University of Alberta

The role of feathermosses in N cycling in lodgepole pine forests

by

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Dedicated to Andrei, my inspiration to begin with,
help and support to carry me through
and nagging power to get me to the finish.

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Conifers and feathermosses: the ecology of the forest

The importance of feathermosses in forest ecology is widely recognized despite the small size and apparent inconspicuousness of the individual moss plants. The reason for this is that under the canopy of conifers, feathermosses often form continuous carpets that cover the entire area. Feathermoss carpets in Alberta coniferous forests are dominated mostly by *Pleurozium schreberi* and/or *Hylocomium splendens*, with frequent inclusions of *Ptilium crista-castrensis* and occasional *Polytrichum commune* and *Dicranium spp.* The total biomass of the continuous feathermoss under the conifer canopy may reach 5 000-10 000 kg/ha (Weetman and Timmer, 1967), and the total photosynthetic potential of the feathermoss of the site is comparable with that of the trees (Martin and Adamson, 2001). In order to support this rate of photosynthesis, feathermosses requires almost as much nutrients, especially N, as trees do (Bates, 1992). As the feathermoss layer covers almost the entire forest floor, it has ready access to nutrient inputs from rainfall or litter fall. The feathermoss layer intercepts most of the nutrients and is capable of fast assimilation of decomposition products and retains the nutrients for extended periods of time (Bates, 1992; Eckstein, 2000).

As a stratum in conifer forest, feathermoss layer plays a complex role in the nutrient cycling. It accumulates nutrients, harbors decomposing and N-fixating microflora, regulates soil temperature and moisture content, and consequently the rate of microbial activity in the mineral soil, and controls the flow of nutrients to the tree roots. In this thesis I am trying to measure the rate and efficiency of nutrient transfer through the continuous feathermoss layer and the effect of the moss on overall productivity in the ecosystem.

Feathermoss: biology and growth requirements

Feathermosses fall in the division of Bryophyta. Mosses do not have a vascular system and lack roots. True mosses (class Bryopsida) are a large and diverse group of plants that usually have small, branching filaments, rhizoids, and reproductive organs that release spores. They mostly grow on tree branches, rocks, and bare soil, but may also grow in freshwater streams or even in deserts. Feathermosses are pleurocarpous (carpet forming) true mosses (Chapman and Sanborn, 1941; Grout, 1932). They are relatively large (10-15 cm), robust mosses occurring in spreading, loose patches. After establishing a continuous carpet, feathermosses continue to grow apically, while the lowermost parts slowly die, presumably from the absence of light, and decompose. After a few years of moss layer development, feathermosses have no rhizoids and no direct capillary contact with the mineral soil and absorb all nutrients and water through the plant surface.

In Alberta, the feathermosses *Pleurozium schreberi* or *Hylocomium splendens* occupy a wide range of sites from poorly drained outwash plains to convex slopes and hill crests. Feathermosses are often associated with bog blueberry (*Vaccinium uliginosum*), labrador-tea (*Ledum groenlandium*), and mountain cranberry (*Vaccinium vitis-idaea minus*) (Foote, 1983; Klinka et al., 1989). Feathermosses often occur in closed to semi-open coniferous forests, in damp woods, swamps, or margins of bogs. Feathermosses require shade and are restricted to areas sheltered by trees and shrubs (Busby et al., 1978). The main stems of feathermosses are perennial and appear to be capable of indefinite growth. Growth is controlled by temperature, rainfall frequency and degree of protection from evaporation stress (Longton and Greene, 1969; Busby et al., 1978). Feathermosses quickly dry when canopy cover is inadequate to prevent high evaporation (Johnson, 1981). Growth rates are highest in habitats protected from evaporation stress, and survival is enhanced in shaded habitats or in environments with high humidity and consistent cloud cover (Busby et al., 1978). It has been shown that tree canopy removal will eventually kill feathermoss, but removal of only the shrub canopy has a less severe effect (Busby et al., 1978). Feathermoss growth is so closely balanced with its microclimate that even the removal of a rather open shrub layer can have a measurable effect on growth rates (Busby et al., 1978).

In Alberta and BC feathermoss is most abundant at the late stages of succession, in the closed, mesic stands. It is also found in dry, nutrient-poor, open, black spruce-lichen stands in suitable areas, such at the base of birch (*Betula spp.*) and black spruce (*Picea mariana*) (Klinka et al., 1989). After canopy closure, feathermoss will generally form a continuous carpet on the forest floor (Carleton and Wannamaker, 1987). Given a shady, undisturbed, humid environment as is found on the cool, basal slopes of conifer stands, feathermoss is an effective competitor against other species. It can quickly spread over and eliminate other ground cover such as lichens (Johnson, 1981).

Feathermoss layer as a sub-ecosystem in the boreal forest

Despite its relatively small vertical dimension, the feathermoss layer can be described as a micro-ecosystem within the boreal forest stand. The feathermoss layer includes autotrophic and heterotrophic organisms, dead decomposable organic matter and mineral particles deposited as dust. It has its own unique microclimate (Busby et al, 1978), internal nutrient cycling (Li and Vitt, 1997) and intricate relationships between organisms inhabiting the organic layer.

The feathermoss layer harbors a large variety of invertebrates, bacteria, algae, fungi, actinomycetes, and protozoa. Favorable microclimate and abundance of organic debris inside the feathermoss layer encourages most microbial species that are usually thought to be characteristic of the mineral soil. In the boreal forest many processes, such as decomposition, N transformation and fixation, occur in the feathermoss layer rather than in mineral soil (Spiess et al., 1990).

Most of the microflora associated with feathermosses is adapted to low pH, high phenol content, and frequent desiccation (Spiess et al., 1990). Microflora can form symbiotic transfer and mineralization of N (Carleton and Read, 1991), be associated with N fixation (DeLuca et al., 2002) or form parasitic relationships with the feathermoss (Prior, 1966). In turn, feathermoss protects itself by producing certain antibiotics (Banerjee and Sen, 1977), which inhibit microbial development.

Interactions of feathermosses with higher plants

Feathermoss carpets dominate only under conifer stands because the leaf litter of deciduous trees covers the moss and blocks the supply of light (Grout, 1932; Klinka et al., 1989). Needle leaf fall is usually trapped between feathermoss shoots and does not interfere significantly with moss growth.

In any ecosystem, the most severe competition between individuals of the same height is between individuals of the same species (Oliver and Larson, 1996). It is assumed that in the boreal forest, pine suffers from the competitive pressure from other pine trees more than from understorey (Drew and Flewelling, 1979). The competition related mortality of the suppressed trees is the most common cause of death of trees in the wild forest (Kimmins, 1996). It is also possible, however, that unfavorable soil conditions such as low nutrient availability, cold soils and potentially allelopathic effects of the understorey vegetation may keep the stands in a near-stagnation phase by preventing development of dominant trees.

Mosses are known to produce phenolic compounds (Rasmussen, 1994) and to capture nutrients and water before they reach root-inhabited zone (Eckstein, 2000). Mineral soil under the feathermoss layer usually has low nutrient availability and the fine roots of trees often grow upwards into the decomposing layer of the feathermoss to access nutrients as they are released from the feathermoss layer. A number of studies have suggested a possibility of moss competing for resources against the higher plants (Eckstein, 2000; Zackrisson et al., 1997; Svensson, 1995) and thus reducing the site productivity.

Another aspect of intraspecific interaction between moss and higher plants may include a complex mutualistic relationship mediated by microflora. In particular, fungi are capable of facilitating nutrient transfer from the moss layer to plant roots (Carleton and Read, 1991).

Feathermoss as an intercepting and retaining layer between crowns and roots

A continuous feathermoss layer can intercept input of organic material and nutrients with litterfall and precipitation (Bates, 1989; Li and Vitt 1997). When pine needles are trapped within the feathermoss, the decomposition rate is often decreased during dry periods (Swift et al., 1979), and when nutrients are finally released, they are first available to the bryophytes closest to the decomposing material (Li and Vitt, 1997).

Nutrients deposited by throughfall or fertilization also tend to be intercepted by feathermoss layer and do not reach mineral soil when applied to the top of the feathermoss layer (Eckstein, 2000). It is not clear if these nutrients will be released to rest of the ecosystem when the bottom of the moss layer dies.

Feathermoss as an insulating layer

The thick feathermoss layer in many lodgepole pine forests of Alberta may have an adverse effect on forest productivity by altering ecosystem processes such as the cycling and availability of nutrients. In forests with a thick organic mat, such as moss, dead grass or needle litter, soil temperature during the summer period can be significantly lower than that without insulating organic layers (Hogg and Lieffers, 1991; Bonan and Van Cleve, 1992; Smith et al., 1998).

