont: The fungal partner.

Natural Abundance: The concentration of isotopes as found in nature.

Nonsorediate: Lichens which are indistinguishable from sorediate species except for their lack of soredia. In these pairs of species the sorediate lichen does not have apothecia while the nonsorediate species produces fruiting bodies.

Old growth: A forest that contains live and dead trees of various sizes, species, composition, and age class structure. Old-growth forests, as part of a slowly changing but dynamic ecosystem, include climax forests but not sub-climax or mid-seral forests. The age and structure of old growth varies significantly by forest type and from one biogeoclimatic zone to another.

Pee Dee Belemnite (PDB): A belemnite from the Cretaceous Pee Dee formation of South Carolina, US which is used as the accepted zero point standard for expression of carbon and oxygen isotope abundance in delta units.

Pendulous: Hanging down from a support.

Per Mil (‰): see Delta Units.

Photobiont: The photosynthetic partner, an alga or cyanobacterium.

Soredia: (sing. soredium) a flour-like or granular ball of cells of the photosynthetic partner surrounded by fungal hyphae; these are produced from cracks or pores in the thallus or on its surface and are asexual propagules.

Species: A singular or plural term for a population or series of populations of organisms that is capable of interbreeding freely with each other but not with members of other species.

Species diversity: An assessment of the number of species present, their relative abundance in an area, and the distribution of individuals among the species.

Stable Isotope: A non-radioactive isotope in which the number of protons and neutrons in the atomic nucleus is constant through time.

Stand: A community of trees sufficiently uniform in species composition, age, arrangement, and condition to be distinguishable as a group from the forest or other growth on the adjoining area.

Thallus: The vegetative part of a lichen, containing fungal and algal cells.

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Chapter 1: Literature Review and Introduction

1.1 Importance of forest canopies

Forest canopies are significant components of the environment because of their importance to forest properties such as productivity, ecosystem health, biodiversity, and to local as well as global climate. Canopy research was overlooked by scientists for years because of the inaccessibility of most forest canopies. However, improvements in canopy access techniques have allowed scientists in recent decades to explore forest canopies - one of the last biotic frontiers on Earth - in order to document the importance of many processes in the forest ecosystem that were previously unattainable (Lowman & Rinker, 2004).

Canopies perform essential functions such as photosynthetic uptake of carbon dioxide (CO_2), release of water (H_2O) through transpiration and uptake and release of nutrients and other compounds (Lowman & Rinker, 2004). Another critical role that canopies play in forest systems is as buffers between atmosphere and soil. They provide physical protection of soils from mechanical impacts of wind and rain and remove particles and pollutants from air and rain that would otherwise directly impact other parts of the forest system. Canopy microclimate can also affect epiphyte occurrence and abundance (Campbell & Coxson, 2001).

It is essential to explore the tree crowns of forests since many environmental, ecological, and societal issues such as environmental change, acid deposition, loss of biodiversity, clean water, timber quantity and quality, pest and disease control, food, and fiber production, are directly influenced by the health of forest canopies (Nadkarni, 2001). New discoveries resulting from canopy research have important implications for forest management, crop production, and medicine. Loss and fragmentation of forest canopies globally has added to the urgency for

increasing our understanding of canopy processes. For example, the annual volume of timber harvested in our very own backyard, British Columbia (BC), has fluctuated around 78 million m^3 in recent decades, peaking at or near 90 million m^3 in 1987, 2005 and more recently in the wake of the mountain pine beetle epidemic (B.C. Ministry of Forests, Lands, and Natural Resource Operations, 2010). Since most of this harvest is still being taken from a one-time legacy of primary forest, it stands to reason that we are losing vast regions of forest canopies yearly that may hold untold ecological legacies.

The forest canopy is home to a wide variety of organisms that interact with one another and their physical environment in ways that impact the entire forest. Canopy ecosystems support approximately 40% of the species that exist in the world today, with 10% of these specific to the canopy (Ozanne et al., 2003). In order to understand the significance of forest canopies to the ecosystem, we must also understand the contributions of all canopy life forms to the forest as a whole. A significant life form that is present in most forest canopies is tree-dwelling plants, or epiphytes. Epiphytes can greatly contribute to the flow and input of nutrients and energy within forest canopies and therefore represent an important and understudied group in forest ecosystems.

1.2 Canopy exploration

Documentation and study of the world's wealth of epiphytes did not truly begin until scientists began exploring the canopies of forests around the world. In the early 1970's, scientists such as William Denison (1973) began studying the inner workings of old growth forests by applying direct-aid mountain-climbing techniques to access tree canopies, thus launching a new branch of forest ecological study. Arborists further developed tree-climbing techniques in the 1980's and forests no longer had to be studied from the ground looking up.

Donald Perry also became a well-known name in canopy research but Perry cites William C. Denison of Oregon State University as the pioneer of canopy-climbing techniques (Perry, 1978). Forest canopies have now been accessed with a variety of approaches including canopy walkways, elevators, cranes, inflatable platforms, tree houses, airships, and traditional tree climbing (Lowman & Rinker, 2004). Many of these methods offer three dimensional access into difficult-to-reach places, however, they can also be expensive and time consuming.

Two basic styles of technical tree climbing that have emerged are tautline-hitch climbing and Single Rope Technique (SRT). The SRT is one of the most popular ways to get into the canopy by using a pair of ascending devices which are hand-held mechanical ascenders that slide up the rope easily. The ascenders grip the climbing rope with cams when weighted and allow for movement up the rope whereas rappelling descenders allow for movement down the rope (Perry & Wiliams 1981, Risley 1981). The SRT method allows climbers to have a wide range of motion in the canopy by moving along branches suspended on a rope attached to an anchor point. The SRT approach was used to gain access to conifer canopies in the present study.

1.3 Epiphytic lichens in the forest canopy

Canopies are home to a wide variety of epiphytes, including vascular and non-vascular plants and lichens. Vascular plant epiphytes are common in many forest types, including tropical and temperate rainforests, but are rare in boreal conifer forests. Lichens and bryophytes are the more common epiphytes of boreal forests, with lichens, particularly chlorolichens, being the more common epiphytes in drier forests of interior BC.

Lichens form the dominant vegetation over approximately 8% of the earth's surface. Of the 20,000 lichen species that exist worldwide, approximately 3,600 species are found in North America. Terrestrial lichens comprise up to 90 percent of the ground cover in northern boreal forests (Brodo et al., 1999). In old-growth spruce and fir forests in sub-boreal BC, forests house as many as 115 different species of epiphytic lichens (Selva, 1996) with 44 different epiphytic macrolichen species observed previously in the wet and cool Sub-Boreal Spruce biogeoclimatic subzone (SBS wk1) (Meidinger & Pojar, 1991) in the Aleza Lake Research Forest (Campbell & Fredeen, 2007). British Columbia's sub-boreal spruce (SBS) forests are home to a wide variety of epiphytic organisms with properties and importance to conifer forest ecosystems that are largely unknown.

Lichens are an association between a mycobiont (most commonly an ascomycete fungi (98%), 1.6% deuteromycetes and 0.4% basidiomycetes (Rai & Bergman, 2002)) and photobiont(s), which can be green algae (commonly *Trebouxia* or *Trentepohlia*, but also *Dichtyochloropsis reticulata* such as in the tripartite cyanolichen, *Lobaria pulmonaria*) and/or cyanobacteria (commonly *Nostoc*). Form is determined by the fungal partner, which provides a living space for the photosynthetic partner, which in turn shares the carbohydrates it produces through photosynthesis. The basic growth forms are crustose (flat, paint-like), fruticose (branched and shrubby), and foliose (leafy with flat thallus). Lichens reflect the conditions of the place they grow and enlarge approximately 1-2 mm per year (Brodo et al., 1999). They are able to colonize rocks, trees, and soil but many factors control the distribution of these mutualistic symbionts. Lichens are poikilohydric organisms that rely directly on the environment for their water but they are capable of surviving with extremely low levels of water content.

Cyanolichens are lichens with cyanobacteria as their primary or secondary photosynthetic partner that contribute significant amounts of nitrogen (N) to forests by converting atmospheric N_2 gas into usable forms such as ammonium (Millbank & Olsen, 1986). There are approximately 500 lichen species where the fungus is associated with both a cyanobacterial and a green algal biont, making them tripartite lichens (Dahlman, 2003). A significant tripartite lichen in sub-boreal conifer forests is *Lobaria pulmonaria*. It has a global distribution and is the most widely distributed and common *Lobaria* in North America. It is also the dominant, and commonly the only, tripartite lichen in lower branches of forest trees in the central interior of BC. Its N_2-fixing symbiont is the cyanobacterium *Nostoc* which is found in pockets within the lichen thallus referred to as cephalodia (Brodo et al., 1999). By contrast, bipartite cyanolichens are relatively diverse at the taxonomic level with many species occurring throughout the lower canopies of central interior forests, but primarily in older forests, being generally absent from younger stands.

1.4 Factors affecting canopy lichen distribution

Canopy lichen abundance and diversity can be influenced by a variety of factors such as climate, age of the forest stand, continuity of forest cover, gradients of temperature, moisture, and light within the canopy, nutrient availability, and tree height.

Lichens have been widely useful as environmental indicators and are valuable for monitoring air quality since they are sensitive to air pollution, with their disappearance often indicative of poor air quality (Boucher & Stone, 1992). Rose (1976) introduced the idea that lichens could be used as historical indicators when he found there was a positive correlation between lichen diversity and forest age, but the complex relationship between lichen species

diversity and stand age varies across ecological gradients. Older and primary forests typically have greater and different lichen diversity than younger forests due to a variety of factors including a more stable canopy environment, which can be more important than the age of the trees to some lichen species (Hilbert & Wiensczyk, 2007). The abundance of epiphytic lichens, specifically alectorioid lichens in coastal BC, has been shown to increase with stand age (Price & Hochachka, 2001). There is an increase in lichen species richness with stand age (Price & Hochachka, 2001). The abundance of N_2-fixing cyanolichens has been shown to increase with forest age in interior cedar-hemlock (ICH) and SBS forests, which has been proposed to represent a significant source of new N in older forest ecosystems of this region (Campbell & Fredeen, 2004; Campbell & Fredeen, 2007).

Forest cover continuity and disturbance can greatly impact the distribution and composition of canopy lichen epiphytes. A lower abundance of desiccation-sensitive lichens was observed along edges of boreal forests than in the interior (Kivitso & Kuusinen, 2000). Epiphytic cyanolichens are often entirely absent in the tree canopies of young *Pseudotsuga-Tsuga* forests (Neitlich, 1993), an absence thought to reflect the limited dispersal abilities of many old-forest associate lichens (Öckinger et al., 2005). Sillett and McCune (1998) showed that old growth lichens did quite well when transplanted into clearcuts on the coast. After a fire or logging disturbance, many lichen epiphytes don't arrive until after colonization by chlorolichens during the first century of stand development (Neitlich, 1993). Major disturbances such as logging can increase irradiance and desiccation, which can cause microclimatic changes in temperature and moisture variables important to sensitive lichen epiphytes (Stevenson & Coxson, 2008).

Vertical gradients in temperature can have distinct effects on the abundance of canopy lichens. Some species are intolerant to long periods of high or excessively low temperatures, which can limit the distribution of these epiphytes in the canopy (Sillett & Mccune, 1998). High temperatures lead to high respiration rates and lichens cannot maintain the high photosynthetic rates to overcome the energy and carbon losses during these warm periods (Gaio-Oliveira et al., 2004). Low temperatures and moisture cause N_2-fixation to cease in cyanolichens, inhibiting sufficient growth and survival of the epiphyte (Denison, 1979; Sillett & Neitlich, 1996). Low temperatures can also affect colonization of regenerating trees in forest gaps by chlorolichens in northern BC (Coxson & Stevenson, 2007). Young stands in cool temperate conifer forests often have limited populations of cyanolichens, despite abundant propagule sources available from adjacent old-growth forests (Benson & Coxson, 2002). Small changes in temperature can also influence the effective moisture content in poikilohydric lichen epiphytes.

Microclimate can vary greatly within a canopy by height and epiphyte abundance. Lichen epiphytes occur along a microclimatic gradient of decreasing humidity and increasing exposure to desiccating wind with increasing canopy height (Campbell & Coxson, 2001). Densely packed epiphytes can reduce air circulation and trap unheated air on the undersides of branches making the air temperature around branches with epiphytes cooler than branches after epiphyte removal (Nadkarni, 2008). Lichens must be able to tolerate frequent cycles of drying and wetting to maintain the symbiosis (Honegger, 1998) and these cycles can in turn influence humidity within the canopy.

As poikilohydric organisms, water content of lichen epiphytes depends on environmental conditions. Lichens become hydrated with the receipt of precipitation and desiccated in its absence. Lichens have no mechanisms to prevent dehydration, but can withstand extended

drought periods and resume physiological processes rapidly (normally minutes) after rehydration (Lange et al., 2001). Lichen species are differentially distributed along moisture gradients. For instance, moist and warm microclimates often support a greater amount of cyanolichen species and dry forests support a greater abundance of chlorolichens (Sillett & Antoine, 2004). Wet and dry periods are required by most lichens in order to maintain symbiosis (Honegger, 1998) because prolonged hydration can reduce lichen photosynthesis by decreasing the amount of CO_2 diffusion to the photobiont (Lange et al., 1993). Chlorolichens can be physiologically activated by water vapour alone but cyanolichens need liquid water in order to be activated (Green et al., 2002). In addition to moisture, light and nutrient availability in forests can also affect lichen abundance and diversity.

The amount of light that is available to lichens depends on their position within the forest canopy and the surrounding canopy cover. In the upper canopy where there are high light conditions, lichens have higher desiccation rates and light saturation points than lower canopy lichens (Demmig-Adams et al., 1990; Proctor, 2000). Upper canopy zones in sub-boreal forests are mainly comprised of hair lichens and chlorolichens that are able to withstand these harsh conditions. Lower canopy positions, though often less prone to desiccation, are often light-limiting for photosynthesis. Gauslaa et al. (2008) found that genera with usnic acid such as *Alectoria* decreased with height while genera with melanic pigments such as *Bryoria* increased with height because dark melanic pigments have higher visible light screening efficiency.

Distribution of epiphytic lichens is also affected by nutrients such as calcium. Spatial variation in soil nutrient content can cause variation in cyanolichen abundance from tree to tree. In one study, more cyanolichen appeared on trees growing in calcium-rich soils than on trees growing in calcium-poor soils (Gauslaa, 1985) suggesting that tree canopies themselves are an

important ion source for epiphytic lichens (De Bruin & Hackenitz, 1986). Another study found cyanolichens to be more abundant on branches within the drip zone of trees such as *Populus* that may leach calcium, allowing cyanolichens to colonize young conifers that would otherwise be too acidic (Sillett & Goward, 1998; Goward & Arsenault, 2000).

Lichen biodiversity in sub-boreal spruce forests changes vertically with each height zone. In previous studies, upper canopies were found to be dominated by nonsorediate *Bryoria* species, the middle canopy by *Alectoria sarmentosa* and *Platismatia glauca* and the lower canopy primarily by cyanolichens and notably, the tripartite cyanolichen *Lobaria pulmonaria* (L.) Hoffm. (Campbell & Fredeen, 2007). In some cases, lower canopy branches of young coniferous forests have been shown to be unfavorable for chlorolichens due to the absence of mature lower branches (Esseen et al., 1996). In some of the wetter sub-boreal spruce forests, *L. pulmonaria* has been found to have greater stand level biomass than all other canopy lichen species combined (Campbell & Coxson, 2001).

1.5 Nutrient dynamics of canopy lichens

Nutrients held in lichens positioned within the canopy are released into the forest system by intermittent leaching (Millbank, 1982). Lichens do not provide energy, nutrients or water directly to the vascular system of their host tree directly. Instead, rain or fog can leach out nutrients held in lichens which can in turn enrich or deplete nutrients in lower epiphytes or become available to other components of the ecosystem. Epiphytes also rely on atmospheric sources for their nutrients, but lichens, particularly those in lower canopy positions, may also gain nutrients from the leaching of host tree tissues (Stewart et al., 1995). The rate at which leaching of nutrients occurs from lichens depends on factors such as morphology of the lichen.

Finely branched hair lichens can facilitate absorption and leaching due to their large surface area to volume ratio relative to flat foliose lichens.

One important nutrient that lichens leach, particularly N_2-fixing cyanolichens, is N. As water passes through the canopy it has been found to decrease in inorganic N and increase in dissolved organic N and anions (Wania et al., 2002), indicating rapid leaching when water is available for leaching. Even free-living N_2-fixers may provide N to epiphytes through leaching (Benzing, 1990), which may be of importance for chlorolichens. The most important forms of N for plant nutrition are nitrate and ammonium but they are highly soluble in water and can be easily lost through leaching. Ammonia volatilization or denitrification also amplifies the loss of N to the atmosphere when ammonium is converted back into N oxides or N_2 (Peoples et al., 1995).

Old-growth forests are highly retentive of nutrients and nutrient release rates from decomposing litter and are dependent on mobility of nutrients in living or dead organic matter. Portable nutrients such as magnesium (Mg), calcium (Ca), and potassium (K) are quickly released during early decay but other elements such as N and phosphorus (P) can be fixed or immobilized in decomposing litter with high initial C:N or C:P ratios (Manzoni et al., 2008). Losses of limiting nutrients, such as N, are low but N can also be released from decomposing litter with low C:N ratios during early decay (Prescott, 2005). Lichens with high N content and low C:N ratios have been observed to have higher decomposition rates and rates were higher in N_2-fixing lichens than in non-N_2-fixing lichens (Crittenden & Kershaw, 1978).

Decomposition rates of lichens are controlled by a variety of factors such as environmental effects, the quality of the litter substrate and the chemical composition of the decomposer community (Cornelissen, 1996). Increases in litter decomposition rates are often

associated with increasing moisture and temperature (Zhang et al., 2008). Decomposition patterns can be strongly influenced by variations in seasonal climate and time of litterfall can highly impact the initial decay rate. Holub and Lajtha (2003) found that cyanolichens had a smaller decay constant ($k = 1.24$ year^{-1}) when placed in the field during the spring than during the fall ($k = 3.1$ year^{-1}) but all lichen was thoroughly decomposed after 1 year.

Studies have found a positive relationship between snowpack and lichen decomposition rate. One study observed slower decomposition rates of cyanolichens at drier sites in sub-boreal forests of central BC and slower decay rates at higher elevation sites, which could be related to deeper and more persistent snowpack (Campbell et al., 2010). The decomposition rate of hair lichen *Alectoria sarmentosa* and *Bryoria* spp. was also influenced by snowpack. Lichen samples lost up to two-thirds of their mass when placed on early winter snowpack as opposed to minor amounts (approximately 10%) in mid and late-winter snowpack (Coxson & Curteanu, 2002).

Decay rates of *L. pulmonaria* can be inhibited by the makeup of its thick thallus and/or its chemical composition. *L. pulmonaria* had higher decomposition rates than *Hypogymnia* spp. and *Platismatia glauca* which both lack the N_2-fixing photobiont (McCune & Daly, 1994). *Lobaria pulmonaria* and *Nephroma helveticum* are both N rich foliose lichen species but *L. pulmonaria* decomposed slower than *N. helveticum* in Pacific Northwest forests (Harmon et al., 2009). The reduced rate in decomposition could be due to the thick outer cortex or chemical resistance of the species (Campbell et al., 2010). *Lobaria oregana* is a species closely related to *L. pulmonaria*. *Lobaria oregana* decomposes slowly because it contains stictic, norstictic and constictic acids (Culberson, 1969), which decrease decomposition rates (Hattenschwiler & Vitousek, 2000) and N mobilization rates from chitin (Greenfield, 1993). The N content of all *L. oregana* samples increased during decay to a peak of around 2.8% N dry mass and then decreased, which

indicated that net N immobilization occurred in the remaining lichen during early decay, followed by net N mineralization in later stages of decay (Holub & Lajtha, 2003).

Fixed N can become available to plants in the immediate area when lichens die and decay or when N compounds leach from living thalli. *Lobaria* species contribute their fixed N to surrounding ecosystems through leaching and decomposition. *Lobaria oregana* contributes 2.5–4.5 kg N $ha^{-1}y^{-1}$ (Pike 1978; Denison 1979), which is approximately 33-67% of total new N coming into old growth Douglas-fir (*Pseudotsuga menziesii*) forest ecosystems (Sollins et al., 1980). Nitrogen made available by *Lobaria* spp. could increase sub-boreal forest ecosystem productivity.

Litter from trees and epiphytes contribute to organic matter on the forest floor and add to the decomposition energy cycle. A recent study by Campbell et al. (2010) determined that the annual above-ground litterfall from wet sub-boreal forests in the interior of central BC was 3448–3978 kg ha^{-1} $year^{-1}$ with lichen litter making up 0.4%– 2.8% of the total litter mass collected. They showed that 0.1%–2.3% of the total above-ground litter biomass was from cyanolichens contributing 0.5%–11.5% of total litterfall N. The decomposition of cyanolichens in this study was estimated to release up to 2.1 kg N ha^{-1} $year^{-1}$ of newly fixed N to mature sub-boreal forests (Campbell et al., 2010) making N-rich macrolichens in the interior of BC a crucial component of the N-cycle. NitrogeN_2-fixing epiphytes capable of biological N_2-fixation are abundant in old-growth sub-boreal forests of central BC and may be a crucial component of the N-cycle. This research was an attempt to quantify the importance of lichen epiphytes, and particularly cyanolichens, to the spruce and fir forest ecosystems of central BC.

1.6 Cyanolichen biological N_2-fixation and its contribution to the N-cycle

Nitrogen is the largest single constituent of the Earth's atmosphere, at 78 percent by volume, and therefore global N pools are largely dominated by the atmosphere. Nitrogen is vital for all life since it is an essential part of proteins and nucleic acids as well as a commonly limiting nutrient for plant growth. It can be deposited onto water and land surfaces through wet deposition or dry deposition, the absorption of compounds by rain or the direct adsorption of compounds to water or land surfaces, respectively. But atmospheric nitrogen (N_2) is inert and not available for use by plants and animals so it is crucial to understand the movement of N through ecosystems.

Humans have altered the global N cycle through the use of N-rich fertilizers and the burning of fossil fuels. Anthropogenic N can especially alter ecosystems by modifying species diversity, plant community composition, and ecosystem function where N supply is limited (Aber et al., 1998). Global N_2-fixation is currently being estimated at ~240 Tg N y^{-1}. In the next 50 years, inputs to the global N cycle are expected to continue to increase to 275 Tg N yr^{-1}. Currently only 40% of all newly fixed N deposited on the Earth's surface each year comes from natural biological and chemical processes, whereas 60% is derived from human sources. This is an unprecedented change on a global scale (Berman-Frank et al., 2003). It is important to understand the N cycle, where this large amount of annually fixed N is being deposited, and if it can be supplemented by biological N_2-fixation from cyanolichens.