Reduced soil temperature under the insulating organic layer may induce a decrease in the number and length of live lateral roots of pine (Haywood et al., 1997). Low soil temperatures inhibit decomposition and the release of the nutrients into the root zone (Swift et al., 1979; Van Cleve et al., 1986; Eckstein, 2000) therefore, contributing to the accumulation of forest floor material and suppression of tree growth in these forests. Finally, a thick feathermoss layer slows soil warming in the spring, which may limit late spring root growth to a relatively thin upper layer of soil.

Most physiological processes slow down with a decrease in temperature. Growth of fine roots of conifer seedlings was noticed to cease below 5° C (Vapaavuori et al., 1992). There are indications that even when water supply is ample, moderate water stress may occur at low soil temperatures because water uptake is restricted (Coates et al., 1991, Wan et al., 1999). At the same time, however, roots of boreal species are adapted to cold climates and show significant respiration at 5° C (Conlin and Lieffers, 1993).

The difference in the air and soil temperature can cause an additional stress on the tree during the spring time, when air is warm enough for photosynthesis, but roots in the frozen soil are unable to supply water and nutrients to the crowns until soil thaws. In some Alberta species, photosynthetic activity ceases at winter time (Man and Lieffers, 1997). Also in the boreal forests, roots are mostly confined to the upper horizons of the mineral

soil and organic matting (Finer et al., 1997) possibly because only upper layers are warm enough for roots to develop in the springtime.

Feathermoss photosynthesis and biomass in the forest

Feathermosses contribute to the flux of carbon into the ecosystem through their metabolism and growth rates. The total photosynthesizing biomass of the feathermoss layer can easily reach 10 t/ha, with net primary production (NPP) ranging from up to 350 g C m^{-2} yr^{-1} (Bisbee et al., 2001, Fenton 1980).

Feathermosses are shade-tolerant and reach light-saturation point at 10% of above-canopy light intensity, compared to *ca* 50% for most C3 plants (Martin and Adamson, 2001). Though the efficiency of photosynthesis is thought to be lower in bryophytes than in higher plants (Turetsky, 2003), with their large biomass and photosynthesizing surface area, feathermoss layers play an important role in C sequestration (Binkley and Graham, 1981). To support its photosynthetic activity feathermoss requires considerable amounts of N and can contain as much as half of the above-ground N in the forest (Wells and Jorgenson, 1975, Turner and Singer, 1976).

Chemistry of the feathermoss layer

Acidity

The products of the conifer needle decomposition usually produces acidic byproducts (Pallant and Riha, 1990), hence soils under boreal forests are usually acidic. Pine can grow on soil with pH as low as 2.7 (MacDonald, 1997. Moss and microflora in the conifer stands are well adapted to the acidic conditions. Also, cations bound to the surface of the moss can contribute to the overall acidity of the site (Cragie and Maass, 1966). Availability of P is generally known to increase with decrease in pH. In acidic soils the dominant form of N is usually NH_4^+, but most plant roots absorb it well. Decrease in pine productivity is expected above pH 6.5 (Marx, 1990) but there are indications that pine grows successfully at pH levels above this (Torbert et al., 1990).

Organic matter

Coarse conifer needle litter can only be decomposed with participation of fungi. The acidic products of this decomposition decrease soil pH to the levels as low as 3.5, which was shown to inhibit bacterial microflora (Bottner et al., 1998). Needle litter has a high C/N ratio. In order to decompose such material, microflora performing decomposition has to take up N released in the decomposition process, thus maintaining N deficiencies for other parts of the ecosystem (Stephenson, 1934).

Feathermoss tissues do not produce lignin (Turetsky, 2003), but dead moss material has a slow rate of decomposition. This might be related to re-uptake of the N by the feathermoss from the senescing tissues (Brown and Bates, 1990). Secondly production of antibiotic compounds by the feathermoss may inhibit bacterial populations (Banerjee and Sen, 1977).

Phenolic compounds in nutrient cycling

One of the characteristics of the chemistry of the moss layer is the presence of phenolic compounds (Rasmussen, 1994; Wilson et al. 1989). Phenols found in mosses come from several sources. Although some aromatic compounds are produced by the moss itself (Rasmussen, 1994), most are accumulated from decomposing debris or from exudates from small shrubs with allelopathic defense mechanisms (Inderjit and Mallik, 1997) and are stored in the moss (Prescott et al., 1989).

Phenolic compounds influence the ecology of the stand in numerous ways. They are phytotoxic to conifer seedlings and saplings; both root (Zackrisson and Nilsson, 1992; Brown and Zhu, 1984) and shoot growth of seedlings were reduced in mineral soils containing aromatic compounds (Inderjit and Mallik, 1997; Gallet and Pellisier, 1997 Gallet, 1994). Phenols also inhibit the soil biological activity and, consequently, nutrient cycling (Prescott et al, 1996; Boufalis and Pellisier, 1994; Pellissier 1994). Polyphenols have also been shown to alter the pathways of nutrient release from the decomposing organic debris (Northup et al., 1995).

N allocation in the feathermoss layer

The nutrients intercepted by the feathermoss layer can be stored in several possible locations (Figure I- 1)

N of trapped particulate matter

Particulate organic matter may shed from foliage and branches, seeds, cones, dust and animal remains and may remain physically trapped between moss plants. The microbial population inhabiting the feathermoss layer decomposes organic matter while particles are still within the level of the living moss. The nutrients released in this process are immediately available for uptake by feathermoss tissues.

The degree of dissolution and decomposition of these trapped particles, especially in acidic environments, is generally unknown (Brown and Bates, 1990), therefore the availability of nutrients derived from particulate matter is little understood.

Soluble N

Nutrients recovered through decomposition of the solid organic matter as well as nutrients deposited on the feathermoss surface in the liquid form (rain water, etc.) are retained in the feathermoss stratum, much like water in a sponge. Once dissolved, nutrients can be recovered from the moss intercellular soluble fraction by the bryophyte tissues and mycorrhiza (Carleton and Read, 1991).

Exchangeable N

The cell wall of bryophytes is the site of substantial cation exchange. Exchangeable properties of the feathermoss are similar to those of soil organic matter (Bates 1989) and reflect the usual established equilibrium between the nutrient concentrations in the solution and the exchange sites of the organic matter. Selective binding depends on the nature of competing cations, their relative concentration and availability of the binding site. The fixed negative sites of the cell wall appear to have a role in uptake of some nutrients (Wells and Brown, 1987; Bates, 1989). For some nutrients the exchange sites might also function indirectly in nutrient recycling (Brown and Bates,

1990) by sequestering nutrient cations from dying or damaged cells, which would otherwise be lost to lower layers.

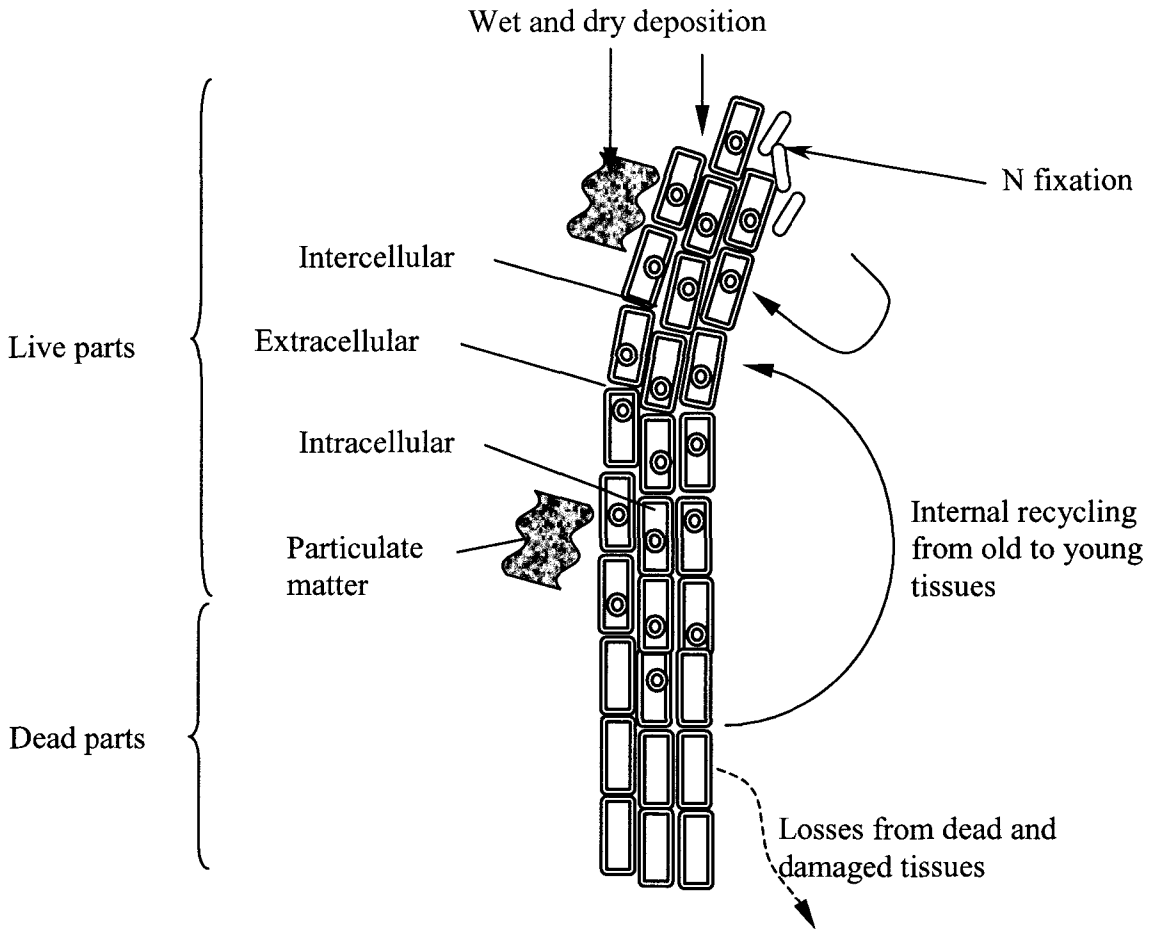
Lower parts of the feathermoss layer constituted of the dead but structurally intact plant remains are also capable of accumulating nutrients due to high cation exchange capacity of the feathermoss surface. Feathermoss tissues have the ability to efficiently take up limiting nutrients such as N (Li and Vitt, 1996). The amount of N remaining in the solutions after filtering through the feathermoss layer is often less than 0.02% of the initial content (Weber and Van Cleve, 1984)

Intracellular N

Intracellular uptake requires passage across the plasma membrane of the cell, using transport sites with various degrees of selectivity (Bates, 1989). Rate of cellular uptake is substantially slower than for extracellular binding and may be controlled by supplies of energy to drive the transport process (Brown and Bates, 1990). For most nutrients, rate of uptake is also regulated by the pre-existing intracellular concentrations.

There is also a significant nutrient pool associated with the microflora (bacteria and fungi) on the surface of the feathermoss layer and underlying duff layer (Carleton and Read, 1991). The feathermoss-covered forest floor stratum of the boreal forest is warm and well-aerated thus creating better conditions for microbiological activity than cold mineral soils. Decomposition and N transformation are essential for ecosystem development and are thought to be associated with mineral soil. However, in the ecosystems with continuous moss cover these processes are likely to take place the forest floor. Carleton and Read (1991) indicated that N transfer between feathermoss and microflora through symbiotic or parasitic interactions can play a significant role in overall nutrient cycling in the boreal forest.

Figure I - 1. N allocation and transfer in a feathermoss layer



N transformations in the feathermoss layer

N transformations in the thick feathermoss layer are somewhat different from those in the mineral soil. The feathermoss layer has an abundance of organic matter and a unique microclimate. Feathermoss is considerably better aerated than mineral soil, is usually warmer from spring to fall, but is susceptible to cyclic wetting and drying during the summer time. The following is a review of the N transformation processes of the feathermoss layer.

Sources of N in the feathermoss layer

Live parts of the established feathermoss have no direct contact with the mineral soil but are able to intercept most of the litterfall and throughfall of the forest (see discussion above). It has been suggested that some of bryophytes, including feathermoss form facultative symbiosis with N_2 fixing cyanobacteria such as *Nostoc* spp. (Rai et al., 2000; DeLuca et al., 2002), providing an additional source of N.

Immobilization

Assimilation of N by moss, microflora and higher plants ties up N in organic form. When available N is limited, immobilization by microbial populations can further decrease N availability for trees and other plants. N assimilated as a NO_3^- is converted to NH_4^+ with the help of nitrate reductase, and then into amino form.

Mineralization

For most plants, mineral forms of N in soil are the only forms available for uptake. In unfertilized soils, the mineral forms of N are derived almost solely from decomposition of organic nitrogenous compounds. The transformation from organic N to NH_4^+ N does not require specific organisms. Most decomposing bacteria and fungi can release NH_4^+ in the environment (Swift et al., 1979). Optimal conditions for microbial mineralization to NH_4^+ are warm, moist soil with adequate aeration. Low soil temperatures in the boreal soils inhibit mineralization rates and decrease rates of N turnover.

Nitrification

Nitrification is a microbial process of oxidation of NH_4^+. This process releases energy that can be utilized by bacteria. It involves first oxidation of NH_4^+ to NO_2 by *Nitrosomonas*, and then further oxidation to NO_3^- by *Nitrobacter*. Both processes require good aeration and pH levels close to or above 6 (Torbert et al., 1990). Since forest soils are usually acidic, the role of nitrification in N cycling is reduced. However, measurable NO_3^- content was reported in feathermoss layers (Carleton and Read, 1991; Smith et al. 1998), suggesting that some nitrifying organisms inhabit the surface of the feathermoss plant in the boreal forest. Nitrification proceeds more rapidly in substrates subjected to alternate wetting and drying than when held permanently moist, resulting in flushes of NO_3^- after re-wetting (Birch, 1958).

Nitrate reduction and denitrification

The term "denitrification" has been used to describe a number of processes. One of the common understandings of "denitrification" is the process of reduction of nitrate (NO_3^-) to N gas or to organic N compounds; in this case "denitrification" is a removal (reduction) of nitrate and an opposite of nitrification. On the other hand, according to the Canadian SIS (Soil Information System) definition, denitrification is the gaseous loss of N by either biological or chemical mechanisms, but exclusive of ammonia volatilization. This includes all processes of conversion of N into a gaseous form, including biological or abiotic nitrification and nitrate-reduction. In this thesis, in order to avoid confusion of terms, I try to avoid using the term "denitrification" whenever possible, instead using terms "gaseous loss of N" in describing conversion of N into gaseous form and escaping to the atmosphere, and "nitrate reduction" when speaking of conversion of NO_3^- to a less oxidized forms of N.

There are two forms of biological nitrate reduction: dissimilative, when NO_3^- is used as an electron acceptor in oxygen-restricted conditions, and assimilative, when NO_3^- is reduced by nitrate reductase in the plant or microbial cell before converting it into nitrate reduction. Dissimilative nitrate reduction is usually conducted by specialized anaerobic microorganisms that use NO_3^- as electron acceptor in the absence of oxygen. In

waterlogged soils NO_3^- is often reduced to N_2 or one of the gaseous N oxides. Gases escape from the soil profile, which results in N losses.

Feathermoss layers in boreal forests are usually well aerated and contain little NO_3^- (Van Cleve et al. 1986). Anaerobic denitrification is assumed to be negligible in the feathermoss. When feathermoss is saturated after rain, however, conditions may be favorable for anaerobic denitrification of the small amounts of NO_3^- that are present in the dead parts of the feathermoss plants.

Possible pathways for N loss

There are several ways in which N can be removed from the forest system. Leaching may result in a substantial loss of nutrients in soils with fine texture and high concentration of NO_3^-. However in fine textured boreal forest soils with thick feathermoss layers, only small amount of N moves to a depth of 50 cm into the soil profile within 100 days after application (Weber and Van Cleve, 1984). Thick feathermoss layers are capable of intercepting fixed N before it reaches mineral soil. There is a possibility of N loss through leaching in forest soils (Nômmik and Möller, 1981; Overrein, 1969) but most estimates of leaching are indirect, based on the difference between amounts of N applied and recovered from the mineral soil and plant tissues (Turner and Singer, 1976).

There is substantial indirect evidence of gaseous losses of N from boreal forest ecosystems, though direct measurements in the field are difficult to obtain. Volatilization of N during urea hydrolysis is one known mechanism of gaseous N loss (Wollum and Davey, 1975, Morrison and Forster, 1977). However, it is unlikely that NH_3 volatilization could be responsible for a significant N loss in unfertilized forests or in those treated with acid-forming fertilizers such as $(NH_4)_2\ SO_4$ (Keeney, 1980). Low pH would minimize NH_3 loss. Only 0.1% of NH_4^+ N is transformed into NH_3 at pH 6 or lower (Wollum and Davey, 1975). Most commonly the pH of the water solution bathing the feathermoss layer varies between 4.6 and 5.5 (Morrison and Foster, 1977)

Emissions of N gases from decomposing plant material and nitrification-denitrification have been well described. Wallace and Smith (1954) found gaseous losses from decomposing leaves. Overrein (1969) detected N_2 emanating from urea-treated plots in the temperate forest under natural conditions. Potential losses of N in the form of NOx

from forest ecosystem are also widely documented (Bremmer and Blackmer, 1978; Black, 1968).