Certain cyanobacteria can be found in a lichenized condition, and these, under the right ecological conditions, can fix atmospheric N into ammonia which can then be utilized by lichen symbionts. A large amount of energy is required to break up the triple bond of N_2 into usable forms of N such as ammonia, so many organisms use sources of fixed N. Nitrogen fixation is an

energy-dependent transformation of atmospheric nitrogen gas (N_2) to ammonia (NH_3) by the enzyme nitrogenase, which can be inhibited by O_2.

Cyanolichens have the ability to fix atmospheric nitrogen that can then be used for growth of higher life forms and can potentially contribute to ecosystem nutrient budgets (Becker, 1980; Forman & Dowden, 1977; (Pike, 1978). Cyanolichens have a higher N content than lichens with green alga as their only photobiont (Rai, 1988). Green et al. (1980) reported the N content to be 3.4% in cyanolichens and 0.5% in other lichens. Hitch and Stewart (1973) reported similar values of 2.2% and 0.85%, respectively. About 10% of lichen species are bipartite and contain cyanobacteria as their primary photobiont (Nash, 1996) and 3-4% are tripartite cyanolichens, which contain green alga as their primary photobiont (Rai & Bergman, 2002). Cyanobacteria found in bipartite lichens are either dispersed throughout the thallus or as a layer below the upper cortex, but in tripartite lichens, the cyanobacteria occur in cephalodia. The cyanobacteria in bipartite lichens have to provide both the fixed carbon (C) and nitrogen to the mycobiont of the lichen but tripartite lichens only have to provide fixed N since the fixed C comes from the algal component of the lichen (Rai & Bergman, 2002).

The process of N_2-fixation in cyanobacteria occurs in microaerobic (low oxygen content) N_2-fixing cells called heterocysts (Brodo et al., 1999) because the enzyme complex responsible for N_2-fixation is extremely sensitive to oxygen (Gallon, 1992). The rates of N_2-fixation are higher in tripartite lichens due to their higher frequency of heterocysts within the cephalodia of cyanobacteria (Rai & Bergman, 2002). Heterocysts have a thick membrane that slows the diffusion of O_2. They contain photosystem I, which supplies ATP, but they lack the water-splitting and oxygen-producing photosystem II (Ernst et al., 1983). About 5-10% of filamentous free-living cyanobacterial cells differentiate into heterocysts under N-limited conditions

(Stewart, 1980). The cyanobiont in the lichen thalli continues to develop heterocysts even in the presence of fixed-N (Bergman et al., 1992) since the cyanobiont does not incorporate the N into its own cells. The development of more heterocysts and the exchange of metabolites are triggered by the presence of N-starved cyanobiont cells and the rapid transfer of NH_4 into the mycobiont, which takes place in the filaments of cyanobacteria.

Vegetative cells provide photosynthate in exchange for fixed N from the heterocysts. Heterocysts lack Rubisco and do not fix C so they must rely on vegetative cells to supply fixed C in order to support fixation of N_2 (Wolk et al., 1994). Once N_2 has been fixed, NH_4^+ ions in isolated free-living cyanobacteria are converted to glutamine in heterocysts by the enzyme glutamine synthetase (GS). Then the ions are exported and catalyzed to glutamate in vegetative cells. Ammonium is then released to the mycobiont and converted to glutamate by the enzyme glutamate dehydrogenase (GDH). The enzyme GDH converts NH_4 to glutamate because glutamine synthase is inhibited (Wolk, 1996). Elevated concentrations of mycobiont GDH have been shown to coincide with rates of N_2-fixation, repression of GS synthesis, and ammonia release by the cyanobiont along a lichen thallus (Rowell et al., 1985).

Mycobiont hyphae and cyanobiont cells in lichen thalli occur in close contact with each other and the hyphae contain a high level of the ammonia assimilating enzyme GDH. The mycobiont effectively absorbs ammonia released by the cyanobiont with negligible ammonia escaping outside the thallus (Rai et al., 2002). Fixed N from the symbiotic tissues is translocated to other parts of the host in the form of amino acids such as alanine in tripartite lichens, which moves from cephalodia to the main thallus (Rai et al., 2000).

Nitrogen-fixing species have a competitive advantage in ecosystems where N is in low supply. However, N_2-fixation is an energy-demanding process and can be costly to an individual

organism so they only fix N when other sources of N are not readily available. When the feather moss *Pleurozium schreberi* (Bird.) Mitt. is exposed to high concentrations of bioavailable N, the putative N_2-fixing moss-cyanobacteria association will use that N instead of expending energy on fixing new N (Zackrisson et al., 2004). Similarly, cyanolichen N_2-fixation only occurs when the N requirements are not met by N content in canopy throughfall. Canopy epiphytes intercepted 8.8% more of total throughfall compared with throughfall collected underneath trees that had their epiphytes removed (Boucher & Nash 1990; Knops et al. 1991).

Biological N_2-fixation represents a significant contributor to the global N cycle. Cyanolichens are a crucial component of this biogeochemical cycle as they have been estimated to contribute 3–4 kg N ha^{-1} $year^{-1}$ to mature conifer forests of the Pacific Northwest (Denison, 1979). Lichens represent a large portion of forest biodiversity and play an important role in the function of the N cycle relative to their small size. The nitrogenase enzyme complex responsible for N_2 reduction represents the single largest contributor to the reductive portion of the global N cycle (Christiansen et al., 2001).

1.7 Stable Isotopes of Nitrogen and Carbon

Stable isotopes can be used to increase our understanding of element cycles in ecosystems and advance our knowledge of relationships between plants and their environment. As elements cycle through the biosphere, the composition of isotopes changes and these changes are being utilized by ecologists and geochemists to understand global cycles of essential elements such as N and C (Peterson & Fry, 1987). Interest in community ecology and ecosystem research using this technique is being facilitated by technological developments making stable

isotope measurements more accessible to researchers (Hobson & Wassenaar, 1999), allowing plant ecologists to address issues that seemed problematic using other methods.

The use of stable isotopes as a tool to investigate ecological research has increased immensely in the last two decades. Enriched ^{15}N tracer studies have been used in agricultural investigations for decades (Bremner, 1965) but natural abundance studies started with Kohl, Shearer, and Commoner (1971) and were rare until the last decade. The use of stable isotopes will continue to increase because they can be used as nondestructive temporal integrators which can determine interactions and responses of plants to their abiotic and biotic environments (Dawson et al., 2002). Natural abundance (NA) is the percentage of an element occurring on earth in a particular stable isotopic form. The abundance of an isotope remains relatively constant in time. Stable isotope methods look at the interactions between organisms and the resources that influence them.

Atmospheric N can only be assimilated by specialized organisms such as cyanolichens but the nitrate and ammonia in soil are forms of inorganic N that can be assimilated by all plants including the cyanolichen host tree. Ammonium produced by cyanolichen N_2-fixation can have several potential fates. It can be assimilated into soil organic microbial biomass, taken up by plant roots, lost by denitrification into the atmosphere, leached into runoff groundwater, or transformed into nitrate through nitrification (Nadelhoffer & Fry, 1994). The distribution of N in forests can be identified by the contents of ^{15}N from N inputs and outputs.

There are two naturally abundant stable isotopes on earth of N and C. The more common N stable isotope is ^{14}N with 99.6337% of the total N in any system as ^{14}N atoms. The rarer but heavier N stable isotope is ^{15}N, and the remaining 0.3663% of ^{15}N atoms (Junk and Svec, 1958). The lighter C stable isotope is ^{12}C with a natural abundance of 98.89% and the heavier C stable

isotope is ^{13}C with a much lower natural abundance of 1.11% (Craig, 1953). The extra neutron in heavier stable isotopes gives the element a greater mass without altering its chemical behavior.

1.8 Stable Isotope Fractionation and Discrimination

Isotopic fractionation is an important factor to consider in natural abundance studies because many reactions can alter the ratio of heavy to light isotopes between a substrate and product (Peterson & Fry, 1987). Delta (δ) values are not absolute isotope abundances but differences between sample and standard values in parts per thousand (i.e. per mil). Differences in the isotope ratios can be very small so we use δ notation which tells us how much the sample deviates from the standard. Processes that result in differences in δ values in products and reactants reflect isotope fractionations and they occur because more energy is required to break chemical bonds involving the heavier isotope than the lighter isotope. Molecules containing ^{14}N react faster with enzymes than molecules containing ^{15}N (Robinson, 2001) because they are atoms of different sizes and weights. In other words, different atomic weights react at different rates. Fractionations in biogeochemical reactions can provide information about elemental processes.

The two common types of isotope fractionations are equilibrium isotope fractionation, which occurs during isotope exchange reactions that convert one phase to another, and kinetic isotope fractionation, which occurs when the reaction is unidirectional. Reaction rates for both types of fractionations are mass dependent (Dawson et al., 2002). Physical processes, such as evaporation, discriminate against heavy isotopes. Enzymatic discrimination, or differences in kinetic characteristics, can result in isotopically heavier or lighter products than their precursor materials. The ^{15}N natural abundance of ecosystem components reflects the isotopic signatures of inputs and outputs within the system. Ultimately, the $\delta^{15}N$ signature of biological material is

the result of $\delta^{15}N$ signatures of the N sources and isotope fractionation during N uptake and N assimilation, N gains and losses, and N pool mixing (Handley & Raven, 1992).

Fractionation between plant and substrate depends on physiological traits of the plant, N form, and external N concentration (Robinson 2001). The extent of isotopic fractionation depends on the substrate to product ratio, temperature, reaction rate, and enzymatic facilitation of the reaction (Nadelhoffer & Fry, 1994). Patterns of ^{15}N abundances in ecosystems can also be influenced by a variety of factors including soil age, N_2-fixing plants, precipitation, biochemical processes, and mycorrhization.

In ecological studies, the degree of fractionation is typically quite small but measurable and it is important to understand for proper data interpretation. However, not every N cycle process always fractionates N isotopes. Fractionation depends on the process, external conditions and the extent to which the N source has been consumed (Robinson, 2001). Fractionation of ^{15}N can be influenced by the N supply rate and when abundant N supplies exist, discrimination against ^{15}N during plant N uptake causes relative depletion in ^{15}N of plant biomass (Kohl & Shearer, 1980). However, under N-limited conditions, plants take up the entire supply of N, which leaves no possibility of potential fractionation (Nadelhoffer & Fry, 1994). It is important to consider isotopic fractionation in forest ecosystems when using the natural abundance (NA) method as a natural integrator and tracer of ecological processes.

1.9 Natural Abundance Method Uses and Advantages

The NA method can be used to make inferences about rates and patterns of the N cycle, the importance of N inputs and soil processes in supplying N for plant uptake, and N retention by forests (Nadelhoffer & Fry, 1994). This method allows ecologists to use stable isotopes as natural integrators and tracers without disturbing the natural behavior of the element in the

system (Handley & Raven, 1992). However, using natural abundance isotopes as tracers requires that the different sources have repeatable and distinct δ values that are more extensive than the natural range of plant δ values measured (Dawson et al. 2002). The NA technique is more efficient if there are just two pools of N available and if the variability of ^{15}N abundance is small compared to the measured difference between these abundances (Boddey et al., 2000). Natural ^{15}N abundance values are also used for improving estimates of N fluxes and N losses from forest ecosystems (Nadelhoffer & Fry, 1994), and are able to show how distributions of N isotopes are linked to N cycling and other biogeochemical processes.

The NA technique has a number of advantages over ^{15}N enrichment procedures. Natural abundance studies are simple, inexpensive, and non-disruptive to the study system and provide a wide assortment of natural experiments at a range of spatial and temporal scales. The ^{15}N enrichment method is costly and often disrupts the system through the addition of trace amounts of a labeled substance such as excess N, limiting its widespread application in N cycle research (Bedard-Haughn et al., 2003). The NA method allows for N_2-fixation to be monitored where both fixing and non-N_2-fixing reference plants are present since there are no additions or disruptions to the system before measurements are taken (Peoples et al., 2002).

1.10 Soil Nitrogen Inputs

Approximately 50% of total N deposition from the atmosphere is comprised of the gaseous species nitrogen monoxide (NO), nitrogen dioxide (NO_2) and NH_3 (Stulen et al., 1998). Ammonium is preferred by the canopy relative to atmospheric nitrate and it is more often retained (Garten & Hanson, 1990). Most of the atmospheric N that reaches the soil surface is in the form of nitrate. Soil nitrate is also preferentially assimilated by tree roots relative to soil NH_4 (Nadelhoffer & Fry, 1988). Nitrogen dynamics in the soil are strongly controlled by biologically

mediated reactions such as assimilation, nitrification, and denitrification. These reactions can result in increases in the $\delta\ ^{15}N$ of the substrate and decreases in the $\delta\ ^{15}N$ of the product.

1.11 Thesis overview

The second chapter of this thesis provides results on the patterns of variation in arboreal lichen communities in sub-boreal spruce forests. Specifically, it focuses on how epiphytic lichen species richness and dominance vary across tree species and height. Few studies have tried to separate the effects of forest structure on species abundance and diversity, both spatially and vertically within the canopy. Most research has compared the extremes of forest structure of managed forests to natural forests. Consequently, little information is available to guide forest managers in assessing natural forests as a potential repository of biological diversity (Pipp et al., 2001). This information from my findings may help guide forest management objectives that relate to conservation of forest epiphytic lichen biodiversity in this region.

The third chapter provides an examination of the importance of N_2-fixing epiphytic lichens to the N budget of SBS forests using the natural abundance of the heavy (stable) isotope of nitrogen (^{15}N) in canopy components as an *in situ* indicator. Cyanolichens such as *Lobaria pulmonaria* are known to contribute fixed N to old-growth forests, but the amounts and significance of these contributions are uncertain as they pertain to the N budget of mature and old-growth spruce and fir forests.

Finally, the fourth chapter gives a brief summary of the overall thesis findings and their significance to sub-boreal forest management. It provides some overall conclusions from this thesis research and recommendations for future management of sub-boreal spruce and fir forests in central BC.

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Chapter 2: Vertical distribution and nitrogen content of epiphytic macrolichen functional groups in sub-boreal forests of central British Columbia

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2.1 ABSTRACT

Vertical distribution, biomass abundance and nitrogen stocks of epiphytic macrolichens were examined in the two dominant tree species, interior hybrid spruce (*Picea engelmannii* Parry ex Engelm. x *glauca* (Moench) and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), in sub-boreal spruce forest ecosystems of central British Columbia (BC). Lichens were contrasted between two different site types containing either high (High Cyano) or low (Low Cyano) epiphytic cyanolichen abundance. A single rope technique was used for canopy access and a 'clump' method was used to estimate the abundance of arboreal lichens at different heights within both canopy tree species for five functional groups: *Bryoria* spp., *Alectoria sarmentosa*, foliose chlorolichens, bipartite cyanolichens, and *Lobaria pulmonaria* (the only tripartite cyanolichen). In this way, the relationship between average lichen biomasses and tree height for each tree species were assessed. We determined that biomass was dependent on tree height and species, with a greater abundance of lichen appearing on fir trees (mean ± SD; 1588 ± 428 g tree^{-1}) than the generally taller spruce trees (917 ± 422 g tree^{-1}). Foliose chlorolichen biomass was more abundant in trees with low abundance of *L. pulmonaria* (777.1 ± 365.9 g tree^{-1}) than those with a high abundance of *L. pulmonaria* (553.6 ± 400 g tree^{-1}). Much of this increase in chlorolichen biomass in trees with low cyanolichen abundance was a result of greater chlorolichen abundances in lower canopy positions where cyanolichens would otherwise predominate. Although bipartite cyanolichens had a higher %N (~3.2 %N) than *L. pulmonaria* (2.3 %N) of dry mass, there was a much higher abundance of *L. pulmonaria* in the canopy of High Cyano sites

containing a larger amount of N (4.04 ± 0.95 kg N ha^{-1}) than in all bipartite cyanolichens combined (2.33 ± 1.11 kg N ha^{-1}) or any other lichen spp. Chlorolichens are poor competitors for lower branch positions when cyanolichens such as *Lobaria pulmonaria* are abundant, the latter of potential importance for N inputs into N-limited sub-boreal forests of central BC.

Key words: Lichen epiphytes, lichen diversity, arboreal lichen abundance, *Lobaria*, nitrogen, *Picea glauca x engelmannii*, *Abies lasiocarpa.*

2.2 INTRODUCTION

Forest canopy research has revealed diverse epiphytic lichen communities in forests of north-central BC (Botting et al., 2008; Campbell & Coxson, 2001; Campbell & Fredeen, 2007; Coxson et al., 2003). In these forests, communities of epiphytic lichens are affixed to canopy branches providing a critical interface between the atmosphere, tree and forest floor. Epiphytic lichen biomass in boreal forests tends to increase with forest structural complexity (Pipp et al., 2001), and species composition varies with temperature and moisture gradients. Sub boreal spruce (SBS) forests of central BC can have significant biomasses of epiphytic N_2-fixing cyanolichens that are usually restricted to lower canopies of primarily old-growth (>140 yrs.) forests where there is reduced stand level disturbance, adequate moisture and lower light levels. Despite substantial epiphytic lichen diversity in sub-boreal forest canopies, a detailed characterization of these lichen communities by host tree species, tree and forest age and in vertical distribution is only recently emerging.

Epiphytic lichens are significant components of boreal forest ecosystems and comprise a large fraction of the forest's total photosynthetic species diversity (Campbell & Fredeen, 2007).

Vertical gradients in epiphytic lichen diversity in forest canopies are structurally complex and have been shown to be affected by the following factors: age of the forest stand (Rose, 1976), continuity of forest cover (Kivitso & Kuusinen, 2000; Coxson & Stevenson, 2007), gradients in temperature (Denison, 1979; Sillett & Neitlich, 1996), moisture (Lange et al., 2001), light (Demmig-Adams et al., 1990; McCune, 1993; Proctor, 2000), nutrient availability (Gauslaa, 1985; De Bruin & Hackenitz, 1986), and relative tree height, host tree species, and soil type (Campbell & Fredeen, 2007). Aspects of lichen ecology are also important. For example, variation in fruticose arboreal lichen accumulation in old-growth balsam fir (*Abies balsamea* (L.) Mill.) forests is speculated to be a reaction to microclimatic gradients, aging of thalli, forest fragmentation, competition, succession, and caribou grazing (Arseneau et al., 1998). Foliose epiphytic lichens also exhibit large changes in community composition along a vertical gradient (Campbell & Coxson, 2001) and these variations have been shown to be related to branch position along tree height (Arseneau et al., 1998). Natural occurrence and growth of the tripartite cyanolichen *L. pulmonaria* in boreal forests appear to be controlled in large part by a compromise between adequate light and moisture availabilities where avoidance of fatal desiccation can be achieved (Gauslaa et al., 2012).

Nitrogen that is held in epiphytic lichens, and most importantly cyanolichens, can be released into the forest system and provide forest canopies and understory plants with N through intermittent leaching (Millbank 1982; Benzing 1990) and decomposition of lichen litterfall biomass (Campbell et al., 2010b). Research has established that nutrient leaching depends on lichen structure, amount of precipitation and surface area to volume ratio, but its quantification has not been properly assessed in sub-boreal forests of central BC. Leaching of soluble materials occurs with mass water transport (Coxson & Curteanu, 2002) during drying and re-wetting

cycles. Pike (1978) showed that organic N compounds are leached from N_2-fixing lichens during laboratory mistings whereas N can be taken up by lichens which do not fix N. Other epiphytes in the canopy may also rely on nutrients leached from N_2-fixing lichens.

Decomposition of lichen is also facilitated by high area to volume ratios (Esseen & Renhorn, 1998) and is highly influenced by seasonal climate variations in temperature and moisture, time of lichen litterfall, chemical characteristics of the lichen litter, and ground cover (Asplund & Wardle, 2013). Nitrogen pools of epiphytic lichens in sub-boreal forests are significant relative to atmospheric N inputs (Campbell & Fredeen, 2007) with decomposition of cyanolichens in these forests releasing up to 2.1 kg N ha^{-1} $year^{-1}$ (Campbell et al., 2010b).

This study had two primary objectives: to describe and quantify the vertical patterns in arboreal macrolichen functional group abundances in sub-boreal spruce forests with varying amounts of cyanolichen, and to quantify the impacts of this variation on the amount of N in aggregate forest canopy lichen. To address these objectives, we assessed all epiphytic macrolichen diversity, with a particular emphasis on cyanolichens given their N_2-fixing properties and the importance of N as a limiting nutrient in SBS forests. The abundance of cyanolichens such as *L. pulmonaria* has been correlated with increased biodiversity (Campbell & Fredeen, 2004), but the effects of epiphytic cyanolichen abundance on chlorolichen functional groups are poorly understood.

Despite the potential ecological importance of sub-boreal epiphytic lichen communities, there are few reliable assessments of its biomass, composition and vertical distribution in sub-boreal forests. Increasing fragmentation and clear-cut harvesting of northern forests may be threatening many epiphytic lichens with limited dispersal ability and old-growth forest preferences. This study attempts to clarify the vertical, spatial and host-tree specificities of

arboreal macrolichens and their biomass and N levels in mature sub-boreal spruce and fir forests of the BC interior.

2.3 METHODS

2.3.1 Study Area

This study was carried out on the north side of the Fraser River near the town of Upper Fraser, BC, located approximately 70 km NE of Prince George, BC (Fig 2.1). According to the BC Biogeoclimatic Ecosystem Classification System, the study sites were situated in Sub-Boreal Spruce (SBS) forest ecosystems across an ecotonal region between the SBS wk1 (Sub-Boreal Spruce Willow Wet Cool) and the SBS vk (Sub-Boreal Spruce Very Wet Cool) subzones (Meidinger & Pojar, 1991). All study sites are characterized by relatively cool, moist summers and cold, snowy winters. Four mature sub-boreal forest sites were chosen based on having trees with predominantly high or low epiphytic cyanolichen abundance and diversity, characterized in greater detail in previous studies (Campbell & Fredeen, 2007; Campbell et al., 2010b). All study sites were approximately 2-3 ha and study trees were located within 1 km of roads for safety reasons. Site locations and spruce and fir stem densities are provided in Table 2.1.

The two dominant tree species, and therefore the primary hosts for lichen epiphytes in all four sites, were interior hybrid spruce and subalpine fir. The two Fraser sites, spanning the SBS wk1 and the SBS vk subzones, had relatively high cyanolichen abundances and diversities (Campbell et al., 2010b) and were termed 'High Cyano' sites. These sites were located at an elevation of 680 m above sea level (a.s.l.) with a mean summer temperature of 11.8 ± 5.3 °C (Campbell et al., 2010b). The two Herrick road sites had relatively low cyanolichens and

diversities (Campbell et al., 2010b) and are termed 'Low Cyano' sites in this study. These sites were located in the SBS vk at an elevation of 850 m a.s.l. with mean summer temperature of 10.8 ± 5.3 °C. Soils at all sites were Orthic Humo-Ferric Podzols formed from sandy-colluvial materials at the High Cyano sites and from sandy–skeletal glaciofluvial materials at the Low Cyano sites. Average precipitation across the ecotonal study area is approximately 897 mm per year (Campbell et al., 2010b). High Cyano 1 and 2 sites and Low Cyano 1 and 2 sites in this study are equivalent to the Hi 1 and 2 and Low 1 and 2 sites in Campbell et al. (2010), respectively.

2.3.2 Sample trees and lichen sampling

Lichen epiphytes were sampled over two summers: June to August 2008 and May to September 2009. Four spruce and four fir study trees were selected at each of the High and Low Cyano sites for a total of 32 trees. Healthy study trees were selected based on having a representative form (height, diameter) and age (ranged between ~50 and 230 years old) of the mature trees on site. All study trees were in excess of 22 m in height and 20 cm in DBH. Needles and lichens were sampled from each canopy height zone starting at the highest accessible point of each of the 32 study trees.

2.3.3 Canopy Access

Access into canopies was achieved through a single rope technique (Denison 1973; Perry & Williams 1981). A pair of ascenders with stirrups were used to climb the tree, and a lanyard system was used to permit movement within the tree canopy. Upon reaching the high point in a tree, a measuring tape was dropped to the ground to measure its height. Ziploc bags were used to collect and label lichen samples by height in the canopy. The climber descended the tree using a

Petzl stop descender (ZI Crolles, Cidex 105 A, Crolles, France), which allowed the hands to be free for biomass assessment and sample collection. Selected trees were rigged, climbed and assessed vertically for epiphytic lichen biomass.

2.3.4 Lichen Biomass Abundance Assessment

Biomass abundance of epiphytic lichens was assessed using the clump method (Stevenson, 1978; Campbell et al., 1999). Lichen biomass was estimated separately for each branch. The clump method relies on a correspondence between visual estimations of standard lichen clumps (area-based for foliose lichens or length-based units for hair lichens) and accurate clump to biomass conversion factors. The heights of all visual estimations of abundance of species were recorded *in situ* so that vertical distributions of lichen biomasses could later be calculated. Hair lichens were separated into *Alectoria sarmentosa* and *Bryoria* spp. by visual estimation of the percentage of *Bryoria* spp. in hair lichen clumps.

Biomass clumps were presumed to represent 2.5 g of hair lichens or *L. pulmonaria* and 1.5 g of foliose chlorolichens and other cyanolichens (Stevenson, 1978). To corroborate the validity of these previously measured clump to biomass conversion factors, we cut down and assessed one percent of total branches that were visually assessed for lichen biomass: a total of 33 representative sample branches collected from 9 randomly selected trees. All lichen was removed from branches, oven-dried and weighed to determine error for lichen biomass estimations. Abundance estimations for hair lichens *Alectoria sarmentosa* and *Bryoria* spp. were quite accurate but still overestimated by an average of 0.45 ±0.80 g and 0.84 ±1.37 g per branch, respectively. Foliose chlorolichens and bipartite cyanolichens were also fairly accurate but underestimated by an average of 1.22 ±2.54 g and 1.01 ±2.25 g per branch, respectively. *Lobaria pulmonaria* biomasses were most underestimated, on average by 9.86 ±30.5 g per

branch. These absolute levels of over- and under-estimation of biomasses were generally small, i.e. 1-10 % of total biomass, for all epiphytic lichens apart from *L. pulmonaria*, which was occasionally underestimated by 10-25%. Because of the large uncertainties in the correction conversion factors, combined with their small impacts overall on biomass estimates, we chose not to adjust the biomass estimates.

2.3.5 Sampling of Lichens for Chemical Analyses

Tree height was measured with a tape from the top of each tree and each tree canopy was divided into three canopy height zones. Upper, middle and lower canopy were designated as the top 1/3, middle 1/3 and lowest 1/3 by height of live tree canopy. Epiphytic lichen and needle samples were collected throughout the tree canopy and 1 sample was collected from the upper, middle and lower canopy height zones if they were present. *Bryoria* species were collected predominantly from upper canopy and *Alectoria sarmentosa* from lower canopy. Samples were placed into plastic Ziploc bags and immediately transferred into paper bags at the lab for biomass oven-drying (55°C for 3 days). Live, green needles were collected from the highest accessible point in the canopy within 1 m of the tree trunk. The needle cohort was exclusively from the previous year's foliage to keep the age of the sampled needles constant. Lichen biomass was separated into five prominent functional groups or species: *Alectoria sarmentosa, Bryoria* spp., foliose chlorolichens, bipartite cyanolichens, and *L. pulmonaria.* The biomass estimates for each site were then extrapolated to a per hectare basis using previously determined canopy tree stem densities (Campbell et al., 2010; Table 2.1). Areal biomass estimates were likely underestimated as snags and small trees were not included in stem density or lichen abundance estimates.

Needle and lichen % N (of dry mass) was analyzed at the University of Saskatchewan Stable Isotope Facilities (Saskatoon, Canada). Samples were analyzed using standardized

protocols with a Costech ECS4010 elemental analyzer (EA) coupled to a Delta V Advantage mass spectrometer with Continuous Flow (Conflo) IV interface (Stocki, 2010).

2.3.6 Statistical Analysis

Statistical analyses were performed using Sigma Plot 11 (Systat Software Inc., San Jose, CA, USA). The estimated biomasses (g dry weight) of *Alectoria sarmentosa*, *Bryoria* spp., foliose chlorolichens, bipartite cyanolichens, and *L. pulmonaria* on each branch of each tree were compared using the Kolmogorov-Smirnov and Shapiro-Wilk tests to determine if the data sets met the assumptions of normality. Differences in total biomass of each functional group among the tree species and two study site types were compared on each study tree using a 2-way analysis of variance (ANOVA) following transformations to normalize the data where needed. *Bryoria* spp. data were square root-transformed while bipartite cyanolichens and *L. pulmonaria* were log-transformed to attain normality prior to analysis. Bonferroni pairwise multiple comparisons were used to determine whether biomasses differed by tree species and site.

For each lichen functional group, the tree height was determined at which peak lichen biomass abundance and median lichen biomass occurred. These two characteristics allowed us to assess patterns of vertical epiphyte distribution. A two way ANOVA was used to analyze the data using site type (High Cyano vs. Low Cyano) and tree species (fir vs. spruce) as factors.

Mean N content was determined for each functional group and a one-way ANOVA with Tukey post hoc test was used to evaluate differences in %N across the six lichen functional groups. One-way ANOVAs with Bonferroni post-hoc multiple comparison tests were subsequently conducted between lichen functional groups within sites and tree species to determine if there was a significant difference in the amount of N (average g N $tree^{-1}$ and average kg N ha^{-1}) in each functional group.

2.4 RESULTS

2.4.1 Epiphytic Lichen Abundance

Fir study trees (~25 m) were on average 5 m shorter than spruce study trees (~30 m), but fir trees carried greater average lichen biomass on a per tree (Table 2.2) and areal (Fig. 2.2) basis. Fir trees also contained greater lichen biomasses for all lichen functional groups than spruce trees at three of the four study sites (High Cyano 1 & 2, and Low Cyano 1), and for foliose chlorolichens and bipartite cyanolichens at the fourth site, Low Cyano 2. Foliose chlorolichens at Low Cyano study sites were in greater abundance than High Cyano study sites, while, not surprisingly, bipartite and tripartite (*L. pulmonaria*) cyanolichens were both in greater abundance at the High Cyano study sites (Table 2.2). There was a noticeable presence of foliose chlorolichens in the upper canopies of both fir and spruce study trees (Fig. 2.2 A & B) but where *L. pulmonaria* was dominant in the middle to lower canopies, we observed a dramatic decline in foliose chlorolichen in these zones.

Hair lichens were found in upper, middle and lower canopy positions at all sites. Among the hair lichens, *Alectoria sarmentosa* was generally found in the mid to lower canopy and the greatest abundance was situated between 10 and 14 m (in the middle canopy zone: Fig. 2.2). *Bryoria* species were found predominantly in the upper canopy and were highly concentrated from 16 to 20 m. The peak abundance of *Bryoria* spp. was higher in the canopy on spruce trees (Fig. 2.2D) than fir trees (Fig. 2.2C). *Bryoria* spp. represented a significant component of the

arboreal lichen in the middle and upper canopy zones with a much greater presence at Low Cyano sites than (Fig. 2.2 C, D) High Cyano sites (Fig. 2.2 A, B).

The dominant foliose chlorolichen that was observed in the lower to upper canopy was *Platismatia glauca* (L.) Culb. & C.F. Culb. and its biomass decreased with increasing tree height within the canopy. When sites had less *L. pulmonaria* (i.e. at both Low Cyano sites, Fig. 2.2C, D), foliose chlorolichens tended to dominate all three height zones and especially the middle and lower canopy height zones on fir trees (Fig. 2.2C). Foliose chlorolichen abundance was noticeably greater on fir (Fig. 2.2C) than on spruce trees (Fig. 2.2D).

Common bipartite cyanolichens that were found throughout the canopy (particularly at High Cyano sites) were *L. hallii* (Tuck.) Zahlbr., *L. scrobiculata* (Scop.) D.C., *Nephroma helveticum* Ach., *Pseudocyphellaria anomala* Brodo & Ahti., and *Sticta fuliginosa* (Hoffm.) Ach. The greatest abundance of bipartite cyanolichens was found in the lower canopy from 10 m to 14 m, similar to *Alectoria sarmentosa* abundance (Fig. 2.2). There was also a greater abundance of bipartite cyanolichens on fir (Fig. 2.2A) than spruce (Fig. 2.2B). *Lobaria pulmonaria* was mainly found in the lower canopy of the tree with highest abundance between 6 m and 10 m. At High Cyano sites, foliose chlorolichens were found primarily in middle and upper tree canopy positions (Fig. 2.2A & B), except when there was little presence of *L. pulmonaria* where foliose chlorolichens became the biomass dominants in both middle and lower canopy positions (Fig. 2.2).

The results obtained from the analysis of the lichen biomass gave information about the variation of lichen loading between tree species and by site cyanolichen abundance. The null hypothesis that there was no difference in the amount of lichen loading between the two tree species was accepted for all functional groups except for foliose chlorolichens which showed a

significant difference between spruce and fir trees ($F_{1,31} = 12.7$, $p = 0.001$). We analyzed this further by looking at the difference in foliose chlorolichen abundance on spruce and fir trees at Low Cyano and High Cyano sites. Using the Bonferroni multiple comparison test we determined that foliose chlorolichen abundance was statistically different in both High Cyano ($t = 2.24$, $df = 31$, $p = 0.033$) and Low Cyano ($t = 2.79$, $df = 31$, $p = 0.009$) sites when controlling for tree species (Table 2.3).

We also wanted to determine if there was a difference in lichen loading between sites. The null hypothesis that there is no difference in the amount of lichen loading between High and Low Cyano site locations was accepted for *Alectoria sarmentosa* and foliose chlorolichens when tree species was not considered as a factor. There was a significant difference for lichen abundance between High and Low Cyano study sites for *Bryoria* spp. ($F_{1,31} = 10.4$, $p = 0.003$), bipartite cyanolichens ($F_{1,31} = 27.3$, $p < 0.001$), and *L. pulmonaria* ($F_{1,31} = 51.8$, $p < 0.001$) (Table 2.3).

2.4.2 Epiphytic Lichen Distribution

The vertical height of peak biomass abundance (i.e. modal maximum biomass, data not shown) and the height where half of the total biomass (i.e. mean maximum biomass) was reached were similar in all study trees for a given functional group of lichen. Measures of vertical peak biomass in the canopy were uniformly lower (in both spruce and fir) at Low Cyano than at High Cyano sites (Fig. 2.3). There was a statistically significant difference between High Cyano (H1 and H2) and Low Cyano (L1 and L2) sites for peak biomass height for *A. sarmentosa* ($F_{1,31} = 10.6$, $p = 0.003$), *Bryoria* spp. ($F_{1,31} = 9.80$, $p = 0.004$), and *L. pulmonaria* ($F_{1,27} = 6.50$, $p = 0.018$) functional groups (Table 2.4). There was also a statistically significant difference between High Cyano and Low Cyano abundance sites for tree height where 50 % of total

biomass was reached for *A. sarmentosa* ($F_{1,31} = 15.6$, $p < 0.001$), *Bryoria* spp. ($F_{1,31} = 7.86$, $p = 0.009$), bipartite cyanolichens ($F_{1,30} = 4.86$, $p = 0.036$) and *L. pulmonaria* within fir trees ($F_{1,27} = 18.2$, $p < 0.001$). In Low Cyano abundance sites, *L. pulmonaria* abundance was significantly different between spruce and fir trees (Table 2.4).

Cyanolichen abundance (bipartite and the tripartite cyanolichen, *L. pulmonaria*) varied greatly across study trees (Fig. 2.4). Matching our High and Low Cyano site visual selection criteria, Low Cyano sites had relatively low cyanolichen abundances (ranging from 0 to 258 g $tree^{-1}$) while High Cyano sites, while having a much larger range of cyanolichen abundances (from 134 to 1313 g $tree^{-1}$), were also on average higher than at Low Cyano sites.

2.4.3 Functional Group Nitrogen Content

There was a higher amount of aggregate epiphytic lichen N in fir versus spruce trees, and higher amounts of epiphytic lichen N in fir trees on High versus Low Cyano sites (Fig. 2.5, Insert). A statistically significant difference in the amount of N coming from *Bryoria* spp. per tree was found between High and Low Cyano sites ($F_{3,40} = 71.1$, $p < 0.001$) but not between tree species. A greater amount of N came from *Bryoria* spp. in Low versus High Cyano site trees. *Alectoria sarmentosa* represented the lowest amount of cumulative N per tree across the five lichen functional groups. There was a statistically significant difference in the amount of N from *A. sarmentosa* between spruce and fir trees at High Cyano sites and spruce trees at Low Cyano sites ($F_{3,49} = 48.7$, $p < 0.001$). A greater amount of N was contained in foliose chlorolichens on fir than on spruce trees and there was a statistically significant difference in the amount of N in foliose chlorolichens between site and tree species ($F_{3,61} = 190$, $p < 0.001$). Bipartite cyanolichens provided a greater amount of N per tree in High versus Low Cyano sites and there was a significant difference between all sites and tree species ($F_{3,98} = 2470$, $p < 0.001$). The

amount of N per tree in *L. pulmonaria* was significantly different between High and Low Cyano sites and between tree species in the High Cyano sites ($F_{3,56} = 987$, $p < 0.001$), but there was no difference in the amount of N in *L. pulmonaria* between tree species in the Low Cyano sites (Table 2.5).

On an areal basis, aggregate N from all lichen functional groups was three times higher at High Cyano sites (8.19 ± 1.52 kg N ha^{-1}) than at Low Cyano sites (2.68 ± 0.97 kg N ha^{-1}) (Fig. 2.6A, Insert). Among the functional groups, the hair lichen *A. sarmentosa* made the smallest N contribution at each site, and *Bryoria* spp. slightly greater amounts. Foliose chlorolichens contained a similar amount of N in High and Low Cyano sites; 1.50 ± 0.09 kg N ha^{-1} versus 1.67 ± 0.10 kg N ha^{-1}, respectively. Bipartite cyanolichens contributed 6-fold more N in High versus Low Cyano sites; 2.33 ± 0.11 kg N ha^{-1} versus 0.39 ± 0.03 kg N ha^{-1}, respectively. Approximately 50 % of the total N at High Cyano sites (4.04 ± 0.12 kg N ha^{-1}) was contained in one species, the tripartite cyanolichen *L. pulmonaria*. By contrast, only 3% of total N at Low Cyano sites was contributed by *L. pulmonaria*.

In addition to site level and tree species effects on area-based N already mentioned, *Bryoria* spp. ($F_{7,40} = 34.2$, $p < 0.001$), *Alectoria sarmentosa* ($F_{7,49} = 62.7$, $p < 0.001$), foliose chlorolichens ($F_{7,78} = 194$, $p < 0.001$), bipartite cyanolichens ($F_{7,114} = 3095$, $p < 0.001$), and *L. pulmonaria* ($F_{7,62} = 710$, $p < 0.001$) contained different aggregate N within all site and tree species combinations (Fig. 2.6B). Combining N from all functional groups, fir trees from High Cyano site 2 had the greatest amount of N (7.56 ± 1.58 kg N ha^{-1}); and fir tree epiphytic lichens from all sites generally contained more N than spruce trees.

2.5 DISCUSSION

Epiphytic lichen species are the primary competitors for branch or stem surfaces within the canopies of sub-boreal forest canopies of the BC interior (Arseneau et al., 1998) where bryophytes are typically absent or minor components of canopy epiphyte assemblages (Botting et al., 2008). Earlier studies of epiphytic lichen species and functional groups in conifer forest canopies have previously documented the unique vertical zonations of lichen species or functional groups (Campbell & Coxson, 2001; McCune, 1993). Färber et al. (2014) found that the vertical gradient of pendulous lichens is consistent with a shift in the type and function of sun-screening pigments. Bryoria 'hair' lichens, for example, are in highest abundance in drier upper canopy positions (Goward, 2003), while cyanolichens, with greater thallus moisture requirements, occupy lower canopy positions (e.g. Campbell & Fredeen, 2007). Our results from this study corroborate these general patterns of vertical distribution of epiphytic macrolichens on both interior hybrid spruce and sub-alpine fir trees in sub-boreal conifer forests of central BC (see Fig 2.2A, B). Since the patterns of lichen functional group biomasses are more similar to the lower maximum heights achieved by subalpine fir than spruce trees, it may be that distance from canopy height, not distance from ground level, is the more critical variable (i.e. Fig 2.2A versus 2.2B). Nevertheless, the higher biomasses in fir versus spruce are consistent with previous studies (Campbell et al., 2007), but a mechanism for this greater biomass is currently lacking.

Of greater interest to this study was the constraining of chlorolichens to mid to high-canopy positions when cyanolichens occupied the lower canopy (i.e. in High Cyano sites). High and Low Cyano sites were clustered (~5-10 km between sites in both cases) and no two sites were more than 20 km apart, with sites differing little in climate, landscape position, host tree

species, composition, or density (Campbell et al., 2010a). Although the underlying reasons for variation in cyanolichen abundance at High and Low Cyano sites were unknown, previous studies provide some general rules. Larger (Öckinger et al., 2005) and old-growth (e.g. Campbell & Fredeen, 2004) conifer trees and conifer under *Populus* spp. (Campbell et al., 2010a; Goward & Arsenault, 2000) have a greater likelihood of harbouring diverse and abundant arboreal cyanolichen communities. Since all High and Low Cyano sites in this study both contained host conifer trees >140 years old and *Populus spp.*, these wouldn't appear to explain the variability in cyanolichen abundances in this study. Limitations in cyanolichen dispersal capacity (Öckinger et al., 2005; Sillett & McCune, 1998) and 'site attributes' (e.g. edaphic characteristics: Campell & Fredeen, 2007) remain possible explanations. Bark pH is another possible factor since under similar climatic conditions, relatively high bark pH is necessary for the development of cyanolichens on conifers (Gauslaa & Goward, 2012; Gauslaa & Holien, 1998). Ultimately, the challenge of deciphering the mechanism(s) explaining variability in cyanolichen diversity and pattern across sub-boreal landscapes is on-going and in many ways related to Hutchinson's 'paradox of diversity' (1957). McCune et al. (1997) have suggested that cyanolichens are constrained to the 'light transition zones', but there were no significant differences in lower canopy light levels between Low and High Cyano sites in this study. Thus, High and Low Cyano sites afforded an excellent opportunity to examine the consequences of naturally occurring (non-manipulative) variation in cyanolichen abundances, which varied by over an order of magnitude from 30 to 86 in Low Cyano sites to 443 to 979 (High Cyano sites) kg $tree^{-1}$ (Table 2.2).

The greatly reduced abundances of bipartite and tripartite (*L. pulmonaria*) cyanolichens at Low Cyano sites (Table 2.3) resulted in a near complete take-over of all canopy zones by

chlorolichens (Fig. 2.2). Foliose chlorolichens dominated much of the upper and middle canopy zones regardless of cyanolichen abundance, but were markedly increased in lower canopy positions in the relative absence of cyanolichens at Low Cyano sites (Fig. 2.2). Chlorolichens extended their realized niches into lower canopy height zones in the absence of cyanolichens, and most notably, the absence of the cyanolichen biomass dominant, *L. pulmonaria*. This was evident by the increases in *Bryoria* spp. biomass in the upper and mid-canopy zones at Low Cyano sites of spruce and fir trees (Fig. 2.2C, D; Table 2.4). Overall, these results suggest that chlorolichens can greatly extend (downward) their realized vertical niches in the absence of cyanolichen competition in sub-boreal conifer stands. The normal preference of *L. pulmonaria* for lower canopy branches of spruce appears to be a general trait (Goward & Arsenault, 2000; Hilmo et al., 2013). However, its presence or relative absence on the distribution of other lichens has, to our knowledge, not previously been documented. Further manipulative experiments such as cyanolichen removals would be helpful in further supporting the phenomenon and uncovering possible mechanisms. We chose not to make these manipulations in this study largely over concerns of extirpating rare bipartite lichens found at High Cyano sites.

Productivity in many conifer forest ecosystems is limited by N availability, and particularly those in northern BC where atmospheric N inputs are relatively low (see Campbell and Fredeen, 2007), potentially making available N from lichen leaching and decomposition important to forest growth and/or health (e.g. Holub & Lajtha, 2003; Knowles, 2004; Caldiz et al., 2007). In our study, the average aggregate N contained in all epiphytic lichen species at High Cyano sites was 22.8 ± 2.0 to g N $tree^{-1}$, more than double that at Low Cyano abundance sites: 9.4 ± 1.3 g N $tree^{-1}$ (Fig. 2.5, Insert). This range of N pool sizes brackets epiphytic lichen N amounts estimated for another sub-boreal spruce and fir forest in central BC (Botting et al.,

2008). Higher cyanolichen abundances resulted in greater canopy lichen N per tree (Fig. 2.5, Insert), largely as a result of the higher N content of bipartite and tripartite cyanolichens. However, foliose chlorolichens represented most of the epiphytic lichen biomass (Table 2.2) and N (Fig. 2.5) at Low Cyano abundance sites. Nevertheless, total forest lichen N was greater when cyanolichens were abundant (i.e. at High Cyano sites) at the tree and stand (area) levels. On average, total lichen epiphyte N totaled 8.2 ± 0.3 kg N ha^{-1} in High Cyano abundance sites compared to only 2.6 ± 0.2 kg N ha^{-1} in Low Cyano abundance sites (Fig. 2.6, Insert).