There is abundant evidence that N_2O can be released from soil through denitrification of NO_3^- under anaerobic conditions. Several studies (Bremmer and Blackmer, 1978; Black, 1968) indicate that this gas is also released from soil during nitrification of NH_4^+ under aerobic conditions. One of the species responsible for the oxidation of NH_4^+ to N_2O was *Nitrosomonas europaea* (Anderson et al., 1993). Nitrification can lead to the formation of NO_2^-, which is rather unstable. The reactions involving NO_2 include:

Spontaneous decomposition of nitrous acid by the following reaction:

$$2HNO_2 = NO\uparrow + NO_2\uparrow + H_2O$$

where $\uparrow$ sign indicates potential loss to the atmosphere.

In the presence of oxygen, nitric oxide (NO) is oxidized rapidly to NO_2 (nitrogen dioxide):

$$2NO + O_2 = 2NO_2\uparrow$$

NO_2 does not spontaneously transform into N_2 or nitrous oxide (N_2O). However, at low pH in the presence of phenols, NO_2 can be reduced directly to N_2 and N_2O:

$$2HNO_2 + 2RH = N_2O\uparrow + H_2O + 2ROH$$

were *R* is low molecular organic substance. Nelson (1967) showed that 25% of added NO_2^- is lost as N_2 or N_2O when incubated for 24 hours at pH 5 in the presence of phenols. Cai et al. (2001) reported 5% gaseous loss of N from soil amended with urea and straw. The experiment they conducted involved incubation of soil with pH 5.8 at 70% water holding capacity for 51 day at 30° C. Emanating N gases consisted predominantly from N_2 and N_2O. Authors suggested that supplemented urea was hydrolyzed to NH_4^+ which later was oxidized by some form of microbial nitrifiers that were tolerant to low pH (Cai et al., 2001).

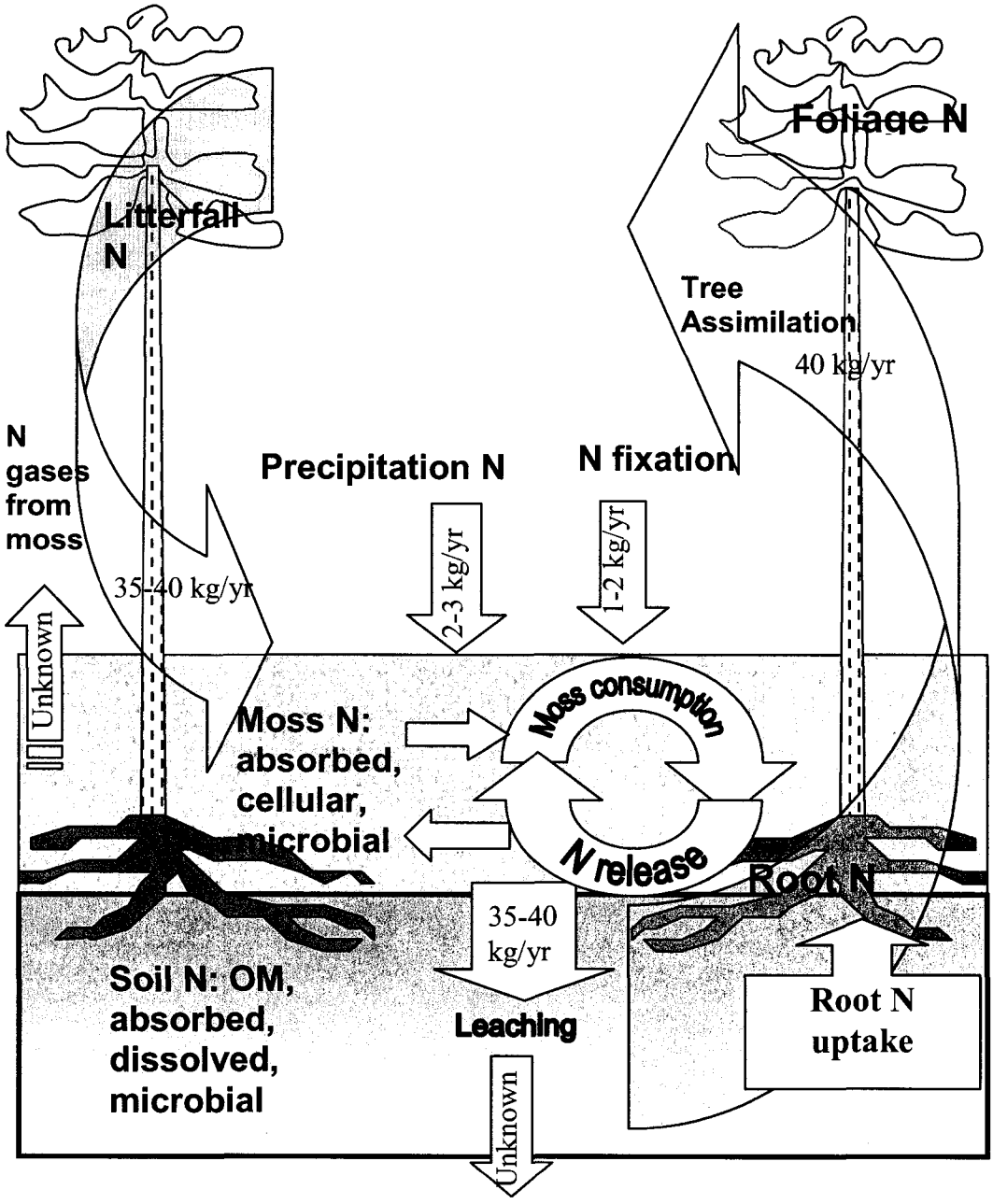
Another reaction requires presence of aliphatic amino groups (Black, 1968):

$$R\,NH_4^+ + HNO_2 = ROH + H_2O + N_2\uparrow$$

Total losses of N to the atmosphere are difficult to estimate. There are no direct field measurements of gaseous N losses reported in the literature. The estimate of N loss have come from by studies of N balance (unaccounted for N in all measured pools after fertilization) can vary from 0 to 70% (Nômmik and Möller, 1981). Most researchers agree that escape of N in gaseous forms can adversely affect stand productivity (Keeney, 1980)

Figure I-2 is a hypothetical diagram of major N pools and potential transfer paths that may occur in boreal forest stands with feathermoss. Common understanding of the nutrient cycling implies that nutrients consumed by roots are eventually returned to soil through litterfall and decomposition and become available again for uptake by the same plants. However, in the ecosystems with continuous feathermoss layers, nutrients can be intercepted by feathermoss and may cycle only in the aboveground organic layer. As a result, the turnover of the N between soil and trees may be disconnected. Tree roots located in or directly under the forest floor are able to access some of this N but feathermoss and its associated microflora compete with the trees for the available N.

Figure I- 2. Schematic representation of annual N transfer (per Ha) in conifer forest with a continuous feathermoss layer



Fate of the supplemental N in the boreal forest

Fertilization is an effective treatment for the improvement of productivity of the forest stands with low nutrient status. Increased biomass production with N fertilization has been widely observed in the forests (Buzykin et al., 1996); ^{15}N recovery studies indicate that the increase in productivity in forests is much lower than in agricultural systems (Keeney, 1980). Uptake by trees is only 15-20% of added N (Tamm, 1964) and most uptake occurs during the first year following application (Nômmik and Möller, 1981). The unaccounted N may be lost due to volatilization, leaching, immobilization or denitrification (Morrison and Forster, 1977). The exact amount and the mechanism of nutrient loss from the ecosystem are not yet clear.

There is little data on the rate of release of nutrients that were absorbed in the feathermoss layer. When fertilizers are applied on top of the moss layer, the assumption is that eventually tree roots in the mineral soil will have access to the additional nutrients. However, only a small fraction of supplied N was recovered in the trees one year after fertilization (Nômmik and Möller, 1981; Li and Vitt, 1997). The remaining portion of the added N was presumed to be retained by the moss or mineral soil. Nutrients are usually expected to be released from the feathermoss within several years, when the enriched feathermoss fragments will begin to die out (Turetsky, 2003). Yet the ability of feathermoss to retain and recycle the limiting nutrients (Li and Vitt, 1997; Bates, 1992; Brown and Bates, 1990) suggests that healthy and undamaged feathermoss is unlikely to release these nutrients.

Nômmik and Möller (1981) have demonstrated that recovery of N fertilizer in the forest ecosystem three years after the application in form of NH_4 NO_3 or urea was less then 50%, sometimes less then 30%. Loss of N apparently increased with the application rate. Although significant amounts of N in urea treatment were lost during hydrolysis, total recovery was greater than recovery following the NH_4NO_3 treatment. NO_3^- may be partially lost to leaching out of the root zone (Vitousek et al., 1979). Nômik and Möller (1981) attributed low recovery of applied N to either plant uptake or gaseous N loss to the atmosphere during the first growing season after fertilization. Simplified experiments using laterally isolated microplots in conifer/feathermoss stands (Nômmik and Popovic, 1971) showed N recovery of 23-76% when fertilizer was applied on top of the feathermoss layer. Caution should be exercised when interpreting data obtained from studies using ^{15}N. N-deficient ecosystems readily assimilate all isotopes without discrimination. However, the process of release of N from cells and engagement in further transformations may be more rapid for lighter N isotopes (Nômmik et al., 1994). For example, in the 5 years following ^{15}N application, new pine needles had greater ^{15}N enrichment than the proportion of ^{15}N in the original fertilizer (Nômik and Möller, 1981). When using a balance method for estimating fertilizer recovery in soil, it is evident that the figures obtained for total N are significantly lower than those for ^{15}N data (Nômmik and Popovic, 1971). Therefore, studies of N cycling using ^{15}N may overestimate the recovery of applied N in the ecosystem.