Translation of lichen epiphyte N into N available for uptake by trees, either through leaching of lichens from throughfall or through decomposition of fallen lichen litter, was recently assessed at the High and Low Cyano sites (Campbell et al., 2010b). They showed that estimated N inputs through decomposition resulted primarily from cyanolichen litter (including all bipartite cyanolichen species plus the tripartite lichen *L. pulmonaria*) and were 2.64 and 0.09 kg N ha^{-1} yr^{-1}at High and Low Cyano sites, respectively. These rates of decomposition represent 31% of the cyanolichen-rich lichen epiphyte N at High Cyano sites, compared to only 3% of the chlorolichen-rich lichen epiphyte N at Low Cyano sites, favoured by low C:N ratios (high N content) of cyanolichen thallus. Leaching of N from epiphytes has yet to be quantified at our sites, but would be expected to be greater from cyanolichen-rich sites than chlorolichen-rich sites. Supporting this notion, previous work at our sites (Campbell et al., 2010b) found that available soil N (NO_3^- + NH_4^+) was 3.3-fold higher and NO_3^- levels >60-fold higher at High than at Low Cyano sites. Older or coastal forests ecosystems have been shown to have even greater N inputs from epiphytic cyanolichens. For example, Denison (1979) estimated the annual N inputs in mature conifer forests of the Pacific Northwest to be 3–4 kg N $ha^{-1}yr^{-1}$. Retention efficiency of the canopy is higher when more inorganic N is available. Lichens from the upper canopy

have also been shown to release minerals less readily than lichens from the lower canopy (Pike, 1978), perhaps in keeping with the low N contents of chlorolichens found in the upper canopy zone relative to high N contents of cyanolichen in lower canopy zones.

Tree (host) species effects on qualitative and quantitative epiphytic lichen loadings have received relatively little examination (e.g. Botting et al., 2008). In a previous study in the same region and general forest type in central BC, epiphyte loadings were greater in abundance on subalpine fir than on interior hybrid spruce (Campbell & Fredeen, 2007), despite the fact that spruce is normally taller than fir in mature forests in our region. We observed the same effects of host species on epiphyte abundance in the present study, with an almost doubling of epiphytic lichens on subalpine fir (317.5 g $tree^{-1}$) as opposed to the canopy dominant interior hybrid spruce (183.5 g $tree^{-1}$) (Fig. 2.2).

Lichen distribution at Low Cyano sites is lower overall than at High Cyano sites but the heights associated with peak biomass and median biomass for individual trees were similar for all functional groups. Peak biomass at the High Cyano sites often occurred approximately 3-5 m higher in the tree canopy than at the Low Cyano abundance sites. This increase in height could be a result of species competition for optimal growth conditions. There are microclimatic gradients in moisture, temperature, light, and nutrient availability which could all have influenced the growth of a certain type of lichen functional groups at certain heights (Renhorn et al., 1997; Campbell & Coxson, 2001; Gaio-Oliveira et al., 2004). Resources such as light, water, and nutrients can be altered in the forest canopy by lichens. It is important to determine how epiphytic cyanolichens interact with these resources to better understand the mechanisms that contribute to the dynamics of canopy diversity. Gauslaa et al. (2006) looked at the natural occurrence of *L. pulmonaria* and reported that biomass growth is controlled by a balance

between light availability and desiccation risk, and that the species is limited to old-growth forests because of a physiological trade-off between growth potential and fatal desiccation damage. Others noted that the differential interception of precipitation within the canopy can affect lichen distribution (Coxson & Stevenson, 2007) when lichen thalli are exposed to forest edges rather than thalli under the established forest canopy. Nutrient availability is also an important factor since lichens are dependent on the deposition of nutrients, including N, directly on the thallus (Nash, 1996), with the exception being N_2-fixing cyanobacterial lichens (Rai, 1988).

Lichen growth strongly depends on the irradiance level during hydration periods (Palmqvist, 2000; Dahlman & Palmqvist, 2003) and hydration during dark periods strongly boosts lichen growth (*L. pulmonaria and L. scrobiculata)* when photosynthesis is high during daytime (Bidussi et al., 2013). A previous study found relationships between lichen growth and site factors reflecting the openness of the canopy (Gauslaa et al., 2008). In that study, main branches were cut down from two height intervals on each tree to quantify epiphytic lichen biomass. They found that total lichen biomass was significantly higher on the lower branches (46.2 g branch^{-1}) compared to the branches at 5–6 m (36.6 g branch^{-1}). The alectorioid/foliose biomass ratio increased significantly from 0.149 at 2-3 m to 0.316 at 5-6 m, suggesting that light can determine the balance between these two growth forms. Our observations showed a similar *Alectoria* to *Bryoria* ratio in our sub-boreal spruce and fir canopies.

Forest stands that grow on similar sites with comparable soil parent material, topographic position, and climate have large differences in species composition attributable to a range of factors including historical events such as fires, droughts, or weather (Pipp et al., 2001). The diversity and abundance of lichen biomass could be an indicator of how long a forest has

developed without a major disturbance or it could give insight to a forest's true age. In the present study, fir trees had much higher *Alectoria* lichen loading than spruce trees. These trees were of similar size but it is unclear whether the variation in lichen abundance was a product of age or structural characteristics that change with age.

Compounding the methodological issues that limited the validity of the mass-estimation-correction models, the initial estimation procedure itself may have been flawed. Using the 'clump method', hair lichen biomasses were slightly overestimated while foliose chlorolichens and bipartite cyanolichens biomasses were underestimated. *Lobaria pulmonaria* biomass was underestimated by the largest extent; thus our estimates of canopy N contributed by L. pulmonaria were likely considerably lower than actual amounts. One possible reason for the greater underestimation in *L. pulmonaria* was that estimation accuracy appeared to decrease with lichen abundance and thallus size. While the clump method gives a relatively accurate estimation of the amount of lichen biomass in the canopy with minimal disturbance of lichen epiphytes, its accuracy and/or precision can vary with researcher, branch length and density. In this study, lichen abundance on tree trunks was not estimated. However, it was determined previously that more than 95% of the biomass of fruticose lichens is generally found on tree branches with the remainder (4–5%) on trunks (Arseneau et al., 1998).

Not all factors were taken into consideration when epiphytic lichen biomass estimates were recorded. There are microclimatic gradients in moisture, temperature, light, and nutrient availability, all which could be influencing the growth of certain types of lichen functional groups at a certain height (Gaio-Oliveira et al., 2004; Gauslaa et al., 2007). Branch length measurements could have been taken, as branch size is an important predictor for epiphytic lichen biomass (Gauslaa et al., 2008). Finally, forest structure, age, site conditions, weather

patterns, disturbance regimes, and ground species composition may also be determining factors for epiphytic lichen biomass presence and abundance (Pipp et al., 2001). All considered, we are still far from understanding the complex ecological conditions that 'cause' presence and abundance of different epiphytic lichen species and communities in forest canopies. Long-term manipulative studies where cyanolichen or chlorolichen abundance are experimentally reduced could be one fruitful avenue to examine these relationships.

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2.7 TABLES

Table 2.1 Latitude, longitude, and tree density with stems >25 cm dbh for four sub-boreal conifer forest study sites in central British Columbia. Data on tree stem densities (stems ha^{-1}) were taken from Campbell et al., (2010b).

Study Site	Location	Latitude	Longitude	Fir (stems ha^{-1})	Spruce (stems ha^{-1})
High Cyano 1	North Fraser	54° 08' 48.8" N	121° 49' 25.8" W	137.5	187.5
High Cyano 2	North Fraser	54° 06' 14.6" N	121° 49' 24.4" W	250	112.5
Low Cyano 1	Herrick Road	54° 07' 09.9" N	121° 40' 50.7" W	150	187
Low Cyano 2	Herrick Road	54° 06' 34.9" N	121° 40' 46.2" W	112.5	150

Table 2.2 Mean (± SD; n = 4) tree diameter base height (cm), tree height (m), tree age (years) and epiphytic lichen biomass at four study sites by functional group abundance (average g dry weight tree^{-1} and average kg dry weight ha^{-1}) on mature subalpine fir and interior hybrid spruce trees in central British Columbia.

Site	Tree Species	DBH (cm)	Tree Height (m)	Tree Age (yrs)	*Alectoria sarmentosa*		*Bryoria* spp.		Foliose chlorolichen		Bipartite cyanolichen		*Lobaria pulmonaria*	
					(g tree^{-1})	(kg ha^{-1})	(g tree^{-1})	(kg ha^{-1})	(g tree^{-1})	(kg ha^{-1})	(g tree^{-1})	(kg ha^{-1})	(g tree^{-1})	(kg ha^{-1})
High Cyano 1	Fir	50.8 ± 13.8	26.9 ± 3.9	103.0 ± 3.6	143.6 ± 125.9	19.7 ± 17.3	172.7 ± 139.1	23.7 ± 19.1	869.7 ± 501.1	119.6 ± 68.9	276.0 ± 135.4	38.0 ± 18.6	587.6 ± 115.5	80.8 ± 15.9
	Spruce	57.7 ± 13.2	30.8 ± 5.6	110.3 ± 7.2	81.3 ± 42.1	15.2 ± 7.9	123.6 ± 106.4	23.2 ± 20.0	582.6 ± 408.8	109.2 ± 76.7	70.5 ± 59.4	13.2 ± 11.1	372.2 ± 473.0	69.8 ± 88.7
High Cyano 2	Fir	49.1 ± 6.1	26.3 ± 1.0	102.0 ± 14.1	47.9 ± 15.3	12.0 ± 3.8	50.2 ± 29.2	12.5 ± 7.3	605.8 ± 151.2	151.5 ± 37.8	373.3 ± 83.1	93.3 ± 20.8	606.1 ± 251.8	151.5 ± 62.9
	Spruce	40.7 ± 3.3	27.5 ± 2.6	77.3 ± 14.2	12.6 ± 5.3	1.4 ± 0.6	13.6 ± 6.3	1.5 ± 0.7	156.2 ± 92.7	17.6 ± 10.4	113.5 ± 81.6	12.8 ± 9.2	414.5 ± 169.0	46.6 ± 19.0
Low Cyano 1	Fir	50.3 ± 5.6	23.6 ± 1.6	134.8 ± 87.9	53.5 ± 20.7	8.0 ± 3.1	182.1 ± 135.5	27.3 ± 20.3	1114.3 ± 392.7	167.1 ± 58.9	69.6 ± 96.8	10.4 ± 14.5	16.2 ± 20.2	2.4 ± 3.0
	Spruce	44.5 ± 22.4	26.8 ± 5.1	159.0 ± 1.4	48.4 ± 23.2	9.0 ± 4.3	179.0 ± 155.5	33.5 ± 29.1	517.7 ± 389.8	96.8 ± 72.9	42.6 ± 38.6	8.0 ± 7.2	11.4 ± 12.9	2.1 ± 2.4
Low Cyano 2	Fir	54.3 ± 7.8	27.3 ± 2.7	119.7 ± 32.7	38.6 ± 15.3	4.3 ± 1.7	212.9 ± 96.8	24.0 ± 10.9	898.5 ± 129.3	101.1 ± 14.5	15.9 ± 13.7	1.8 ± 1.5	15.3 ± 23.2	1.7 ± 2.6
	Spruce	59.6 ± 15.3	30.1 ± 6.6	163.0 ± 76.9	74.1 ± 61.3	11.1 ± 9.2	248.0 ± 151.6	37.2 ± 22.7	577.9 ± 180.1	86.7 ± 27.0	11.7 ± 12.6	1.8 ± 1.9	17.8 ± 19.2	2.7 ± 2.9

Table 2.3 Two-way analysis of variance for the amount of lichen loading (total lichen grams tree $^{-1}$) between spruce (S) and fir (F) and site type (High Cyano versus Low Cyano). Asterisks represent significant difference *p $\leq$ 0.05, **p $\leq$ 0.01, ***p $\leq$ 0.001.

Lichen Functional Group	Source	Sum of Squares	df	Mean Square	F
Alectoria sarmentosa	Site (H vs. L)	2511.17	1	2511.17	0.717
	Tree (S vs. F)	2255.66	1	2255.66	0.644
	Site*Tree	8175.71	1	8175.71	2.333
	Residual	98119.24	28	3504.26	
	Total	111061.78	31	3582.64	
Bryoria spp.	Site (H vs. L)	252.92	1	252.92	10.388**
	Tree (S vs. F)	10.60	1	10.60	0.435
	Site*Tree	13.36	1	13.36	0.549
	Residual	681.71	28	24.35	
	Total	958.58	31	30.92	
Foliose chlorolichens	Site (H vs. L)	900260.63	1	900260.63	2.982
	Tree (S vs. F)	1367754.63	1	1367754.63	12.688***
	Site*Tree	118830.71	1	118830.71	0.394
	Residual	2415234.25	28	100634.76	
	Total	4802080.22	31	154905.81	
Bipartite cyanolichens	Site (H vs. L)	7.18	1	7.18	27.278***
	Tree (S vs. F)	0.65	1	0.65	2.479
	Site*Tree	0.89	1	0.89	3.391
	Residual	7.11	27	0.26	
	Total	15.92	30	0.53	
Lobaria pulmonaria	Site (H vs. L)	1842450.08	1	1842450.08	51.805***
	Tree (S vs. F)	83793.95	1	83793.95	2.356
	Site*Tree	81949.23	1	81949.23	2.304
	Residual	995831.01	28		
	Total	3004024.27	31		

Table 2.4 Two-way analysis of variance for study tree height at peak biomass and 50% of total biomass by lichen functional group between site type: High Cyano (H) versus Low Cyano (L) and tree species: spruce (S) versus fir (F). Asterisks represent significant difference *p $\leq$0.05, **p $\leq$0.01, ***p $\leq$0.001.

Lichen Functional Group	Source	Sum of Squares	df	Mean Square	F	Sum of Squares	df	Mean Square	F
		Peak Biomass Height				50% Total Biomass Height			
Alectoria sarmentosa	Site (H vs. L)	86.46	1	86.46	10.63**	80.01	1	80.01	15.56***
	Tree (S vs. F)	0.02	1	0.02	0.002	1.28	1	1.28	0.249
	Site*Tree	9.25	1	9.25	1.137	13.01	1	13.01	2.529
	Residual	227.75	28	8.13		143.98	28	5.14	
	Total	323.48	31	10.44		238.28	31	7.69	
Bryoria spp.	Site (H vs. L)	130.82	1	130.82	9.803**	88.11	1	88.11	7.862**
	Tree (S vs. F)	24.33	1	24.33	1.823	15.26	1	15.26	1.362
	Site*Tree	26.83	1	26.83	2.010	7.13	1	7.13	0.636
	Residual	373.66	28	373.66		313.81	28	11.21	
	Total	555.63	31	555.63		424.31	31	13.69	
Foliose chlorolichens	Site (H vs. L)	47.05	1	47.05	2.618	30.03	1	30.03	3.134
	Tree (S vs. F)	5.95	1	5.95	0.331	12.50	1	12.50	1.304
	Site*Tree	0.85	1	0.85	0.047	3.51	1	3.51	0.366
	Residual	503.14	28	17.97		268.34	28	9.58	
	Total	556.98	31	17.97		314.38	31	10.14	
Bipartite cyanolichens	Site (H vs. L)	44.99	1	44.99	2.952	62.98	1	62.98	4.856*
	Tree (S vs. F)	9.98	1	9.98	0.655	1.36	1	1.36	0.105
	Site*Tree	16.83	1	16.83	1.104	0.11	1	0.11	0.008
	Residual	411.46	27	15.24		350.19	27	12.97	
	Total	483.86	30	16.13		415.25	30	13.84	
Lobaria pulmonaria	Site (H vs. L)	25.25	1	25.25	6.504*	32.07	1	32.07	18.152***
	Tree (S vs. F)	5.18	1	5.18	1.333	8.61	1	8.61	4.876**
	Site*Tree	3.18	1	3.18	0.820	9.81	1	9.81	5.551**
	Residual	93.15	24	3.88		42.40	24	1.77	
	Total	125.75	27	4.66		90.59	27	3.36	

Table 2.5 One-way analysis of variance of functional group nitrogen content across both tree types between High Cyano versus Low Cyano sites. Asterisks represent significant difference *p $\leq$ 0.05, **p $\leq$ 0.01, ***p $\leq$ 0.001.

Source	Sum of Squares	df	Mean Square	F
Avg. g N tree $^{-1}$				
A. sarmentosa	0.712	49	0.237	48.7***
Bryoria spp.	9.206	40	3.069	71.1***
Foliose chlorolichens	209.202	61	69.734	190***
Bipartite cyanolichens	934.065	98	311.355	2470***
L. pulmonaria	1124.708	56	374.903	987***
Avg. kg N ha^{-1}				
A. sarmentosa	0.0466	49	0.00665	62.7***
Bryoria spp.	0.340	40	0.0485	34.2***
Foliose chlorolichens	9.457	78	1.351	194***
Bipartite cyanolichens	60.025	114	8.575	3095***
L. pulmonaria	84.013	62	12.002	710***

2.8 FIGURES

Figure 2.1 Map of study area including four sampling sites (High Cyano 1 and 2, Low Cyano 1 and 2) to the east of Prince George (Large inset) near Upper Fraser, British Columbia, Canada (Small inset).

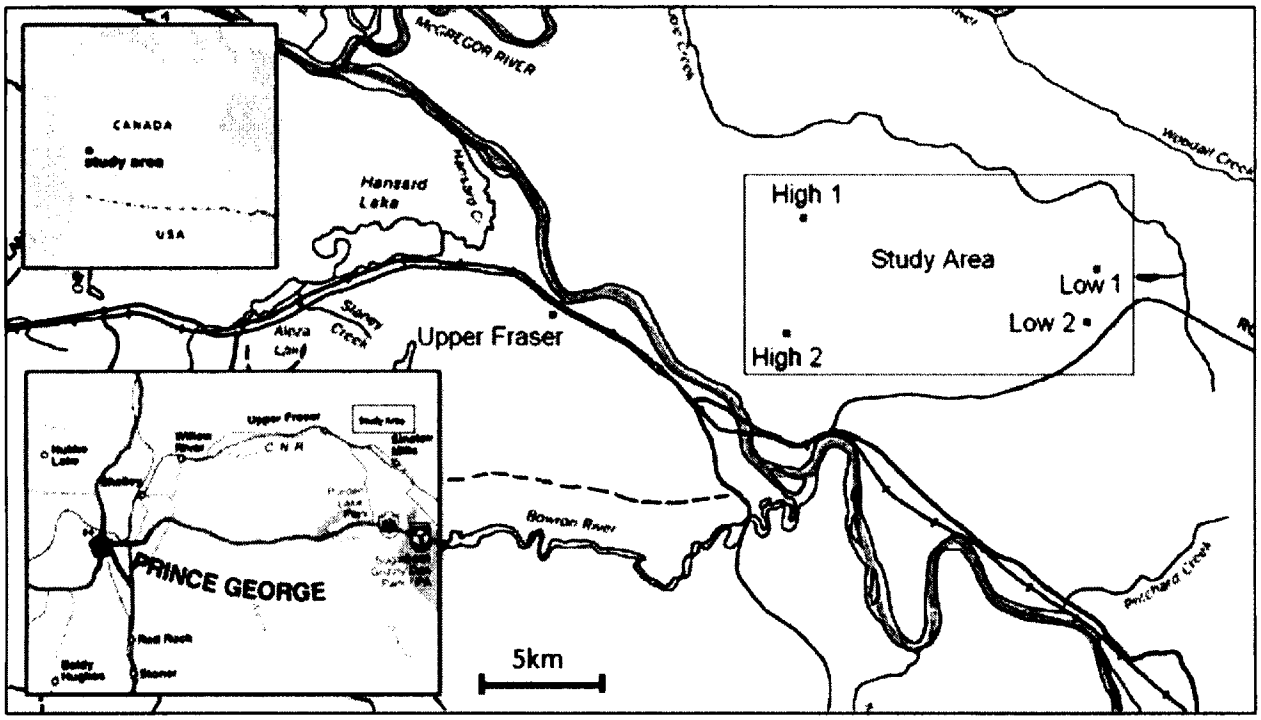


Figure 2.2 Vertical gradients in mean lichen functional group abundances (g dry weight m^{-1} tree height) on mature subalpine fir and interior hybrid spruce trees versus tree height (meters) in High Cyano and Low Cyano lichen abundance sites in sub-boreal conifer forests in central British Columbia.

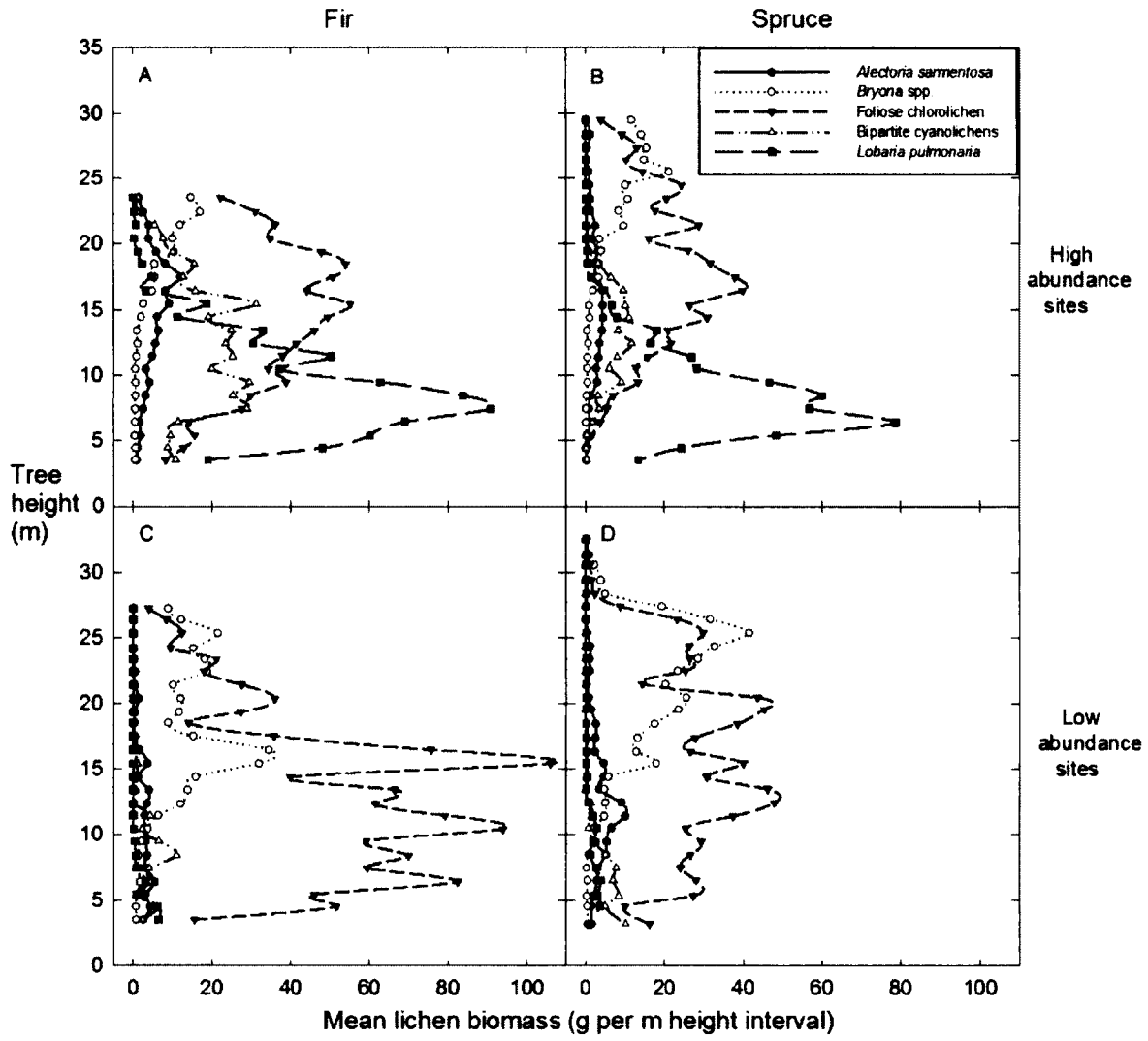


Figure 2.3 Median height (± SD) where half of the total epiphytic lichen biomass is reached for each functional group on mature fir and spruce trees at High Cyano (large symbols) and Low Cyano (small symbols) sites in sub-boreal conifer forests of central British Columbia.

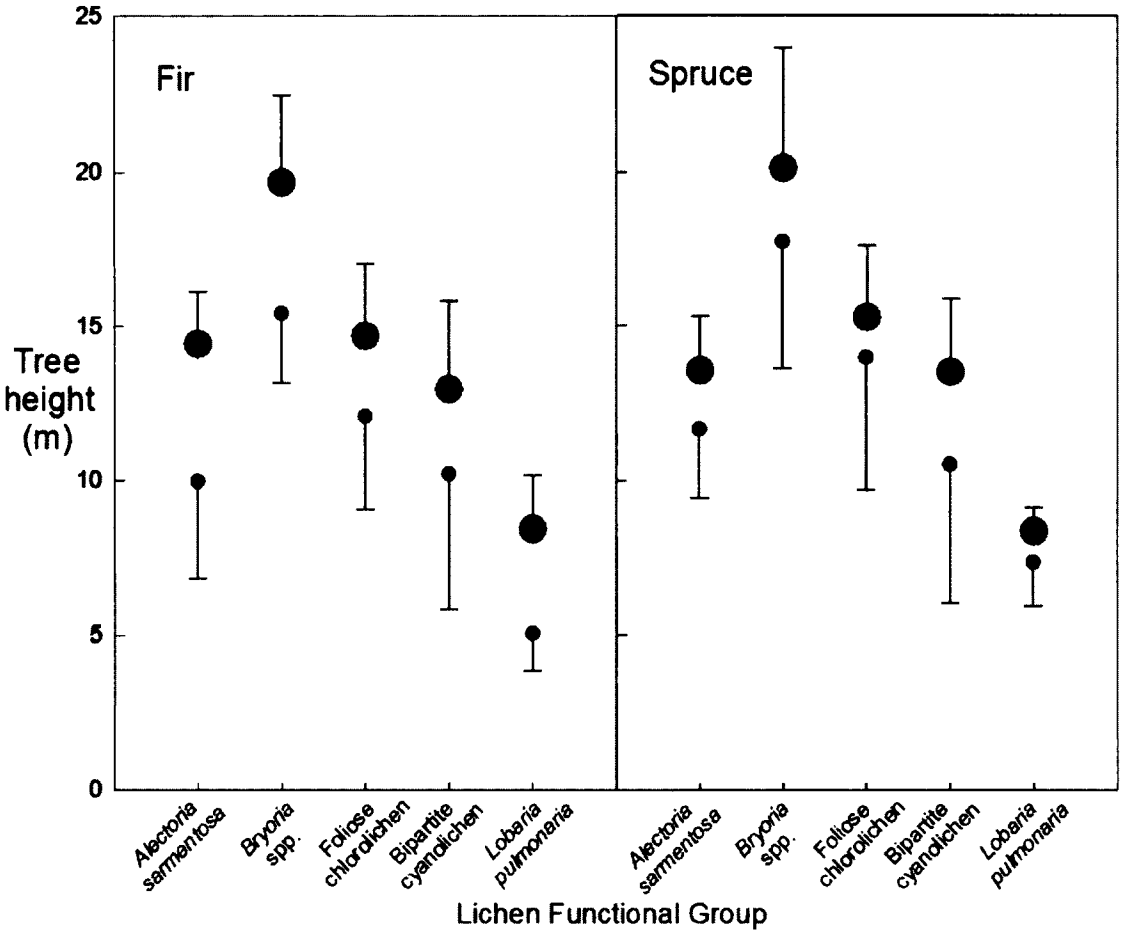


Figure 2.4 Total cyanolichen (bipartite + tripartite cyanolichen) abundance (g dry weight tree^{-1}) on Fir (F) and Spruce (S) study trees arranged from lowest to highest cyanolichen abundance at High (H) and Low (L) Cyano sites. Mean cyanolichen biomasses tree^{-1} ± SD are shown for fir and spruce trees at all sites.

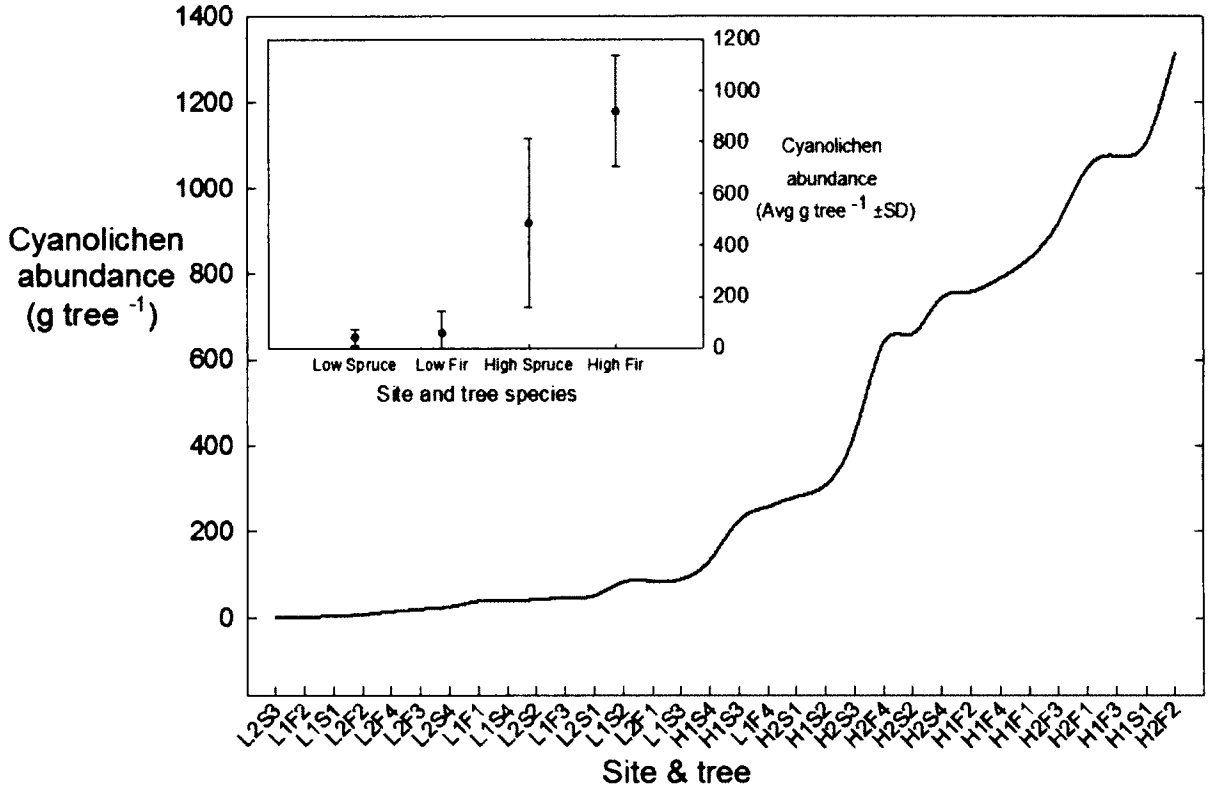


Figure 2.5 Mean epiphytic lichen nitrogen (N: g of N tree^{-1} ±SD) across High and Low Cyano sites for fir and spruce study trees in central British Columbia. Different letters indicate significantly different mean values for each functional lichen group across the sites (Bonferroni multiple comparison tests: $p < 0.05$). Aggregate tree-level epiphytic lichen N is shown for study trees at both High and Low Cyano sites (Figure 2.5 Inset).

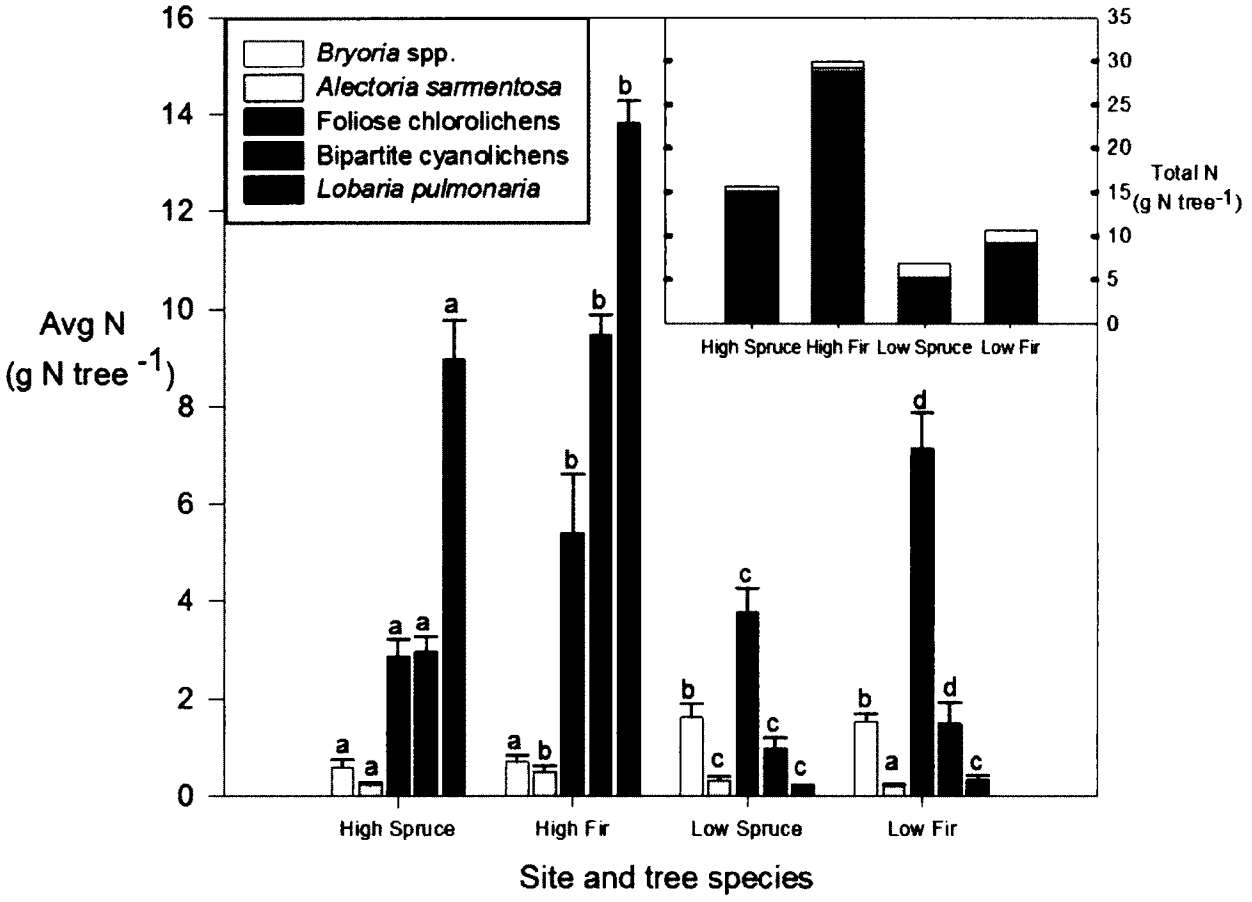
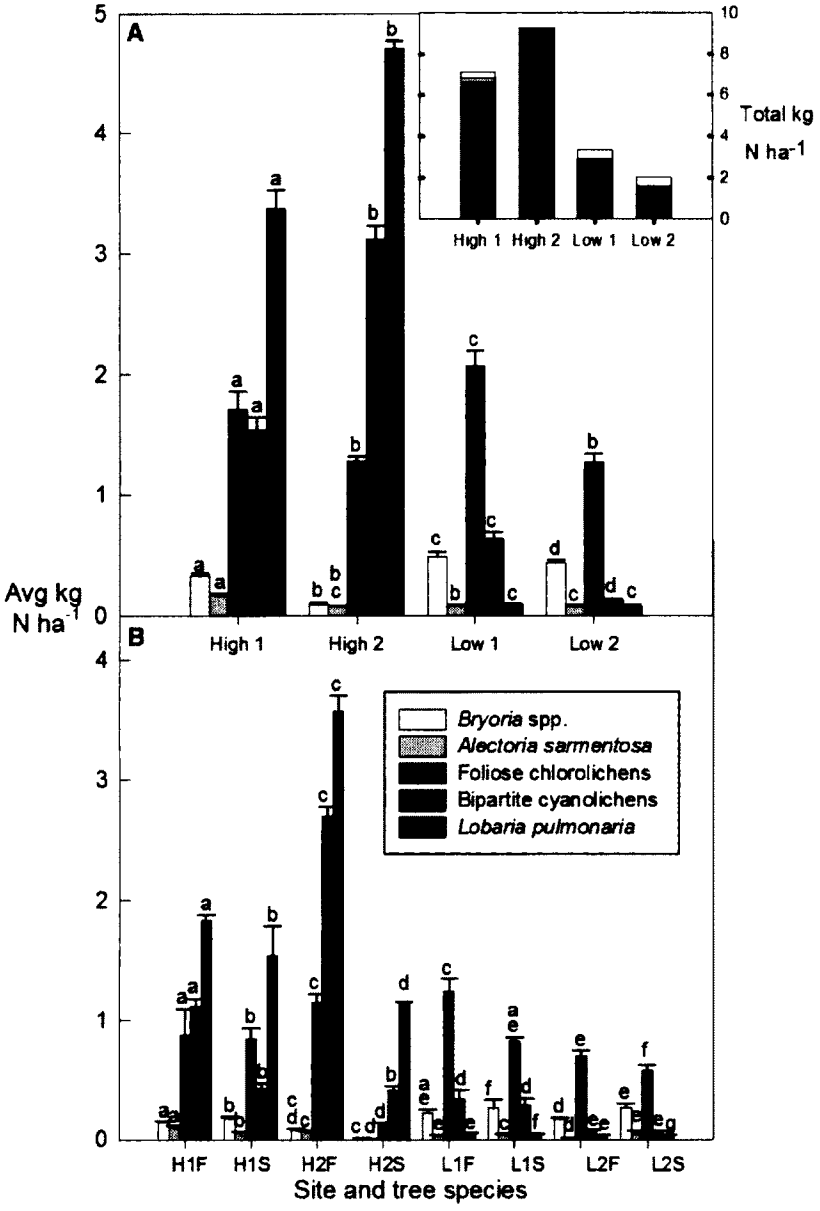


Figure 2.6 Mean epiphytic lichen nitrogen (N: kg of N ha^{-1} ± SD) at sites with (A) high (High Cyano 1 or 2) versus low (Low Cyano 1 and 2) cyanolichen abundance on (B) either Spruce or Fir by lichen functional group in central British Columbia. Aggregate N by functional group is also shown (Figure 2.6A Inset). Dissimilar letters indicate significantly different mean values (Bonferroni multiple comparison tests: P>0.05).



Chapter 3: Using stable isotope ratios $^{15}N:^{14}N$, $^{13}C:^{12}C$ to determine the effects of epiphytic lichens on the nitrogen (N) and carbon (C) cycles of wet sub-boreal spruce and fir forests of central British Columbia

3.1 ABSTRACT

Forest canopy research has revealed rich epiphytic lichen communities that represent a critical interface between the atmosphere and soil. In mature sub-boreal forest ecosystems of central British Columbia (BC), epiphytic cyanolichens make important contributions to biological diversity, but their importance to nitrogen (N)-cycling in these forests, particularly with respect to N_2-fixing cyanolichens, has not yet been adequately quantified. Variations in the ratios of the natural abundance of stable isotopes of N and carbon (C) (i.e. $^{15}N:^{14}N$, $^{13}C:^{12}C$) in forest ecosystem components represent an important *in situ* method to estimate N inputs from cyanolichens. Four mature sub-boreal spruce and fir dominated forest sites were chosen based on relative abundance of cyanolichens: two high (High Cyano) and two low (Low Cyano) abundance sites. Interior hybrid spruce (*Picea glauca x engelmannii*) and subalpine fir (*Abies lasiocarpa*) trees were randomly selected at High and Low Cyano sites and access into canopies achieved through a single rope technique. Lichen, conifer needle, and soil samples were obtained and their $^{15}N:^{14}N$, %N, $^{13}C:^{12}C$, %C contents determined using continuous flow gas isotope ratio mass spectrometry. We hypothesized that High Cyano sites would elevate N levels in host tree foliage and have isotopically heavier N (i.e. more ^{15}N), indicative of greater inputs of cyanolichen fixed-N with isotopic compositions similar to atmospheric N_2. Our results did show a modest positive correlation between host tree foliage (conifer needle) N concentrations and cyanolichen abundance. Cyanolichen abundance was a statistically significant but not an effective predictor of lichen functional group delta (δ) ^{15}N. By contrast, tree branch height above

ground level was a statistically significant predictor of needle $\delta^{15}N$. However, site-level cyanolichen abundance did not significantly affect $\delta^{15}N$ or %N of lichen or soil. Stable isotopes of C can be used to gain further information about lichen performance across ecological gradients in both space and time. Lichen functional groups and tree foliage fell into three distinct groups with respect to $\delta^{13}C$; *L. pulmonaria* (lightest), needles (intermediate), and bipartite cyanolichens, hair and chlorolichens (heaviest). Carbon isotopes were used to determine that tree branch height was an effective predictor of needle δ13C. We were able to show how the isotopic composition of two elements changed based on various conditions such as changes in cyanolichen abundance and canopy height. While our work did find enhancements in host tree foliage N with increasing cyanolichen abundance, further studies are required to determine if these result in greater tree growth.

Key Words: $\delta^{15}N$, $\delta^{13}C$, stable isotope natural abundance, arboreal lichen, sub-boreal forest, cyanolichen, *Lobaria pulmonaria*

3.2 INTRODUCTION

3.2.1 Lichen Characteristics

Tree foliage, branches, and arboreal epiphytes all intercept and regulate inputs from the atmosphere contained in wet or dry deposition, with the amount of interception depending on relative abundances and properties of these canopy elements (Nadkarni & Sumera, 2004). Arboreal lichens, with large specific surface areas, can play a significant role in mediating the exchange of water and depositional constituents such as nutrients through interception, absorption and/or assimilation processes (Pypker et al., 2006).

Nitrogen (N) is an essential growth-limiting macronutrient in plant systems and generally limiting in forests where ambient atmospheric deposition of N is low, e.g. northwest BC, Canada. In N-limited conifer forests, biological N_2-fixing species have been shown to make important contributions of N to forest by intercepting, retaining and releasing this growth-limiting nutrient (Coxson & Nadkarni, 1995). Epiphytes on lower branches within a tree are exposed to throughfall of dry and wet deposition and N sources from upper canopy epiphytes. This interaction between upper and lower canopy zones can be further altered by vertical gradients in the epiphyte guild to create significant variation in N concentration and $\delta^{15}N$ values of epiphytes from a communal tree (Bergstrom & Tweedie, 1998). Although the presence of epiphytic lichens has been shown to enhance a tree's ability to absorb and enhance atmospheric N deposition and inputs, boosting nutrient cycling fluxes, the removal of lichens in an oak forest system had no effects on tree growth (Knops et al., 1997). Lichens are also nutritionally specialized fungi that derive C from algal and/or cyanobacterial photobionts (Honegger, 1993).

3.2.2 Stable Isotope Techniques

Stable isotopes have been used experimentally to infer many properties about elemental cycles of important nutrients (most notably C and N) at a variety of temporal and spatial scales and ecological contexts. The ratio of the heavier less common stable isotope to that of the lighter abundant isotope (e.g. ^{15}N:^{14}N and ^{13}C:^{12}C) can be expressed as atom %. In practice, isotope levels are typically represented in delta (δ) notation which is not the absolute isotope ratio, but the difference between the sample measurement and an internationally accepted reference standard (Robinson, 2001) in parts per thousand or per mil (‰).

With respect to nitrogen, the natural abundance of ^{15}N in air is constant at 0.3660% gas and is therefore used as the reference standard for N isotope analyses. Specifically, $\delta^{15}N$ stable isotope results were reported as the per mil (‰) difference (δ) between the ^{15}N:^{14}N isotope ratio of each sample compared to the same isotope ratio of the N_2 in air defined as 0.36637. By contrast, the common reference material for ^{13}C is a limestone Pee Dee Belemnite (PDB). Specifically, $\delta^{13}C$ stable isotope results were reported as the per mil difference (δ) between the C isotope ratio of each sample and the ^{13}C:^{12}C isotope ratio of PDB limestone. The absolute isotope ratio (R) is the ratio of ^{15}N:^{14}N for N and ^{13}C:^{12}C for C isotope abundance. Once the R of the sample and standard are measured, the δ is calculated. Positive δ values indicate the sample contains more of the heavy isotope than the standard and negative δ values indicate the opposite. Therefore, higher levels of ^{15}N or ^{13}C enrichment correspond to less negative (or more positive) $\delta^{15}N$s and $\delta^{13}C$s, respectively (Dawson et al., 2002).