Intermittent desiccation of the feathermoss layer

Drought-tolerance

Forest feathermosses are known to undergo several wetting and drying cycles during summer months (Dilks and Proctor, 1976). In its desiccated form the feathermoss lose 80% of its fresh weight, when biological activity virtually stops (Tucker et al., 1974). Feathermoss tissues have developed an efficient drought tolerance mechanism that allows it to recover within 24 hours after complete dehydration (Noailles, 1978). However, feathermoss suffers dramatic changes in its physiology and cell structure as a result of the

dehydration (Tucker et al., 1975; Noailles, 1978). Cell membranes are damaged, and some of the nutrients leak out of the cell wall. Carlton and Read (1990) showed that upon submersion of dried feathermoss in water, leaching of N is 5-10 times greater than following submersion of fresh feathermoss. The schematic illustration of cellular changes in the feathermoss is shown in Figure I-3. The proposed sequence is based on descriptions and microphotographs by Noailles (1978), Tucker et al., (1975), Krochko et al., (1978), Brown and Bates (1990) and Bates (1997).

Compared to higher plants, bryophytes generally have a low level of tissue differentiation. Consequently, nutrients can be assimilated by any part of the feathermoss plant. At the same time, the limited development of a cuticle means that all cells are potentially in contact with bathing solutions. As shown in Figure I-3, the protoplasm of dehydrated moss cells drastically shrinks in volume. The concentration of internal solutions will increase and may reach toxic levels. Desiccation increases permeability of the plasma membrane (Brown and Bates, 1990). As a result, intracellular nutrients are susceptible to leaching by the next rain event.

Changes in the moss cell microstructure following re-wetting

Existing microscopic and biochemical studies of in the moss tissues during desiccation and after re-hydration point to a number of cellular changes. The following summary of cytological processes in the moss tissues is based on the studies of Tucker and co-authors (Tucker et al., 1975; Noailles, 1978; Brown and Bates, 1990; Dilks and Proctor, 1974, 1976; Richardson, 1981).

Desiccation phase:

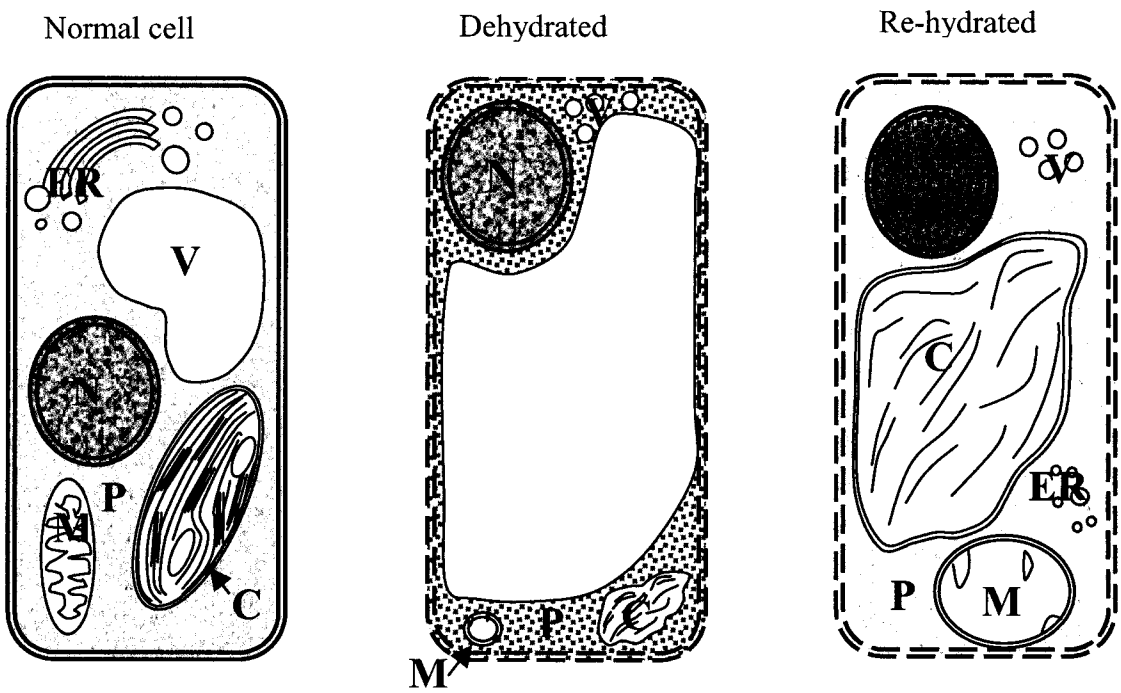
- Protoplasm condenses at distal and proximal cell ends become gel-like; the cell either collapses or if the breakage of membranes permits free entrance of the atmospheric air, a void forms in the central portion of the cell.
- Membrane permeability increases.
- Chloroplasts and mitochondria become more rounded and compact; vesicles in chloroplasts become very small or absent.

- Nucleus remains normal-looking.

Immediately after re-hydration:

- Cytoplasm expands and fills entire volume of the cell; gas pockets shrink and disappear.
- Irregularities and clefts appear on the surface of the chloroplasts.
- Chloroplasts appear swollen; outer membrane wrinkled; thylakoids are no longer compacted; chloroplasts appear internally split; starch grains disappear from chloroplasts.
- Mitochondria are round and swollen; internal structure change and cristae nearly disappear.
- Ribosomes are dispersed; dictyosomes and endoplasmic reticulum either non-visible or appear as clusters of numerous small bubbles.
- Vacuoles are small and numerous (instead of 1-4 large ones).
- Nuclei are not changed but are pushed and distorted by surrounding swollen organelles; internal nuclear material has grainy appearance.

Figure I- 3. Schematic representation of the internal changes in the moss cells subjected to desiccation and following re-hydration



In the first hours after re-wetting, feathermoss has damaged cellular components, particularly mitochondria (Noailles, 1978) (Figure I-3). We can reasonably assume that, as a consequence, tissues might be temporarily depleted of ATP and moss may not be able to re-assimilate nutrients through active membrane transfer (Bates, 1997). Temporarily elevated N availability in the surrounding solution increases the probability of N loss to atmosphere in gaseous form as a result of nitrification and denitrification processes.

Pleurozium schreberi is capable of complete recovery of most of its cellular structures and photosynthetic ability in 4 to 7 hours after re-hydration (Noailles, 1978; Dilks and Proctor, 1974). Presumably, the N content of photosynthetically active parts of the feathermoss should also recover. It is unclear, whether feathermoss can re-assimilate the expelled N from its surface, or has to replenish the deficit from decomposing organic matter. In the boreal system the likelihood is that rewetting of the surface of the feathermoss by the rain would be expected to lead to a flush of nutrient release and potential loss of N (Carleton and Read, 1991).

Leakage of nutrients and photosynthate movement from the feathermoss following re-hydration

Feathermoss tissues leak out nutrients (Carleton and Read, 1991) and photosynthates (Gupta, 1977) rapidly after re-hydration. This leakage is possible because membranes are damaged during desiccation and become permeable to the solutes. Gupta (1977) demonstrated that feathermoss tissues are able to eventually recover some of the leached photosynthates after cell recovery if the solution remains in the vicinity of the moss surface.

Increase in respiration following re-hydration of the moss tissues

Recovery of feathermoss after the desiccation damage is associated with considerable increase in the CO_2 production (Dilks and Proctor, 1976). Displacement of absorbed gas from the voids in the desiccated cells (Figure I-3) with water was suggested as one of the possible mechanisms of the increased CO_2 emanation from the feathermoss after re-hydration. It is likely that increased energy requirements of re-hydrated tissues for

membrane repair, as well as rapid decomposition of microbial and feathermoss cells that did not survive the desiccation could substantially contribute to the total CO_2 flux. Together with leakage of photosynthates, increased respiration after re-wetting of the feathermoss tissues can deplete energy stores of the feathermoss tissues (Richardson, 1981)

Synthesis

The role of a continuous feathermoss cover in N cycling in the boreal ecosystems is not completely understood. Effects of intermittent desiccation on the feathermoss layer and its significance for the boreal forest require further research. Apparently, feathermoss tissues can have drastic losses in the water content but recover upon rewetting; this process, however, consumes energy and depletes feathermoss reserves.