In theory, natural abundances of isotopes can be used to make inferences about the contributions of N_2-fixing species to forest trees by exploiting the naturally occurring differences in ^{15}N:^{14}N ratios between plant-available mineral N sources in the soil and that of atmospheric

N_2 utilized by N_2-fixing species (Boddey et al., 2000). Non-N_2-fixing plants receive their entire N supply from soil N pools and can be expected to be isotopically heavier than N_2-fixing plants and lichens, which derive a large component of their N directly from the atmosphere. Consequently, natural abundances of stable N isotopes have frequently been used to estimate direct or indirect contributions of N_2-fixation to plant N content (Vitousek et al., 1989).

The distributions of stable isotopes of C and N have been widely investigated. The naturally occurring $\delta^{13}C$ values for biological carbon compounds range from ~0‰ to -110‰ relative to the Pee Dee Belemnite standard. However, observed lichen $\delta^{13}C$ values occur over a much narrower range (-14 to -35 ‰: Lange et al., 1988), and considerably narrower still in temperate tree species with C3 physiology (e.g. -24‰ to -33‰ range: O'Leary, 1988). Carbon isotopes can inform us about interactions that occur between lichens and other organisms. A previous review by Ehleringer & Rundel (1988) revealed that C isotopes can inform about how species adjust their gas exchange metabolism, strategies of resource acquisition and use, and life history patterns to ensure competitiveness as well as survival.

Most terrestrial materials have $\delta^{15}N$ compositions ranging between -20‰ and +30‰ (Junk & Svec, 1958), but as before, lichens have been observed to contain a more restricted range of compositions (-21.5‰ (Fogel et al., 2008) to +18‰ (Huiskes et al., 2006)), and plants even more so (e.g. -5‰ to +2.9‰: Amundson et al., 2003). The natural abundances and ratios of stable isotope compositions are potentially useful to the researchers quantifying these processes in that they can shift due to isotopic fractionation.

Isotopic fractionation can occur from a variety of processes. For example, N_2-fixation in organisms such as epiphytic cyanolichens does not discriminate between the ^{15}N and ^{14}N and therefore would normally represent N isotope ratios close to the atmospheric $\delta^{15}N$ standard of

0‰. By contrast, non-N_2-fixing elements and processes of forest systems such as ectomycorrhizae (ECM), mineralization of organic nitrogen, nitrification, microbial assimilation of inorganic N, and denitrification can fractionate N stable isotopes below ground. Nutrient uptake in temperate and boreal trees is predominantly dependent on ECM hyphae growing into the soil from the mycorrhizal root tips (Wallander et al., 2004). In a 1996 study, Högberg et al. found that ECM roots of Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) were 2‰ enriched in ^{15}N relative to non-mycorrhizal roots. The study also found ECM fungi were enriched in ^{15}N compared to their host plants, further suggesting that ^{15}N ECM discrimination was fungal in origin. ECM fungi were also more enriched in ^{13}C relative to total soil C (Högberg et al., 1999) demonstrating ^{13}C ECM discrimination. Biologically mediated reactions that control elemental dynamics in soils can result in ^{15}N and ^{13}C enrichment or depletion, and therefore inferences can be difficult in complex ecosystems with a multitude of important sources and sinks for nutrients.

3.2.3 Soil N Inputs

Soil $\delta^{15}N$ values are higher than atmospheric N, which is isotopically lighter than biological material, suggesting that there is discrimination against the lighter isotope during decomposition (Peterson & Fry, 1987). Soil $\delta^{15}N$ values are usually positive and increase with depth of organic horizons ('L', 'F', and 'H'), and with mineral horizons having higher $\delta^{15}N$ than surficial organic horizons (Mariotti et al., 1980). This pattern occurs because there is a faster loss of ^{14}N than ^{15}N during decomposition in soils, which results in ^{15}N increases of ~5-10‰ with increasing depth (Peterson & Fry, 1987). Thus, fractionation of isotopes during litter decomposition in forests causes surface soils to have lower $\delta^{15}N$ values than deeper soils

(Nadelhoffer & Fry, 1988). Even slight fractionations occurring over decades of transfers of N from mineral soil to forest biomass can be sufficient enough to increase $\delta^{15}N$ soil organic matter by ~6-8‰ (Billings & Richter, 2006). Surface soils located beneath trees have also been found to have lower $\delta^{15}N$ values than those in open areas as a result of litter deposition (Shearer & Kohl, 1986) making decomposing epiphytic lichens important to soil nutrient isotope composition.

Since soil N is typically more abundant in ^{15}N than the atmosphere, non-N_2-fixing plants typically have higher $\delta^{15}N$ values, and N_2-fixing plants lower $\delta^{15}N$ values (i.e. closer to atmospheric N_2). The difference between these values forms the basis of the ^{15}N natural abundance technique for estimating fixed-N contributions to plants (Shearer & Kohl, 1986). However, attempting to trace fixed N through the ecosystems can be complicated by a myriad of fractionations, which are caused by numerous and often serial pathways of mineralization, nitrification, immobilization, and denitrification within the soil, plant root and mycorrhizal fungus mediation of soil N uptake (Lajtha & Schlesinger, 1986).

3.2.4 Forest Ecosystem Nutrient Cycling

Nutrient cycling processes in the soil are known to vary with plant community composition (Ehrenfeld et al., 2005). The processes associated with litter decomposition and enzymatic transformations of organic substrates in particular can vary because of differences in a variety of factors such as chemistry of the litter material, soil biota and soil chemistry associated with different plant species (Aerts, 1997). Both litter accumulation and stem flow can deliver N to epiphytes from various N pools (Bergstrom & Tweedie, 1998), making the abundance and branch position of lichens on a tree significant. Lichens may play an important role in nutrient cycling in forest ecosystems, but the relative impact of lichens compared to other ecosystem

components is not well known. The effect of lichens on the pattern of mineral cycling could range from major in ecosystems where lichens are abundant and contribute to the bulk of the biomass, to minor where lichens are only present in trace amounts (Pike, 1978).

3.2.5 Research objectives

Chapter 3 builds on the findings contained in Chapter 2 by examining the importance of the vertical distributions of epiphytic lichen species compositions and biomasses to the N contained in wet sub-boreal spruce and fir forest ecosystems of the central interior of BC. To do this, we measured the ^{15}N:^{14}N, %N, ^{13}C:^{12}C, and %C contents of host tree (conifer) foliage (needles), host tree epiphytic lichen functional groups and organic and mineral soil horizons under the tree crowns at sites containing variable amounts of cyanolichen tripartite (i.e. *L. pulmonaria)* and bipartite lichens from relatively low to high levels.

We hypothesized that sub-boreal forest trees with high cyanolichen abundance should have isotopically lighter N (i.e. more ^{14}N) and higher %N in foliage, indicative of greater inputs of biologically fixed-N into these systems. Among epiphytic lichens, cyanolichens should have a $\delta^{15}N$ closer to zero, whereas other non-N_2-fixing lichens (i.e. chlorolichens) should have a more negative $\delta^{15}N$ (Freyer, 1991). Knowledge obtained from this canopy study provides valuable information on the ecological function and biodiversity of epiphytic lichens on SBS forest ecosystems in central BC.

3.3 METHODS

3.3.1 Study Area

The study area was previously described in Chapter 2. In brief, this study was carried out on the north side of the Fraser River near the town of Upper Fraser, BC, located approximately 70 km NE of Prince George, BC. All study sites were characterized by having relatively cool and moist summers and cold, snowy winters. Four mature sub-boreal forest sites were chosen based on having trees with predominantly high (high sites) or low (low sites) epiphytic cyanolichen abundance and diversity, characterized in greater detail by Campbell et al. (2010b).

The two dominant tree species, and therefore the primary hosts for lichen epiphytes at all four sites, were interior hybrid spruce and subalpine fir. The two Fraser sites were selected because of a high cyanolichen abundance and diversity and were termed 'High' sites that spanned the SBS wk1 and the SBS vk subzones (Campbell et al. 2010b). These sites were located at an elevation of 680 m above sea level (a.s.l.) with a mean summer temperature of 11.8 ± 5.3 °C and relative humidity of 78% (Campbell et al. 2010b). The two Herrick road sites were determined to have low cyanolichen abundance and diversity and were termed 'Low' sites (Campbell et al., 2010b). These sites were located in the SBS vk at an elevation of 850 m a.s.l. with mean summer temperature and relative humidity of 10.8 ± 5.3 °C and 78%, respectively. Soils at all sites were Orthic Humo-Ferric Podzols formed from sandy-colluvial materials at the High sites and from sandy–skeletal glaciofluvial materials at the Low sites. Average precipitation in the ecotonal study area is approximately 897 mm per year (Campbell et al., 2010b).

3.3.2 Sample Trees

Sample tree selection was previously described in Chapter 2 with the exception of the fact that trees adjacent to Sitka alder (*Alnus viridis*) were excluded from the study given that annual N_2 fixation by Sitka alder can be substantial (Sanborn et al., 2002) and would potentially confound ^{15}N natural abundance interpretation. In brief, all study trees were in excess of 22 m in height and 20 cm in DBH. Needles and lichens were sampled from each canopy height zone at the highest accessible point of each of the 32 study trees.

3.3.3 Canopy Access

Canopy access was previously described in Chapter 2. In brief, access into canopies was achieved through a single rope technique (Denison, 1973; Perry & Williams, 1981). Selected trees were rigged, climbed and assessed vertically for epiphytic lichen biomass.

3.3.4 Epiphytic Lichen and Needle Sampling

Lichen and needle sampling was previously described in Chapter 2. In brief, epiphytic lichen and needle samples were collected over two summers: June to August 2008 and May to September 2009 at various heights throughout the tree canopy. One random sample was sent for isotopic analysis from the upper, middle and lower canopy height zones if they were present. Lichen biomass was separated into five categories: *Alectoria sarmentosa, Bryoria* species, foliose chlorolichen, bipartite cyanolichens, and *Lobaria pulmonaria,* the only tripartite cyanolichen at the site. The needle cohort was exclusively from the previous year's foliage to keep the age of the sampled needles constant.

3.3.5 Composite Soil Sampling

Soil samples were collected from the base of each sample tree between June and August 2009. We extracted soil cores using a soil auger (7.5 cm in diameter) by rotating the auger while applying downward force and lifting out the full blades. Soil core samples were extracted on a 1-m distance around each sample tree base from each of the cardinal directions. Samples were separated into three distinct layers: F – folic layer composed of organic matter rich in mycelia (~5 cm $\pm$ 2 cm deep), Ae – grayish surface soil layer (~2-10 cm thick) and Bf – yellowish brown to reddish brown subsoil layer (~10-20 cm). All layers were distinct and could be easily separated from each other; the soil auger was wiped down between cores. The three layers of the four subsamples were air dried on aluminum (Al) pie plates for two days at room temperature (~22-25 °C). Next, roots were removed from soil and samples were sieved through a 2-mm sieve. Then, F layer samples were separated twice more (0.85 mm and 0.3 mm sieves) to remove small pieces of woody debris before composite samples were prepared. Composite samples were also made from the four Ae and Bf subsamples from each site. A dry weight of 2 g from each of the three layers of the four samples was mixed into one composite sample per soil layer (8 g dry weight), for a total of 96 soil samples.

3.3.6 Stable Isotope Analyses

Sample preparation of lichen, needle and soil samples took place at the University of Northern British Columbia (UNBC) before samples were shipped to the Stable Isotope lab at the University of Saskatchewan (U of S) in Saskatoon, Canada for analysis. Lichen and needle samples were oven dried for three days at 55 °C in paper bags. Samples were ball-milled (Retsch MM301) to a particle size of less than 250 μm and inspected for fibrous matter or visible granules that could reduce precision of isotopic analysis. All samples were transferred, pressed

and encapsulated in 8 x 5-mm tin capsules (Catalog # D1008, Elemental Microanalysis Limited, Okehampton, UK) before combustion. Each capsule was tightly crimped so that it did not leak. The final shape of the wrapped sample was spherical or cylindrical. A list of sample weights (accurate to 0.01 mg) was used later for mass-specific calculations. Samples were stored in scintillation vials and weighed to an optimum weight of ~2 mg $\pm$ 0.2 mg. Ball chambers were cleaned with deionized water between samples, wiped with kimwipes, and air dried to remove all water droplets.

Soil, needle and lichen samples were analyzed using a Costech ECS4010 elemental analyzer (EA) coupled to a Delta V Advantage mass spectrometer with Continuous Flow (Conflo) IV interface. The continuous flow gas isotope ratio mass spectrometer was used to measure % N, % C and the relative abundance of isotopes (^{15}N and ^{13}C) in needle, lichen, and soil samples.

Isotope ratio analysis, ^{15}N:^{14}N and ^{13}C:^{12}C, of solid phase samples started with transformation to gas phase by extremely rapid and complete flash combustion of the sample material. Ionized combustion product was then mass-analyzed by means of differing mass:charge ratios among the various isotopic species of N_2 and carbon dioxide (CO_2). The $\delta^{15}N$ and $\delta^{13}C$ results were optimized for maximum accuracy by the application of a 'correction curve' to the results. However, if two different $\delta^{15}N$ results were available then the $\delta^{15}N$ result which had an N_2 beam area closest to 50 was used. Likewise, if two different $\delta^{13}C$ results were available then the $\delta^{13}C$ result which had a CO_2 beam area closest to 70 was used. When the stable isotopes react with the particle beam, a nuclear reaction occurs between the protons and the target atoms creating radioactive isotopes.

Samples were stored in a desiccator prior to analysis and then placed into a Zero Blank autosampler where atmospheric gases containing C and N were purged by ultra high purity helium (He) carrier gas. The He flow rate was set to 70 ml min^{-1}. The autosampler dropped samples into a chromium oxide (CrO) and silvered cobaltic oxide combustion reactor set at 1040 °C in the elemental analyzer. Two seconds prior to sample entry, a pulse of high purity oxygen (O_2) was released to the top of the combustion column. The combustion of the O_2 pulse and high temperature caused a flash combustion of the sample which raised the temperature to 1700 °C. The flash ensured complete sample combustion while the CrO ensured complete oxidation of C gases to CO_2. Nitrogen oxide gases produced were then converted to N_2 in a reduction reactor filled with copper at 650 °C. Finally, a water trap dried out the sample gas and a 3-m gas chromatography column separated N_2 from CO_2 before gases were sent to an open split in the Conflo III interface and then to the mass spectrometer. Isotope ratio calibrations were performed with IAEA-N1, IAEA-N2, IAEA-NO-3, IAEA-CH6, and USGS-24 standards (International Atomic Energy Agency, 1995). Standards were encapsulated and periodically placed within the run of samples; two within-run reference samples were placed between every five samples (Stocki, 2010).

3.3.7 Natural Abundance Method

The δ values for ^{15}N and ^{13}C were calculated as follows:

$$\delta\ ^{15}N\ [‰\ \text{relative to atmospheric}\ N_2] = (R_{sample}/R_{standard} - 1) \times 1000 \qquad \text{(Eq.1)}$$

$$\delta\ ^{13}C\ [‰\ \text{relative to PDB}] = (R_{sample}/R_{standard} - 1) \times 1000 \qquad \text{(Eq.2)}$$

The ^{15}N natural abundance method was used to infer the fractional contribution of biologically fixed N to any forest system component. This temporally integrated assessment is based on small but measurable differences in ^{15}N abundance between atmospheric N_2 and other sources of N. The value of 0.366 atom % ^{15}N was determined by Nier (1950) and established to be constant by the work of Junk and Svec (1958). Measurements of $\delta^{15}N$ provide time-averaged insights into the N cycle without disturbing the system.

3.3.8 Statistical analyses

Data were analyzed using Sigma Plot 11 (Systat Software Inc., San Jose, CA, USA). Ordinary least square (OLS) multiple linear regression analyses were performed to address the following research questions. Was there a statistically significant difference in $\delta^{15}N$ and $\delta^{13}C$ between the lichen functional groups and needles controlling for tree branch height, tree species, and lichen abundance? Was there a difference in $\delta^{15}N$ and $\delta^{13}C$ with tree branch height controlling for the lichen functional groups and needles? Was there a difference in $\delta^{15}N$ and $\delta^{13}C$ between High and Low lichen abundance sites controlling for the lichen functional groups, needles and soil? Was there a difference in $\delta^{15}N$ and $\delta^{13}C$ between tree species (Spruce and Fir) controlling for the lichen functional groups, needles and soil? Did %N in foliage (needles) increase at sites with higher abundances of cyanolichen? Six regression models were created to predict: i) $\delta^{15}N$ in lichen functional groups; ii) $\delta^{15}N$ in needles; iii) $\delta^{13}C$ in lichen functional groups; iv) $\delta^{13}C$ in needles; v) $\delta^{15}N$ in soil; and vi) $\delta^{13}C$ in soil. Correlation analyses were conducted between all the variables in each model to check for potential collinearity. Additional correlations were also conducted to look at the relationships between needle $\delta^{15}N$ and cyanolichen abundance, % N and cyanolichen abundance, and soil $\delta^{15}N$ and cyanolichen abundance.

Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine normality of data. Both tests showed lichen and needle $\delta^{15}N$ were not normally distributed ($KS = 0.191$, $p < 0.001$ and $W = 0.886$, $p < 0.001$). Mass distributions across all lichen functional groups also failed normality tests. In addition to violating the normality assumption all variables were equally heteroscedastic on the independent variable. Due to the bimodal distributions of the dependent variable, data transformations were not used. Both normality tests also showed that lichen and needle $\delta^{13}C$ were not normally distributed ($KS = 0.122$, $p < 0.001$ and $W = 0.949$, $p < 0.001$). No transformations could be used to fix this multimodal distribution. The distribution of lichen and needle $\delta^{15}N$ and $\delta^{13}C$ but not soil $\delta^{15}N$ and $\delta^{13}C$ showed signs of heteroscedasticity and non-linearity across all independent variables. Since the lichen functional group and needle data violated two of the three assumptions required by linear regression, the results of these models may be dubious. Soil $\delta^{15}N$ and $\delta^{13}C$ data were normally distributed.

All ordinary least square (OLS) linear regression outcomes show an unstandardized coefficient (B) which was the estimated change in the dependent variable associated with a one-unit change in the identified independent variable controlling for all other independent variables.

3.4 RESULTS

Nitrogen (N) contents varied by over 6-fold among lichen epiphytes sampled. At the extremes, *Alectoria sarmentosa* had the lowest % N (0.50 ± 0.13) and bipartite cyanolichens had the greatest % N (3.24 ± 0.52) on a dry weight basis (Fig. 3.1). *Bryoria* spp. and foliose chlorolichens had very similar %N contents of 0.77 ± 0.18 and 0.73 ± 0.19, respectively. Needles had a higher %N content (1.11 ± 0.14) than hair lichens and foliose chlorolichens but not as high as *L. pulmonaria* (2.26 ± 0.30). The differences in mean %N between functional

groups were significant ($F_{5,404} = 950.3$, $p < 0.001$: Tukey test) except for *Bryoria* spp. and foliose chlorolichens (Fig. 3.1).

All lichen functional groups or species and conifer foliage (needle) had mostly unique $\delta^{15}N$ signatures relative to their %N (Fig. 3.2). *Bryoria* spp. hair lichens at the top of the canopy had the lowest $\delta^{15}N$ while the hair lichen *Alectoria sarmentosa* found lower in the canopy had slightly lower N content and higher $\delta^{15}N$ than *Bryoria* spp. Host tree needles and foliose chlorolichens had $\delta^{15}N$ values that were intermediate between hair lichens and cyanolichens but similar N contents. While *L. pulmonaria* and bipartite cyanolichens both had $\delta^{15}N$ values close to atmospheric N_2 ($\delta^{15}N = 0$), *L. pulmonaria* had 1.5% less N on average (Fig. 3.2). Mean $\delta^{15}N$ isotope values were slightly higher at Low abundance sites for all lichen functional groups and host tree needles, but there was no significant difference in the $\delta^{15}N$ values or N contents of lichens or needles on trees with high (black symbols) versus low (white symbols) abundance of cyanolichens (Fig. 3.2).

When we compared $\delta^{15}N$ of living conifer foliage (needle) on each of the 32 study trees to their actual cyanolichen abundance, there was a small, but ultimately not significant ($r = -0.16$, $p = 0.121$) negative correlation between mean needle $\delta^{15}N$ and increasing cyanolichen abundance (Fig 3.3A). Host tree foliage %N showed a significant but weak positive correlation to site-level cyanolichen abundance ($r = 0.24$, $p = 0.019$; Fig 3.3B).

There were no issues with collinearity but there were some significant correlations between variables. Only weak to moderate relationships existed between independent variables so we proceeded with regression analyses.

When lichen species, tree branch height, and tree species were controlled for, cyanolichen abundance in the canopy was a statistically significant but ineffective predictor of

$\delta^{15}N$ ($B < 0.001$, $p < 0.001$, Table 3.1). The regression coefficients of the dependent variable $\delta^{15}N$ are displayed in Table 3.1 and suggest that lichen species and cyanolichen abundance but not tree species or tree branch height were significant predictors of $\delta^{15}N$ in lichen functional groups. The regression analysis showed that *A. sarmentosa* ($B = -3.657$, $p < 0.001$), *Bryoria* spp. ($B = -4.838$, $p < 0.001$) and foliose chlorolichens ($B = -0.482$, $p = 0.002$) were negatively related to $\delta^{15}N$ levels in lichen functional groups. Conversely, bipartite cyanolichens ($B = 0.561$, $p < 0.001$) and *L. pulmonaria* ($B = 1.608$, $p < 0.001$) were positively related to $\delta^{15}N$ levels in lichen functional groups. Both tree species ($B = -0.166$, $p = 0.095$) and tree branch height ($B = 0.010$, $p = 0.238$) were not significantly related to $\delta^{15}N$ levels in lichen functional groups (Table 3.1). However, tree branch height was a significant predictor of $\delta^{15}N$ in needles ($B = 0.028$, $p = 0.002$) but both tree species ($B = -0.146$, $p = 0.267$) and cyanolichen abundance ($B < 0.001$, $p = 0.061$) were not significantly related to $\delta^{15}N$ levels in needles.

Mean soil horizon $\delta^{15}N$ values were consistently higher at Low (means: F = 1.340, Ae = 5.130, Bf = 6.262) than at High (F = 0.782, Ae = 4.765, Bf = 5.383) cyanolichen abundance sites and soil $\delta^{15}N$ decreased with increasing cyanolichen abundance in soil F, Ae and Bf horizons (Fig. 3.4A-C), respectively. The abundances of cyanolichens and $\delta^{15}N$ were negatively correlated in all three soil horizons, with correlations being nearly significant for the uppermost horizons (F: $r = -0.341$, $p = 0.056$; Ae: $r = -0.263$, $p = 0.146$) and significant for the Bf horizon ($r = -0.356$, $p = 0.045$). Mean soil horizon %N was also greater at Low than at High Cyano abundance sites (Fig. 3.4D-F) and the uppermost soil horizon had the highest %N (Fig. 3.4D).

Cyanolichen abundance, tree species and soil horizon were all significant predictors and all contributed to the overall relationship of both soil $\delta^{15}N$ (Table 3.3) and soil $\delta^{13}C$ (Table 3.4). The regression analysis showed that there was a statistically significant difference in both $\delta^{15}N$

(B = -0.001, $p < 0.001$: Table 3.3) and $\delta^{13}C$ (B = 0.000, $p < 0.001$: Table 3.4) between High and Low cyanolichen abundance sites. There was also a statistically significant difference in soil $\delta^{15}N$ (B = -0.525, $p < 0.001$, Table 3.3) and soil $\delta^{13}C$ (B = -0.221, $p = 0.009$, Table 3.4) between host tree species (spruce and fir).

When controlling for all independent variables, tree branch height was a significant predictor of $\delta^{13}C$ for lichen functional groups and needles (Fig. 3.5A, Table 3.2). There was evident clumping of $\delta^{13}C$ into three distinct groups. Specifically, $\delta^{13}C$ values of the lone tripartite cyanolichen (*L. pulmonaria*) were lower than host tree needles which were less than hair lichens (*A. sarmentosa* and *Bryoria* spp.), bipartite cyanolichens and foliose chlorolichens. Tree branch height was not a significant predictor of $\delta^{15}N$ for lichen functional groups but was a significant predictor of $\delta^{15}N$ for needles (Fig. 3.5B, Table 3.1). Nevertheless, hair lichens *A. sarmentosa* and *Bryoria* spp. had uniformly negative $\delta^{15}N$ values that were also more negative than all other functional groups.

A plot of $\delta^{15}N$ versus $\delta^{13}C$ resulted in a visual separation of most biomass types (Fig. 3.6). Host tree foliage was intermediate to *L. pulmonaria* (high ^{15}N and low ^{13}C) and all other lichen functional groups. *Lobaria pulmonaria* had the lowest $\delta^{13}C$ and the highest $\delta^{15}N$ values. Bipartite cyanolichens and foliose chlorolichens had similar ranges in both $\delta^{15}N$ and $\delta^{13}C$. Hair lichens *A. sarmentosa* and *Bryoria* spp. had a similar range of $\delta^{13}C$ values as both bipartite cyanolichens and foliose chlorolichens, but $\delta^{15}N$ values that were more negative (from -3 to -12 ‰) than all other biomass types analyzed.

Lobaria pulmonaria had a uniquely distinctive $\delta^{13}C$ values (-32 and -36 ‰) relative to other lichen groups/species (-22 and -27 ‰) despite relatively consistent carbon contents (Fig. 3.6). Host tree needles had $\delta^{13}C$ values intermediate to *L. pulmonaria* and all other lichen

functional groups (Fig. 3.6), but at distinctly higher C contents than all fungi (Fig. 3.7). Lichen species, cyanolichen abundance, tree branch height and the interaction between cyanolichens and site cyanolichen abundance (but not tree species) were significant predictors of $\delta^{13}C$ in lichen functional groups. The regression analyses showed that *A. sarmentosa* ($B = 4.899$, $p < 0.001$), *Bryoria* spp. ($B = 4.513$, $p < 0.001$), foliose chlorolichens ($B = 3.969$, $p < 0.001$), and bipartite cyanolichens ($B = 4.324$, $p < 0.001$) were positively related to $\delta^{13}C$ levels in lichen functional groups (Table 3.2). Conversely, *L. pulmonaria* ($B = -4.358$, $p < 0.001$) was negatively related to $\delta^{13}C$ levels in lichen functional groups. Both cyanolichen abundance ($B = -0.001$, $p < 0.001$) and tree branch height ($B = 0.093$, $p < 0.001$) were significantly related to $\delta^{13}C$ levels in lichen functional groups. There was also a statistically significant interaction between cyanolichens and site cyanolichen abundance and $\delta^{13}C$ increased slightly as the site cyanolichen abundance increased ($B = 0.001$, $p = 0.005$, Table 3.2). There was no significant difference in $\delta^{13}C$ between sample tree species spruce and fir in lichen functional groups ($B = 0.048$, $p = 0.594$, Table 3.2). Alternatively, both tree species ($B = 0.622$, $p < 0.001$) and tree branch height ($B = 0.148$, $p < 0.001$), but not cyanolichen abundance ($B < 0.001$, $p = 0.942$), were significant predictors of $\delta^{13}C$ in needles ($B = 0.028$, $p = 0.002$).

3.5 DISCUSSION

Nitrogen-fixing lichen epiphytes can be abundant in conifer forests of central BC and are conjectured to be an important component of the N-cycle in these forest locations (Campbell & Fredeen 2004, 2007; Campbell et al., 2010a). Although lichen N pool sizes and decomposition rates in these studies were indicative of enhanced mineral N flux rates as observed in other forests types (e.g. Knops et al., 1997)), direct links between cyanolichen abundance and improved host tree N status have been elusive. In our study, gradients in cyanolichen abundance

across host trees (interior spruce and subalpine fir) in two generally high cyanolichen abundance sites and two generally low cyanolichen abundance sites (Campbell et al., 2010b) provided us with an opportunity to examine the potential for enhanced cyanolichen N-inputs into these forests in central BC. We hypothesized that foliar (needle) N contents would be positively correlated with cyanolichen abundance, and in fact this was found to be the case (Fig. 3.3b). We further sought to more directly link increased host tree foliar N with cyanolichen N through the unique ^{15}N:^{14}N stable isotope ratios of cyanolichen N.

Our results corroborate previous findings of $\delta^{15}N$ values in lichen functional groups and or species. It is already known that cyanobacterium-containing lichens (cyanolichens) that fix N_2 typically have $\delta^{15}N$ values close to atmospheric N_2, i.e. 0‰ (Macko et al., 1987; Virginia & Delwiche, 1982). This was confirmed for both bipartite and tripartite (*L. pulmonaria)* cyanolichens in our study. By contrast, foliage, hair and chlorolichen epiphytes all had $\delta^{15}N$ values that were negative and less enriched in ^{15}N than cyanolichens. Hair lichens had the lowest $\delta^{15}N$ values, while chlorolichens and host tree foliage had intermediate $\delta^{15}N$ values between cyanolichen and hair lichens. Interestingly, *A. sarmentosa*, occurring lower in the canopy than *Bryoria*, and therefore overlapping with cyanolichen canopy zones (see Chapter 2), had $\delta^{15}N$ closer to atmospheric $\delta^{15}N$ (0) (Fig. 3.2). This is consistent with *Alectoria* receiving more leached-N from cyanolichens than *Bryoria*. This was supported by the fact that in High sites, a notable increase in *Alectoria* $\delta^{15}N$ was observed, while relatively little change in $\delta^{15}N$ was observed in *Bryoria* with increased cyanolichen abundance. In both hair lichens, relatively low N contents and low $\delta^{15}N$ (~ -6 ‰ in Low Cyano sites) were consistent with low atmospheric N inputs in central BC (e.g. Hope, 2001) and the negative $\delta^{15}N$ of precipitation N measured in other studies (Freyer, 1991).

Although biological N_2-fixation may be a significant N input into ecosystems, it can be difficult to identify since it lacks a unique $\delta^{15}N$ signature over and above that of background $\delta^{15}N$ values due to its relative isotopic effect (Vitousek et al., 1989). Nitrogen isotope signatures of epiphytes varied with canopy position in our study, but the reason for lichen $\delta^{15}N$ variability in most studies remains unexplained. The $\delta^{15}N$ of hair lichens, with single sources of N (i.e. atmospheric fixed-N), may be relatively easy to define, in contrast to other lichens (cyanolichen and chlorolichen) lower in the canopy. At least 10 processes have been identified that can alter $\delta^{15}N$ values, none of which can currently be separated out in field studies (Robinson, 2001). A few explanations for $\delta^{15}N$ variability are: i) the preference for the lighter ^{14}N isotope in uptake may lead to $\delta^{15}N$ enrichment, ii) fractionation of N isotopes in gaseous phase ammonia is greater than in the liquid phase of nitrate, and iii) transfer of organic N could result in increased ^{15}N depletion of the photobiont and less depletion in the mycobiont (Hobbie & Hobbie, 2008). Variations of $\delta^{15}N$ values are also attributed in part to altered ^{15}N discrimination during N acquisition and to changes in partitioning of N isotopes (Wania et al., 2002). In general, our results were in agreement with previous work showing that lichens with a green alga as their photobiont (including all lichens but bipartite cyanolichens) also showed the greatest ^{15}N depletion (Riera, 2005) (see Fig. 3.1).

Soil $\delta^{15}N$ values increased with soil depth in our study, consistent with results of Gebauer and Schulze (1991). In keeping with our expectations, we also observed decreasing trends in $\delta^{15}N$ with increasing cyanolichen abundance in all soil horizons (Fig. 3.4A-C) equating to less positive soil horizon $\delta^{15}N$ values, more proximal to the $\delta^{15}N$ of cyanolichen biomasses which were uniformly close to zero. There are many reasons why soil $\delta^{15}N$ wouldn't necessarily be closely correlated with tree $\delta^{15}N$. First, measured $\delta^{15}N$ of soil pools may not represent the true

isotopic composition of N available to plant roots, since most of the N in soils is bound in forms that are not immediately available to plants (Jansson, 1958; Binkley & Hart, 1989). Second, only a few % of soil total N becomes available in a year (Högberg, 1997), and symbiotic fungi can alter the $\delta^{15}N$ of the N transferred from soil to host plant. Nevertheless, a similar downward trend in tree foliage $\delta^{15}N$ at our sites with increasing abundance of cyanolichen (Fig. 3) was consistent with the downward trend in soil $\delta^{15}N$ (Fig. 3.4A-C). Explaining the increased %N in host tree foliage with increased cyanolichen abundance is more difficult to reconcile with total soil N. If greater cyanolichen inputs of N increased soil N concentrations, then a straightforward mass action of greater plant N uptake could explain enhanced foliar %N. However, soil N decreased in all soil horizons, though non-significantly, with higher cyanolichen abundance (Fig. 3.4D-F). Since total soil N does not in any way equate with amounts and forms of available inorganic N, it is possible that more readily available N fractions were more available at high abundance cyanolichen sites, even though total %N was not. Further work on soil N at these sites would be required to test this hypothesis.

Foliar (needle) $\delta^{15}N$ values in our host trees ranged between 0.6 and -2.8 ‰ and did not differ significantly between High and Low lichen abundance sites or tree branch height. However, there was a trend of decreasing foliar $\delta^{15}N$ with increasing cyanolichen abundance (Fig. 3.3A). Gebauer and Schulze (1991) reported much lower $\delta^{15}N$ values of conifer needles (between -2.5 and -4.1‰) which varied according to stand and age, with foliage from the healthiest site having the lowest $\delta^{15}N$. It is unclear why $\delta^{15}N$ values observed by Gebauer & Schulze were more negative than ours, but one possibility would be the presumed greater atmospheric N inputs in these forests when compared to the N-limited forests of central BC. Gebauer and Schulze (1991) also reported a similar trend of $\delta^{13}C$ values of needles, which

ranged between -26.2 and -32.2 ‰ and did not differ between lichen abundance sites but did change with canopy height. Similar to our study, needle $\delta^{13}C$ values were not significantly different between abundance sites (Table 3.2), were more negative in the lower canopy and increased with tree height (Fig 3.5).

Lichen $\delta^{13}C$ values were previously found to vary widely over a large range of habitats and species (Batts et al., 2004). Our lichen functional groups and tree foliage (needles) fell into three distinct groups with respect to $\delta^{13}C$ values: the tripartite cyanolichen *L. pulmonaria* with the lowest $\delta^{13}C$ values (-36 to -31), host tree foliage with intermediate $\delta^{13}C$ values (-33 to -26), and all other lichens including bipartite cyanolichens, hair lichens and chlorolichens with the least negative $\delta^{13}C$ values (-28 to -23). Our values are entirely consistent with those of Maguas et al. (1995), namely that tripartite lichen associations typically have the lowest and bipartite lichens the highest $\delta^{13}C$. Carbon isotope discrimination in lichens (Riera, 2005) has been attributed to the species of photobiont present.

Interestingly, we observed positive relationships between tree branch height and $\delta^{13}C$ values for both *L. pulmonaria* and foliage. Possible causes that may be responsible for the observed height-specific differences in these $\delta^{13}C$ values are CO_2 source, light level and factors influencing diffusion resistance such as water availability. Carbon dioxide source influences the $\delta^{13}C$ value of lichens (Farquhar & Ehleringer, 1989; Lakatos et al., 2007) since lichens growing in the canopy or close to the soil will more readily assimilate CO_2 from plant/atmosphere and soil respiration, respectively (Broadmeadow et al., 1992; Máguas et al., 1993; Máguas et al., 1995). Light levels influence photosynthesis and can alter the CO_2 gradient inside the lichen thallus. Inorganic carbon acquisition in lichens is accomplished by the photobiont and depends

on moisture and light availability (Palmqvist, 2000). Lange et al. (1988) found that water content influences $\delta^{13}C$ values. Increased $\delta^{13}C$ values have been reported for lichens in drier habitats (Batts et al., 2004) and more positive $\delta^{13}C$ values in epiphytes on thin versus thick branches are generally used as indicators for desiccation stress (Hietz et al., 2002). Water stress would be expected to increase with branch height. Therefore, differences in $\delta^{13}C$ likely exist due to different microclimatic conditions in the canopy and/or the uptake of isotopically different CO_2 sources.

The major limitation of our study was that the lichen abundance data violated the basic assumptions of the model due to methodological flaws in estimating the abundance of lichen on tree branches (see Chapter 2). Due to this methodological estimation flaw, we could not reliably assess that any significant relationship was present between any of the dependent and independent variables as it pertains to lichen abundance. Although our model was inconclusive, our results do suggest that cyanolichen abundance was a significant predictor of $\delta^{15}N$ in lichen functional groups. There were significant findings using the soil isotope data which suggest that further exploration with lichen data is warranted. The sample size of 32 trees in this study only provided enough power to detect large main effects. Increasing sample size of trees, though not feasible in this study, would have provided greater power to detect moderate to small effects.

3.6 LITERATURE CITED

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3.7 TABLES

Table 3.1 Linear regression (OLS) coefficients with standard error for the regression model predicting $\delta^{15}N$ in lichen functional groups ($F = 220.501$, $p < 0.001$, $R^2 = 0.817$), and needles ($F = 4.363$, $p = 0.006$, $R^2 = 0.126$) based on tree species (spruce and fir), cyanolichen abundance, tree branch height, and lichen species.

	Lichen Functional Groups (Model i)		Needles (Model ii)	
	Unstandardized coefficient (B)	Std Error	Unstandardized coefficient (B)	Std Error
Intercept	-1.126***	0.165	-1.499***	0.171
Tree species (Spruce)	-0.166	0.099	-0.146	0.131
Cyanolichen abundance	<0.001***	<0.001	<0.001	<0.001
Tree branch height	0.010	0.008	0.028**	0.009
Alectoria sarmentosa	-3.657***	0.168		
Bryoria spp.	-4.838***	0.183		
Foliose chlorolichen	-0.482**	0.156		
Bipartite cyanolichen	0.561***	0.137		
Lobaria pulmonaria	1.608***	0.165		

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

Table 3.2 Linear regression (OLS) coefficients with standard error for the regression model predicting $\delta^{13}C$ in lichen functional groups ($F = 711.517$, $p < 0.001$, $R^2 = 0.942$) and needles ($F = 79.545$, $p < 0.001$, $R^2 = 0.724$) based on tree species (spruce), cyanolichen abundance, tree branch height, the interaction between cyanolichens and site cyanolichen abundance and lichen species.

	Lichen Functional Groups (Model iii)		Needles (Model iv)	
	Unstandardized coefficient (B)	Std Error	Unstandardized coefficient (B)	Std Error
Intercept	-30.523***	0.154	-31.868***	0.204
Tree species (Spruce)	0.048	0.089	0.622***	0.156
Cyanolichen abundance	-0.001***	<0.001	<0.001	<0.001
Tree branch height	0.093***	0.008	0.148***	0.011
Cyanolichen X Abundance interaction	0.001*	<0.001		
Alectoria sarmentosa	4.899***	0.151		
Bryoria spp.	4.513***	0.164		
Foliose chlorolichen	3.969***	0.140		
Bipartite cyanolichen	4.324***	0.166		
Lobaria pulmonaria	-4.358***	0.149		

***p < 0.001, **p < 0.01, *p < 0.05

Table 3.3 Linear regression (OLS) coefficients with standard error for the regression model (v) predicting $\delta^{15}N$ in soil based on cyanolichen abundance, tree species, and soil horizon ($F = 314.044$, $p < 0.001$, $R^2 = 0.932$).

	Unstandardized coefficient (B)	Std Error
Intercept	1.586***	0.14
Cyanolichen abundance	-0.001***	0.000
Tree species	-0.525***	0.123
Mineral surface soil (Ae)	3.887***	0.145
Mineral subsoil (Bf)	4.777***	0.145

***p < 0.001, **p < 0.01, *p < 0.05

Table 3.4 Linear regression (OLS) coefficients with standard error for the regression model (vi) predicting $\delta^{13}C$ in soil based on cyanolichen abundance, tree species, and soil horizon ($F = 117.897$, $p < 0.001$, $R^2 = 0.838$).

	Unstandardized coefficient (B)	Std Error
Intercept	-26.923***	0.094
Cyanolichen abundance	0.000***	0.000
Tree species	-0.221**	0.062
Mineral surface soil (Ae)	1.523***	0.097
Mineral subsoil (Bf)	1.961***	0.097

***p < 0.001, **p < 0.01, *p < 0.05

3.8 FIGURES

Figure 3.1 Mean percentage of nitrogen (%N of lichen biomass ± SD) in functional groups of lichen and foliage (needle) from 32 fir and spruce study trees at four study sites with varying amounts of cyanolichen abundance. Different letters above error bars indicate significant ($p < 0.001$) differences between lichen functional groups (Tukey multiple mean comparison test).

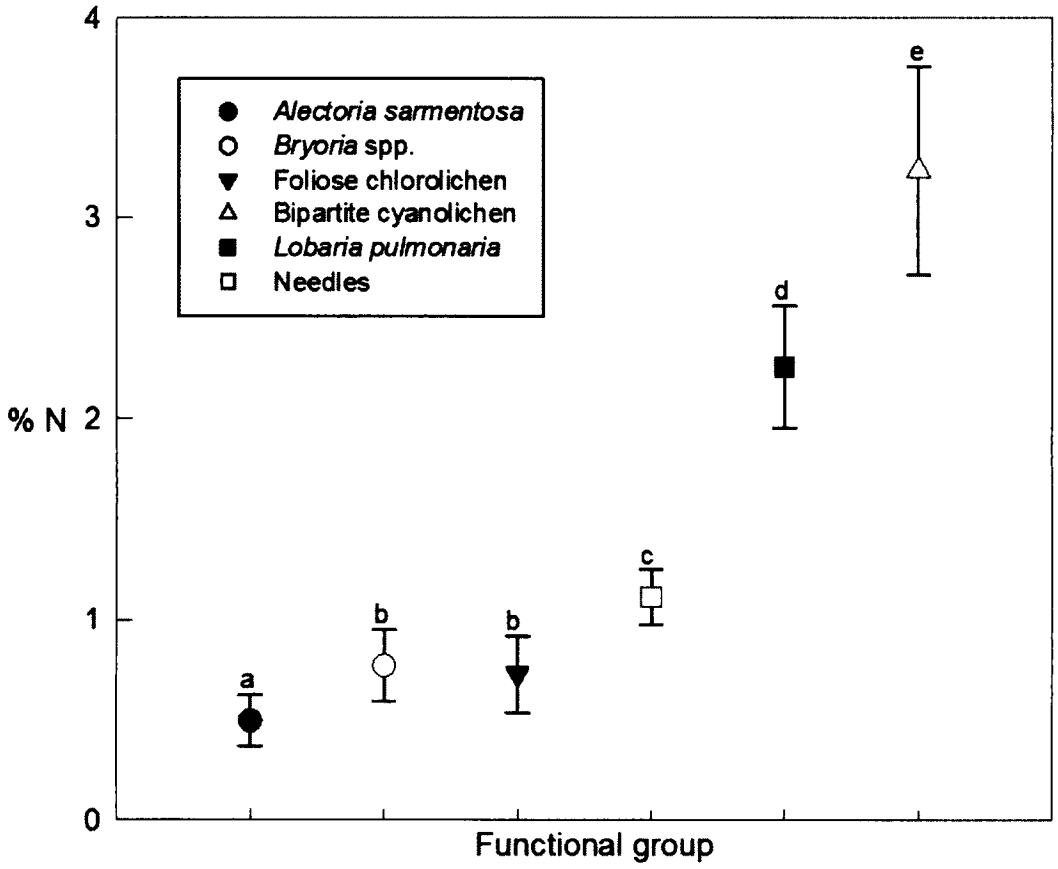


Figure 3.2 Mean (±SD) N concentrations and $\delta^{15}N$ signatures of five epiphytic lichen biomasses and the host tree foliage (needle) biomasses across four sites and two host tree species from sub-boreal spruce and fir forests in central interior BC. Half of the sites had relatively high cyanolichen abundances (black symbols) and the other half had low cyanolichen abundances (white symbols).