Moss tissues release N to the solution after re-hydration of the feathermoss, but the mechanism of restoration of moss nutrients status after re-hydration is unclear. Apparently, moss tissues must recover lost nutrients, but neither the source of these nutrients nor the time frame of such recovery was ever studied.

Increase in CO_2 production after desiccation was described before, but the carbohydrate sources and ecological significance of such increase in respiration were not described. Desiccation/re-hydration of the feathermoss are associated with certain changes in the chemistry and oxidative properties in the extra-cellular space of the moss; although these changes might theoretically have an effect on the nutritional status and N transformations in the feathermoss layer, the details of this phenomenon and possible volatilization of N were not explored.

The physiological effect of low soil and air temperature is most often described using seedlings or young trees. The physiology of mature trees in boreal forests is less studied and the data on stand productivity, as a function of the soil temperature, are contradictory. It was noticed that certain stand manipulations such as thinning and forest floor removal could lead to a change in soil microclimate and drainage properties. Limited data are available on the effect of modification of the nutrient cycling and uptake as a result of these changes.

I hypothesized that the moss as a stratum in the conifer forests regulates and controls the soil microclimate and the flow of nutrients downwards to the roots. N from precipitation, N fixation, throughfall and litter deposition is being mineralized within the moss, and it is retained and assimilated by the moss tissues before it would be available to the plant roots. Later the fate of the N and the rate of its release to the soil layers inhabited by tree roots is determined by the peculiarities of the moss physiology, in particular its susceptibility to desiccation and ability to recover upon rehydration. I suggest that moss plays a complex role in N cycling by capturing and processing N input, assimilating and recycling N within its strata, and rationing N release to the other components of the ecosystem. Frequent drying of the moss layer, changes of temperature and multiple stages of N transformation may lead to a partial volatilization of gaseous forms of N, thus decreasing the productivity of the ecosystem.

Objectives of this thesis were to:

- Estimate the role of continuous feathermoss layer in the N transport and cycling in the conifer forest and determine the effects of moss removal on the tree productivity.
- Assess effects of nutrient status and desiccation followed by rewetting of feathermoss, on N transfer between moss tissues and external water solutions.
- Study the effects of desiccation on gas exchange of the feathermoss layer, including respiration and N volatilization.
- Assess the rate of N release from the feathermoss layer, N retention and transfer between feathermoss and soil and to compare the effects of moss removal, with thinning or fertilization in lodgepole pine stands.

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Chapter 2. Dynamics of mineral nitrogen released from feathermosses after dehydration and handling stress

Summary

The capacity of feathermosses to release mineral N to water and eventually re-capture it back from the solution was periodically measured in several 64-hour experiments. Mosses were collected from 13 locations in western Alberta, Canada, and given several pretreatments in the days leading up to the submersion of mosses in aerated distilled water. In a factorial experiment, the pre-extraction conditions were fertilized or left as controls and, kept moist or allowed to dehydrate. The concentration of mineral N in the solution was monitored by withdrawing small samples of the solution for colorimetric analysis at pre-determined time intervals. To assess the effects of microflora and handling damage to the moss tissues on the rate of N exchange between moss and solutions, the experiment was repeated firstly using solution of antibiotics instead of water and secondly using mosses that were not given time to recover from handling. No perceptible leakage of N was recorded from fully hydrated moss tissues. Dehydrated mosses lost as much as 8% of their total N content to the solution within two hours after re-hydration, but had recovered two thirds of it within the next 16 hours. Mosses with higher N content released more N but also recovered more N in the following hours. Bruised moss tissues released approximately 0.7% of it total N and recovered it at the same rate as the desiccation-damaged mosses. Application of antibiotics affected neither leakage nor re-absorption rate. The ability of mosses to quickly re-absorb released N from surrounding solutions suggests that leakage of N from dried moss after rewetting, as a source of N to the ecosystem, is not as large as suggested by previous literature.

Introduction

Feathermosses are widely distributed across the boreal regions of the world and in many forests feathermosses maintain as much or more photosynthesizing biomass as the trees on the same sites (Richardson, 1981). Feathermosses are thought to play a significant role in the nutrient cycling of these forests. The moss layer insulates the mineral soil underneath (Bonan, 1992); this slows biological activity and decreases evaporation from the soil. Feathermosses are also known to capture a large part of the N input from litterfall; only a small fraction of N deposition on the surface of the moss layer passes through to the mineral soil (Weber and Van Cleve, 1984). In pristine environments, feathermosses may host N fixing algae (DeLuca et al., 2002), thereby adding N to the ecosystem.

While feathermosses are known to trap N, there is also an assumption that they release N after a period of time (Berg, 1986), likely through the death and decomposition of older portions of the moss stems at the base of the moss layer. A second possible mechanism for release of N may be activated several times during a summer. Unlike higher plants, mosses do not have roots or cuticle, and they are subject to wide swings in tissue moisture content depending upon weather; feathermosses may vary from fully hydrated, to dry and back to hydrated again in a few days, without suffering any apparent harm. Carleton and Read (1991) demonstrated that there is a release of nutrients from the moss layer upon re-wetting of the desiccated moss. This release of nutrients into solution is likely due to the membrane damage during drying. The nutrient leakage from dried moss is considered to be a potentially important source of nutrients for the forest ecosystem (Coxon, 1990; Carleton and Read, 1991; Gupta, 1977). It is yet unclear if the released N is readily available to other components of the ecosystem and if so, how moss is able to compensate for the loss of such vital nutrients.

After rewetting, mosses must eventually recover nutrients to continue photosynthetic activity. Such recovery may be accomplished by re-capture of released nutrients from the moisture bathing the surface of the moss. The quantity of N that is released after dried mosses are rewetted and how quickly is the moss able to re-capture N from the surrounding solution is unknown. Unless moss can re-assimilate lost nutrients before they are taken up by tree and shrub roots or moved to a deeper soil layer by rain

water, it will have to recruit a fresh supply of N from other sources such as decomposition of trapped litterfall or N-fixation (DeLuca et al., 2002).

The ability of moss tissue to recover released cations and photosynthates has already been noted (Richardson, 1981). However it is not known if N compounds can also be quickly taken up after release. There are indications (Dilks and Proctor, 1974, 1979; Gupta, 1981) that photosynthesis begins within one hour after re-hydration. This implies that there is enough N present in the tissues to establish a base level of photosynthesis. Other functions, including full mitochondrial activity require as long as 24 hours to recover (Dilks and Proctor, 1974; Noailles, 1978), especially after prolonged periods of desiccation. A resumption of shoot extension of the moss may be delayed even longer (Busby et al., 1978; Hanslin et al., 2001).

Upon re-wetting of the moss layer, N present outside the moss cells in the dissolved mineral form would be readily assimilated by many components of the ecosystem, first by microflora associated with the moss surface. Whether microflora competes with moss tissue for released N or serves as a mediator in re-absorption is another unknown issue.

It is reasonable to suggest that the amount of N present in the moss prior to drying in the moss tissues may determine how much N may leak out after re-hydrating. We can hypothesize that moss well-supplied with N would lose more N to solution than moss suffering from N deficiency. There is no information if N-saturated moss would be able to recover lost N as efficiently as N-deficient moss.

As a null hypothesis tested in this study I assumed that there will be no differences between mineral N concentration in the water extracts from the moss subjected to fertilization, desiccation and mechanical damage, and that the concentrations of mineral N in the aerated bathing solutions surrounding moss tissues will remain stable over time.

The objectives of this study were:

- To measure the amount of leakage of mineral N from living moss tissue as a function of time, as affected by fertilization, desiccation and mechanical damage.
- To document re-absorption (if any) of mineral N by moss and factors affecting it.

- To determine the role of bacterial activity in the competition for N uptake of in moss leachate.
- To establish the nature of N transformations in the moss layer in relation to gradual desiccation.

Materials and Methods

Release and uptake of mineral N from feathermoss

Moss samples were collected from 13 natural pine stands in north and western Alberta, representing a wide variety of ecological and soil conditions (Figure II -1, Table II-1). The moss cover on the research sites was dominated by *Pleurozium schreberi* (60-90% of moss cover). Other species in the samples, ranked in declining importance were: *Hylocomium splendens*, *Ptilium crista-castrensis* and occasionally *Dicranium polysetum*. There was no obvious link between moss species, soil type and stand condition at the study sites (Table II-1).

Sample collection and pre-treatment

Intact moss layers (21 x 50 cm rectangular mats, approximately 7 cm deep) were cut out, placed in flats and transported to a growth chamber. Photoperiod was set at 16 hours at a light intensity of 50 μmol $m^{-2}s^{-1}$ at the moss surface level. Day and night temperature was set at 20°C and 16°C, respectively. Mosses were watered by misting 6 times a day for 14 days. After this adaptation period, flats were divided in half and placed in 21 x 21 cm trays. Half of the trays were fertilized with 6 grams of crystallized ammonium nitrate, which roughly corresponds to a dose of 100 kg N/ha. Mosses were grown for another 3 weeks before they were sub-sampled for analysis. During this pre-treatment and post fertilization period, the total amount of water passing through the moss layer was approximately 70 mm.

Water extraction

For each incubation (see below), two sub-samples of 20 g of fresh moss (green parts only) were separated from the moss mat and carefully placed in 500-ml Erlenmeyer flasks. Samples were held for 2 days in the growth chamber so that the moss could recover from the handling damage under the same misting and light conditions as during the adaptation period. After that, one of the two flasks was positioned under a ventilation fan in a greenhouse (20°C) and dried to a constant air-dry weight. Drying took at least 3 additional days.

Both fresh and dried samples were placed on an orbital shaker platform in the growth chamber with similar light intensity and temperature as the chamber used to grow moss. Each moss source served as a replicate in each experiment.

For the first experiment, 300 ml of distilled water was added to each flask, plus an additional 15 ml for the dry samples to compensate for the water absorption by the tissue. The time of addition of water was registered as the beginning of the experiment. Flasks were covered with loosely fitted lids to prevent contamination but still allow air circulation. The shaker was adjusted to approximately 100 rpm to ensure a gentle agitation and aeration of the solution. Concentration of dissolved O_2 in the solution was periodically measured using an O_2 probe (CellOx 325). At no point during the experiment did O_2 concentration in the solution drop below 90% of full saturation.

A similar protocol was used in two other related experiments. The second experiment used fresh unfertilized moss samples to test N leakage and rate of recovery related to handling the moss (such as isolation, sorting and insertion of fresh moss shoots into Erlenmeyer flasks). Moss sub-samples were handled in the same way as above, but no recovery time was allowed and moss was immediately submerged in distilled water. The third experiment evaluated the role of microorganisms in nutrient re-absorption. Here unfertilized but dried moss was rehydrated with a wide spectrum antibiotic solution containing 500 mg of amoxilin trihydrate, 200 mg of trimethoprim and 200 mg of sulfamethoxazole per liter of distilled water. This antibiotic mixture was designed to suppress the activity of gamma-positive and gamma-negative bacteria and actinomycetes. The base level of NH_4^+ concentration in antibiotic solution due to the cleavage of amino-groups was accounted for by subtracting the reading of blank antibiotic solution from the

readings of the samples treated with antibiotic. Adjusted readings for antibiotic-treated samples were compared to re-hydration with distilled water of unfertilized and dried moss.

Analysis of N dynamics in the solution

The bathing solution was sampled for analysis at pre-determined time intervals, by pipetting 1.5 ml of the extract in Technicon™ cups. Bathing solution was sampled at 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64 hours after the beginning of the experiment. Samples were covered with Parafilm™ and stored at 4°C for 1 to 5 days before analysis. NO_3^- and NH_4^+ content in the solution were analyzed using a Technicon™ analyzer II and the results were expressed in mg of dissolved N per g of dry moss tissue. At the end of the 64 hours, the remaining solution was drained from the moss, the moss sample was dried at 60°C for 48 hours and weighed. Total N content in the moss was analyzed separately using peroxide digestion of freeze-dried samples.

N transformations associated with desiccation

For this second experiment, an additional lodgepole pine/feathermoss site was selected approximately 30 km east of Edson, Alberta. In order to measure the effect of drying rate in N state in the moss tissues, two sub-samples were collected from each of five replications, one was immediately freeze-dried, and another allowed to dry naturally for 7 days and then also subjected to freeze-drying to an equal amount of residual water in the samples. These treatments were called "freeze-dry" and "air-dry" respectively. Approximately 0.5 g of the green freeze-dried moss shoots was placed in the 50 ml extraction vial. An aliquot of 15 ml of de-ionized water was added to the moss samples and the vial was shaken for 10 seconds, then decanted and the solution analyzed for NH_4^+ and NO_3^-. The results were calculated in mg of released N per kg of dry moss tissues.

Data analysis

In the first experiment, the response variable was the amount of N released per unit mass of dry moss. The effect of fertilization and drying was analyzed in a 2x2 factorial experiment. The effects of antibiotic application and mechanical damage on the amount of released N were compared using t-tests. Changes in N concentration in the solution were

plotted against time elapsed since re-hydration and the best-fit equation was found for each sample. Fit parameters of the different treatments were compared using ANOVA.

Results

N leakage from the moss tissues

Total N content in unfertilized moss varied from 0.9 to 1.4% with a mean of 1.2%. After fertilization the amount of N in the moss increased on average by 30%. There was no apparent increase in shoot growth or biomass production in fertilized moss, compared with unfertilized control (data not shown).

If given two days to recover from handling prior to addition of water, the majority of fresh moss samples did not release any significant amount of mineral N (Table II-2) when submerged in water. Fresh fertilized moss also did not have any appreciable leakage of either NO_3^- or NH_4^+. However, a measurable amount of N was released by samples that were bathed with distilled water immediately after handling. There was a high variability in the amount of N released from newly-handled moss possibly because it was difficult to standardize the handling procedures, especially untangling of the moss stems. Most of N released from newly-handled moss was in the form of NO_3^- (Table II – 2).
All samples of dried moss released significant amounts of mineral N after re-hydration (Tables II-2, II - 3). Peak amounts of N released from moss positively correlated with total concentration of N in the moss tissues (Figure II-2). Concentration of N in the bathing solution reached its maximum about 2 hours after the addition of the water (Figures II-3, II- 4). After 8 hours, concentrations in the solution decreased steadily, in some cases reaching immeasurably low levels by 16 hours. The samples re-absorbed on average 2/3 of released mineral N through the duration of the experiment.

Most of soluble N released from dehydrated moss was in the form of NH_4^+; moss bruised by handling had greater ratio of NO_3^- to NH_4^+ concentrations in the solution compare to desiccated moss ($p<0.05$) (Table II-2). Dried fertilized moss released almost twice the amount of mineral N compounds compared to dried unfertilized moss. Mosses fertilized prior to drying and re-wetting released 3- to 5-fold more NO_3^- than unfertilized mosses (Table II-2) and had a greater rate of re-absorption of N compared to unfertilized moss (Figure II-3).

The addition of wide spectrum antibiotics did not change the rate of re-absorption of N after re-hydration of the unfertilized and desiccated moss, compared to the control. I have found no indications that microflora played a significant mediating or competitive role in re-absorption of the released mineral N.

N transformations associated with desiccation

Amount of mineral N extracted from freeze-dried moss was almost twice that from air-dried moss ($p<0.01$) (Figure II-5). Significant reductions of N leaching from the tissues were recorded for both NH_4^+ ($p<0.1$) and NO_3^- ($p<0.01$) forms, but decrease of NO_3^- portion of the mineral N was most noticeable (Figure II-5). The ratio between NO_3^- and NH_4^+ leaking out of freeze-dried mosses was 0.43 compared to 0.03 from air-dried mosses ($p<0.01$).

Discussion

Leakage of soluble N from the moss tissues

I found that dehydrated moss released appreciable amounts of mineral N within the first 2 hours after re-wetting; up to 8% of the total N content of moss samples leaked into the solution in the form of NH_4^+ or NO_3^-. It is possible that more N was released in organic forms (Carleton and Read, 1991). Mineral N leaked readily from the dried moss tissues likely because the cell membranes were damaged during desiccation (Simons, 1974; Dilks and Proctor, 1974) and presumably soluble compounds leaked through the damaged membrane into the surrounding solution were driven by an osmotic gradient.

In contrast, very little soluble N was extracted from undamaged hydrated moss tissues. Cell membranes of healthy moss tissues, even without a cuticle, apparently provide adequate protection for the intracellular supply of nutrients. At the same time, I found that bruising from ordinary handling caused moss tissues to release some N to the solution, likely due to cell membrane damage. Leakage due to the handling damage might explain the apparent contradiction between my data and a previous report by Carleton and Read (1991) where there was noticeable leakage of N from non-desiccated moss, but there

is no indication if in their study any time was allowed for moss to recover following handling. Moss is likely to be susceptible to mechanical membrane damage because of the absence of a protective cuticle. Though in my experiment, handling damage allowed for a comparatively small amount of N to be released as compared with desiccation effects, release of N after mechanical damage might still be a valuable source of N for the system. Mechanical damage of the moss layer by animals, falling debris, raindrops and hail can potentially contribute to the N transport to the roots, especially during rainy periods.

I recorded noticeably higher proportion of NO_3^- in the leachate from bruised moss tissue compared to re-hydrated moss (Table II-2). The reasons and possible implications of the difference in the composition of N extracts from moss tissues subjected to the different forms of membranes damage will be discussed below.