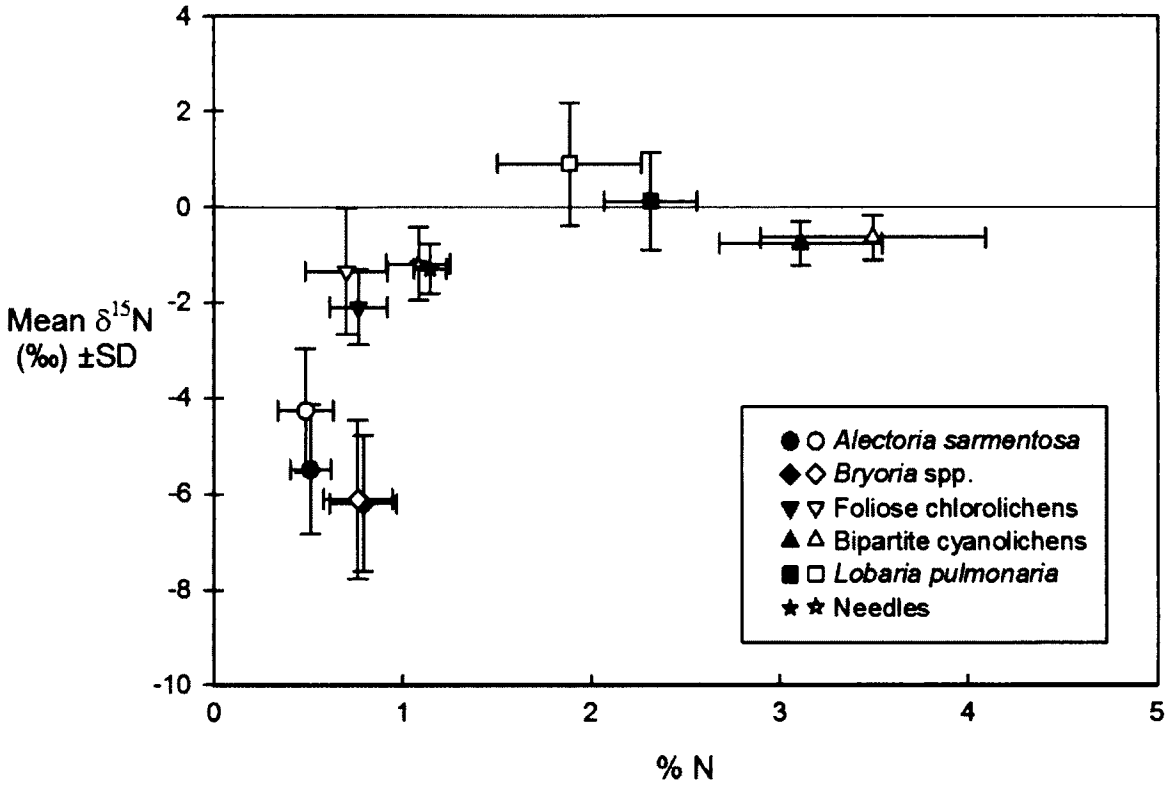


Figure 3.3 Mean (A) $\delta^{15}N$ and (B) %N of host tree foliage (needle) from lowest to highest cyanolichen abundance (total g tree $^{-1}$).

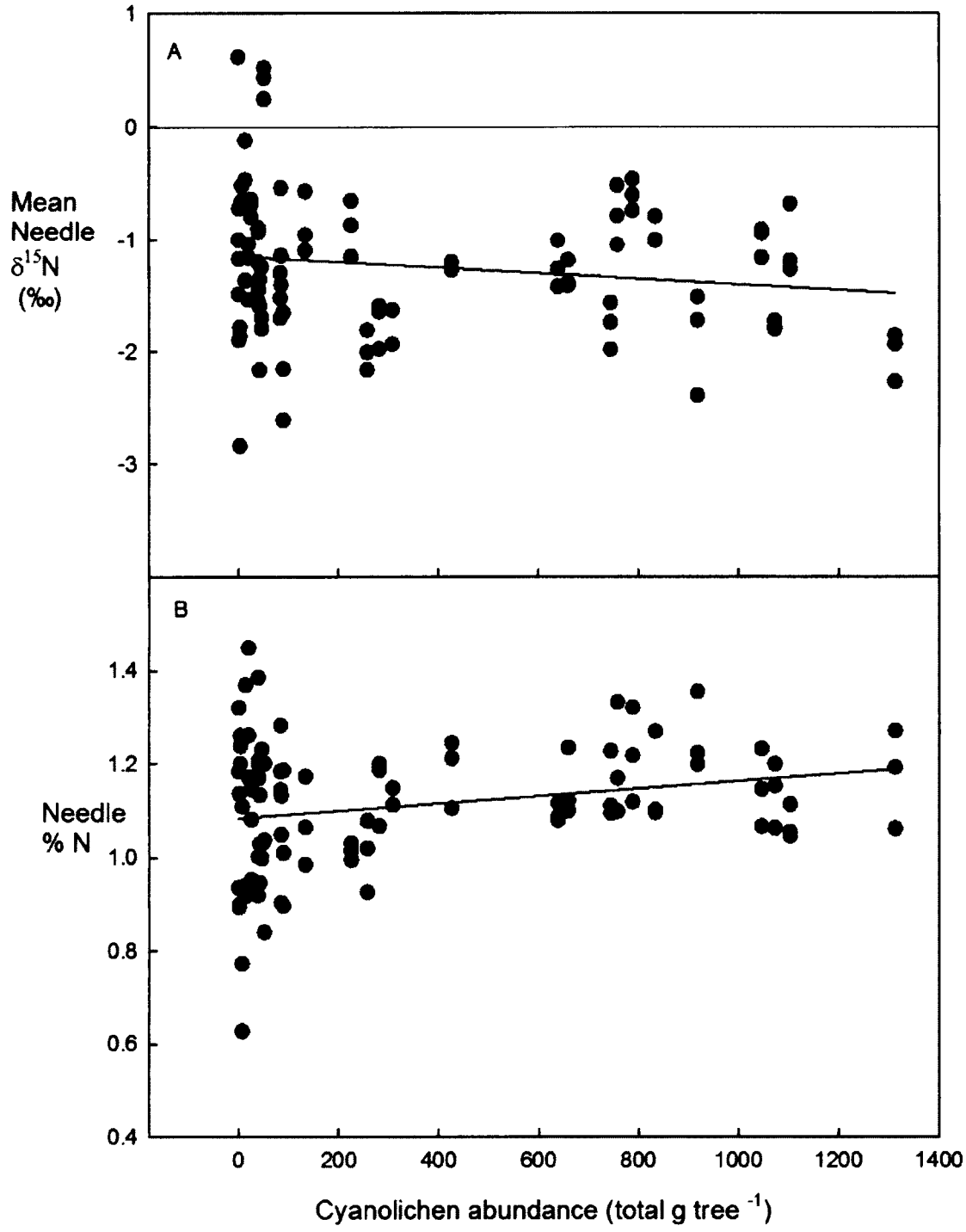


Figure 3.4 Mean $\delta^{15}N$ of each soil horizon, surface organic layer (F), upper most mineral horizon (Ae) and next mineral horizon (Bf) at sites with lowest to highest cyanolichen abundance (total g tree $^{-1}$).

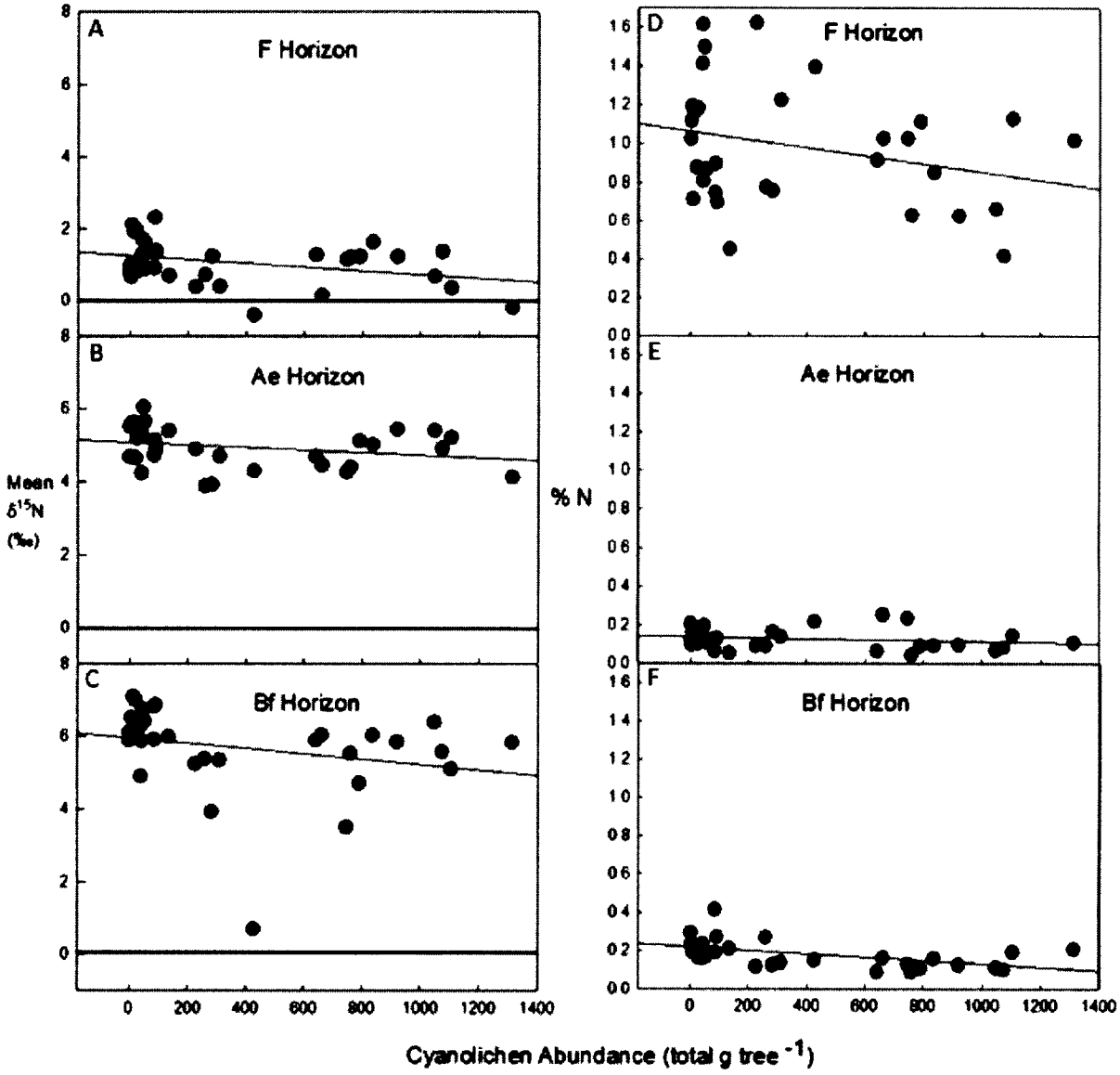


Figure 3.5 Variation in the natural abundances of (A) $\delta^{13}C$ and (B) $\delta^{15}N$ of five epiphytic lichen biomasses and the host tree foliage (needle) biomasses according to tree branch height above-ground-level for all sites.

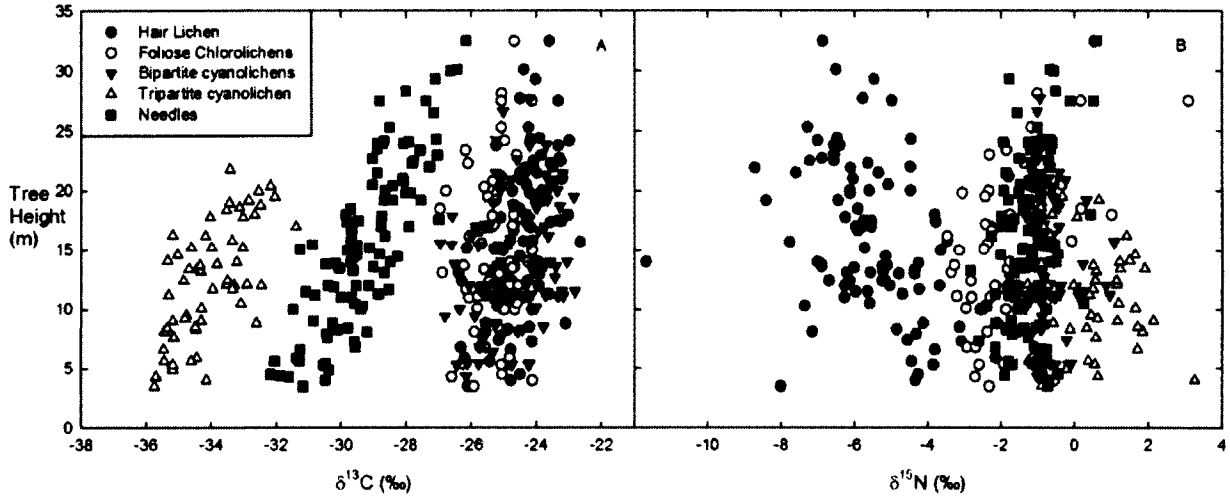


Figure 3.6 Dual natural abundance isotope plot of $\delta^{15}N$ and $\delta^{13}C$ of the five epiphytic lichen biomasses and host tree foliage (needle) biomasses across four sites and two host tree species.

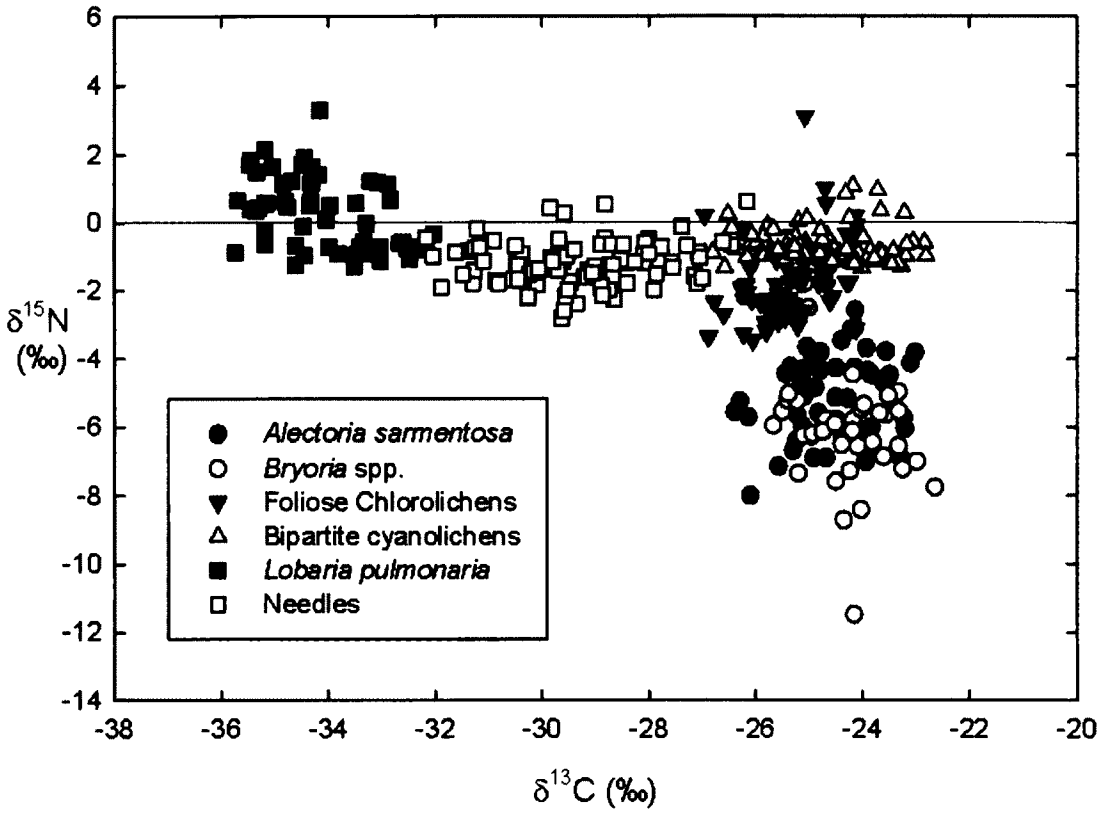
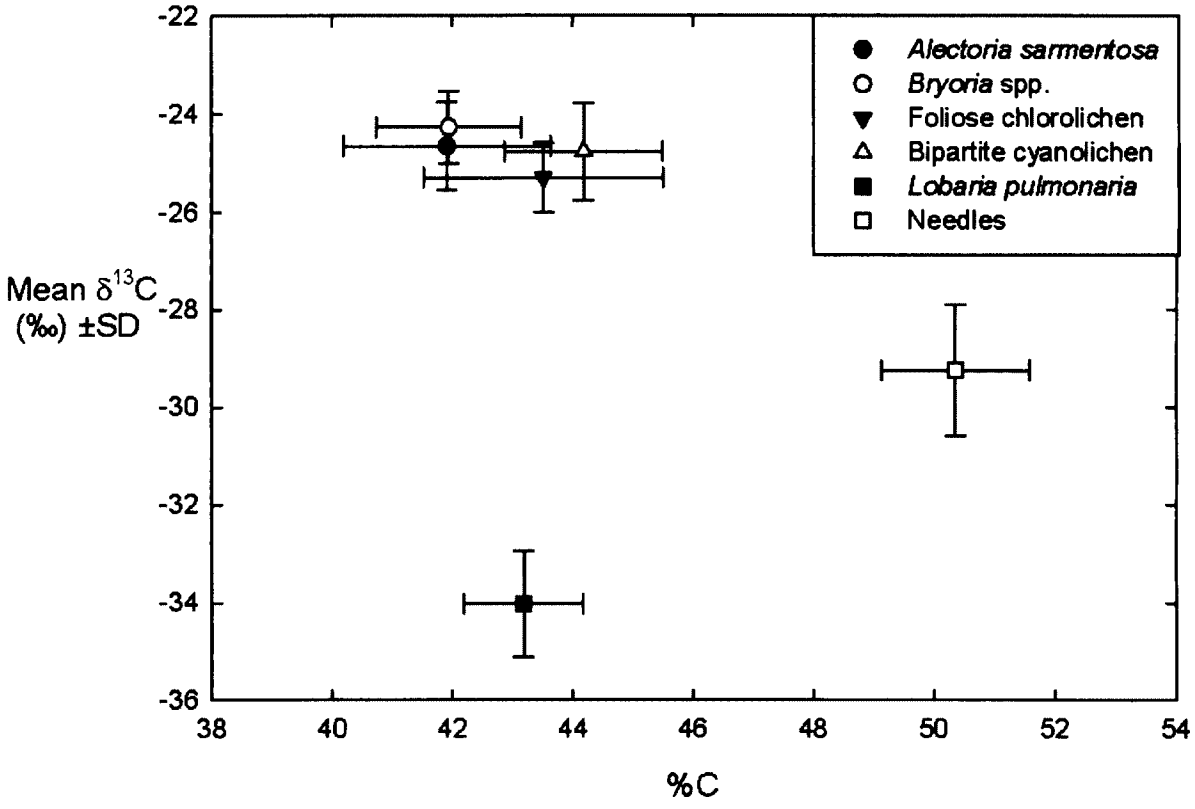


Figure 3.7 Mean (±SD) C concentrations and $\delta^{13}C$ signals of five epiphytic lichen biomasses and host tree foliage (needle) biomasses across four sites and two host tree species.



Chapter 4: Conclusions

This research provides new insights into how epiphytic lichen species and functional groups are distributed within canopies of sub-boreal spruce- and fir-dominated forests of central BC where cyanolichen epiphytes are found at high or low amounts. In forest sites where cyanolichens were in low abundance, typified by the absence of *L. pulmonaria* in its normal lower-canopy positions, a flourishing canopy zone takeover was observed by another lichen species, a foliose chlorolichen, *Platismatia glauca*. When cyanolichens including *L. pulmonaria* were absent, the reordering of lichen communities in the bottom part of the forest canopy had an effect on N contained in total epiphytic lichen biomass. Epiphytic lichen N was more than three times as great in High Cyano sites as Low Cyano sites. Lichen biomass estimates of the five lichen functional groups had distributions of growth that indicated that these epiphytes are non-uniformly distributed across the canopy. Fir trees had higher lichen loading than spruce trees making the former tree species more favorable for colonization than the latter species. Finally, the tree height below which half of the total lichen biomass was found was the same as the height of peak lichen biomass across all lichen species.

Vertical distribution and quantities of lichens emphasize the importance of biodiversity conservation in SBS forest systems. Lichens are ecologically important as food for herbivores, shelter, nesting materials for a variety of animals and they provide substrate and microhabitats for other species (Ozturk et al., 2010). Epiphytic cyanolichens play an important role in regulating the link between nutrient pathways, thus determining where N_2-fixing cyanolichens are present in the forest canopy contributes to understanding where biomass is being added to the system. The vertical distribution of lichens within forest stands also enhances an understanding

of ecosystem function, nutrient cycling and changes in microclimatic conditions in the forest ecosystem (Ellis, 2012).

The use of natural abundance of N stable isotopes in this sub-boreal canopy study has shown that the abundance of cyanolichens in the canopy can be a useful ecological indicator in determining $\delta^{15}N$ of lichen functional groups and that tree branch height is valuable in determining the $\delta^{15}N$ of needles. $\delta^{13}C$ of lichen functional groups can be delineated by site cyanolichen abundance, tree branch height, or the interaction between cyanolichens and site cyanolichen abundance. Both tree species and tree branch height could be used to determine the $\delta^{13}C$ value of needles. Cyanolichen abundance, tree species and soil horizon were all significant predictors of both soil $\delta^{15}N$ and $\delta^{13}C$. Mineral subsoil was the only soil horizon that showed a significant decrease in $\delta^{15}N$ with an increase in site-level cyanolichen abundance. Stable isotope techniques have become important tools for ecophysiology and ecosystem research (Högberg, 1997; Dawson et al., 2002) but have rarely been applied to lichen research. Determining explicit values of stable isotopes is important for establishing unusual N isotopic ratios (Fogel et al., 2008) of epiphytic lichens. Stable isotope abundances of lichens can be used for monitoring environmental quality and change (Batts et al., 2004).

Future research should focus on improving the estimation method and investigate these relationships with a larger sample size. Using the stable isotope approach is advantageous in showing how components of ecosystems are connected and which processes are most sensitive to ecosystem-level changes such as clear cutting, fertilization or burning (Peterson & Fry, 1987). It could be beneficial to look at isotope abundances in disturbed locations to determine if stable isotope ratios change drastically in lichen, soil, and needles surrounding clear-cut forest sites or sites affected by atmospheric pollutants.

Despite the significant limitations of this study, results suggest epiphytic lichens, particularly those that can fix atmospheric N_2, may provide important N-inputs into northern interior forests of BC. Nitrogen is the most limiting nutrient for these forest ecosystems, and the sustainability of current forest communities may depend on the conservation of N_2-fixing epiphytic lichens, which are generally most abundant in mature and old growth stands. Sustainability of social communities may also depend on the conservation of epiphytic lichens relative to forestry. Study results could influence logging areas in the central interior of northern BC. Composition of epiphytic lichens should be considered in forest management practices to maintain greater species abundance and richness in the forest system in order to increase diversity.

Cyanolichens could also be introduced to nitrogen-deficient forests. Management of forests and lichen biodiversity influence the storage of N in sub-boreal landscapes. Disturbances to these sub-boreal forests can have an impact on forest diversity and function and should be monitored closely. Shorter lived and statured subalpine fir supports larger biomasses of all species relative to the canopy-dominant interior hybrid spruce. The mixture of spruce and fir may provide for greater lichen epiphyte abundances as well as cyanolichen N-inputs into these sub-boreal forests of central BC.

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