After drying and re-hydration, fertilized moss released almost twice the amount of N compared to unfertilized moss. Extracted N in both fertilized and unfertilized dried moss was mostly in the form of NH_4^+, but the presence of NO_3^- increased considerably in fertilized samples, possibly owing to the moss cells storing excess N in the less toxic form of NO_3^-.

Re-assimilation of soluble N from the solution

The most interesting result of the experiment is that I had clearly observed the ability of dehydrated moss to reabsorb the lost nutrients from the solution. My experiment showed that N levels in solution had significantly declined 16 hours after re-hydration, suggesting that membranes were relatively quickly repaired (Figure II-4) and cells are ready for active transport of nutrients against an osmotic gradient. The notion of moss being able to recover released compounds has been suggested before by Gupta, (1977) and Richardson (1981), who had shown that mosses have the ability to re-capture photosynthates after rewetting. Micro-structural studies of *Pleurosium shreberi* recovering from desiccation (Noailles, 1978) indicated that moss could restore the outer membrane within several hours after re-hydration. The apparent ability of the moss to recapture N from solution appears to be consistent with the time of membrane repair. I have observed that after 16 hours, 2/3 of the released N was assimilated and would no longer be available for uptake by other organisms and that despite the absence of a

cuticle, moss demonstrated an ability to reduce leakage of soluble nutrients away from the moss layer.

Despite significant increases of tissue concentration of N in the fertilized moss, feathermosses were as efficient in absorption of released N, on a weight proportional basis, as unfertilized samples. The ability of moss tissue to re-assimilate and retain greater amounts of N from the solution could potentially limit N flow between moss and trees, thereby decreasing efficiency of fertilization of forests.

The nature of membrane damage did not seem to affect the process of re-absorption of N. Though the composition of solutes released from mosses bruised by handling differed from solutes released after desiccation, in both cases the time required and efficiency of re-absorption was the same regardless of the nature of damage to the moss tissue (Figure II-4). This suggests similar membrane repair mechanisms for desiccated and mechanically damaged moss tissues.

Certain amounts of dissolved N remained in the solution at the end of 64 hours of the experiment. Regardless of the amount of N released to the solution, fertilized or unfertilized moss tissues re-assimilated on average approximately 2/3 of the leached out N; incomplete re-absorption of N allows for a range of explanations. Some of N dissolved after addition of water might be of non-cellular origin, such as organic debris or animal waste, or liberated from dead microbial biomass. Klieft and co-authors (1987) indicated that 60% to 90% of bacterial population dies back during desiccation; presumably soluble N contained in the dead bacterial biomass would be dissolved after addition of the water. It is also plausible to suggest that some moss cells and tissues do not survive an extreme desiccation and therefore, do not participate in the re-assimilation of N upon re-hydration. Recovery from desiccation damage creates a considerable strain on moss tissues and depleting of energy sources (Noailles, 1978). Conceivably, moss cells that suffered desiccation damage in a moderate degree are likely to recover initially only N essential for the normal functioning, because N assimilation is an energy-consuming process. At the same time, irreversibly damaged moss cells would disintegrate after re-wetting and would not be able to recover any N. N released from senescing tissues would not be re-captured by the recovering moss for several days; this would explain the relative consistency of the 2/3 ratio between re-assimilated N and total N release, across all treatments.

N remaining in the solution at the end of 64 hours would be effectively lost for the moss tissues, as it would likely be either absorbed by the vascular plants (Turetsky, 2003), fungue giphae (Emmerton et al., 2001) or leached down to the mineral soil, where it becomes available to the tree roots. Considering the ability of the moss layer to intercept and retain nutrients, this residual N not taken back up by the moss is likely to be the only source of this nutrient for the roots. Therefore prediction of this value can serve as a tool of estimation of site productivity.

Bacterial activity and availability of nutrients in moss extracts

I found that the antibiotics had no apparent effect on the rate of N re-absorption (Figure II-4). Thus despite the fact the feathermoss layer has a rich microflora (Spiess et al., 1990) it is unlikely that this microflora plays a significant role in the N uptake, at least under extreme drying conditions.

Effect of the rate of drying on the amount and composition of the released mineral N

Feathermosses have demonstrated an ability to conserve N in their tissues despite desiccation damage. Samples that were allowed to gradually dehydrate over a prolonged period of time released significantly less N into the extract as compared to the samples that were freeze-dried. The composition of the N forms extracted from the moss also changed depending on the speed of the drying process. One of the most noticeable changes in the chemistry of N was the reduction of the NO_3^- concentration in the extract (Figure II-5). This observation is in agreement with the previously described data on increased levels of NO_3^- extracted from the bruised moss compared to desiccated moss tissues (Table II-1). In both experiments I have noticed greater NO_3^-/ NH_4^+ ratio in the extracts from moss tissues that were not subjected to a gradual desiccation. This indicates presence of significant amounts of NO_3^- inside normal hydrated mosses (Figure II-5) while practically none was found in the slowly dehydrated moss tissues.

Taking into account the facts that moss suffers desiccation stress regularly, it must be assumed that re-hydrated moss tissues will return to initial levels of NO_3^- through oxidation of NH_4^+ after recovery from desiccation stress. The origin of NO_3^- in the fully hydrated feathermoss tissues, however, is not clear. The NO_3^- found in most natural soils or

plant tissues, is usually produced by nitrifying microflora; but the activity of nitrifiers is suppressed in acidic environment of conifer forests, and generally most of the mineral N is present in form of NH_4^+ (Birch, 1959; Robertson and Tiedje, 1987; Van Cleve et al., 1986). It appears puzzling why more than 40% of the mineral N in the fresh moss tissues is represented by NO_3^-. Possibly, NO_3^- is produced by microflora closely associated with the moss and forms an important storage function, or is an intermediate product in the biochemistry of hydrated feathermosses.

NO_3^- is highly soluble and, as an anion, is unlikely to be retained passively through absorption on the surface of the moss plants, and therefore would be easily leached from the moss layer upon re-hydration. The fact that more N in desiccated samples was in the NH_4^+ form suggests that under the osmotic stress the NO_3^- in the moss was shifted to an organic N form, perhaps as a mechanism to conserve N during the period of membrane damage and repair. Thus, though the integrity of cellular membranes of the dehydrated moss is regularly compromised by dehydration so that protoplasm content leaks into the surrounding solution (Carleton and Read, 1991; Gupta, 1977), mosses have developed defense mechanisms to minimize nutrient loss (Figure II-5). Under gradual onset of osmotic stress moss tissues apparently undergo series of changes in order to protect content of the cells from leaking out, including conversion of valuable nutrients such as N into less soluble forms.

Role of N leakage and re-absorption by the moss layer in forest N cycling

The question still remains, how much of the nutrients released during a dry-wet cycle might be made available to other components of the ecosystem? The previous studies used to determine the amount of nutrients leaked from dried or fresh moss usually involve a one-time water extraction from moss for 30 minutes or 1 hour (Coxon, 1991; Carleton and Read, 1991). If concentration of the surrounding solution is measured only once within 2 hours after submerging of the dry moss tissues in the water, the reading of released soluble N would be of near the all-time peak and overestimate the amount of N that can be potentially available to other organisms. It should be noticed that in all of the experiments moss was exposed to a large amount of water (an equivalent of at least 100 mm of rain in

my experiment) instantaneously. Under natural conditions, water flow would usually be much slower and would cause a smaller amount of nutrients to be extracted from the moss tissues. My data show that if the water is in close contact with the moss, most of the leached nutrients are quickly recaptured. Only about 1/3 of released N that remains in the solution would potentially become available to the microbial and vascular plant consumption.

The fact that some N remains in the solution does not necessarily mean that it will reach the tree root system. Residual N can be assimilated by small shrubs and other vegetation that has roots directly inside the moss layer, or be consumed in microflora, unless the rain is intense enough to wash N down in a relatively short time. The rain intensity alone is not a sufficient condition to deliver N to the mineral soil. First, the moss layer must be reasonably desiccated before the rain event to release appreciable amounts of N to the surrounding water. Therefore, I believe that it would take a large and rapid rainfall following desiccation to move nutrients past the moss layer. My reasoning is as follows:

Desiccated feathermosses can absorb as much as 15 times their weight in water (Dilks and Proctor, 1979). Assuming an average of 1.4 kg of dry moss/m^2 (observed at the study sites is typical for most lodgepole pine forests), this translates into up to 22 mm of precipitation that would be needed to re-wet the moss layer. Thus, a dried moss layer would need a heavy rainfall in a relatively short period (*ca.* 24 hours) for the N to be both leached out and washed away from the moss tissue and active absorbing surfaces and transported into the mineral soil. These relatively rare conditions might occur during mid summer dry periods broken up by heavy thunderstorms. The significance of feathermosses as a storage/exchange source of N for the forest ecosystem (Oechel and Van Cleve, 1986; Carlton and Read, 1990) therefore, probably has been overestimated.

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Figure II- 1. Locations of the moss collection sites.

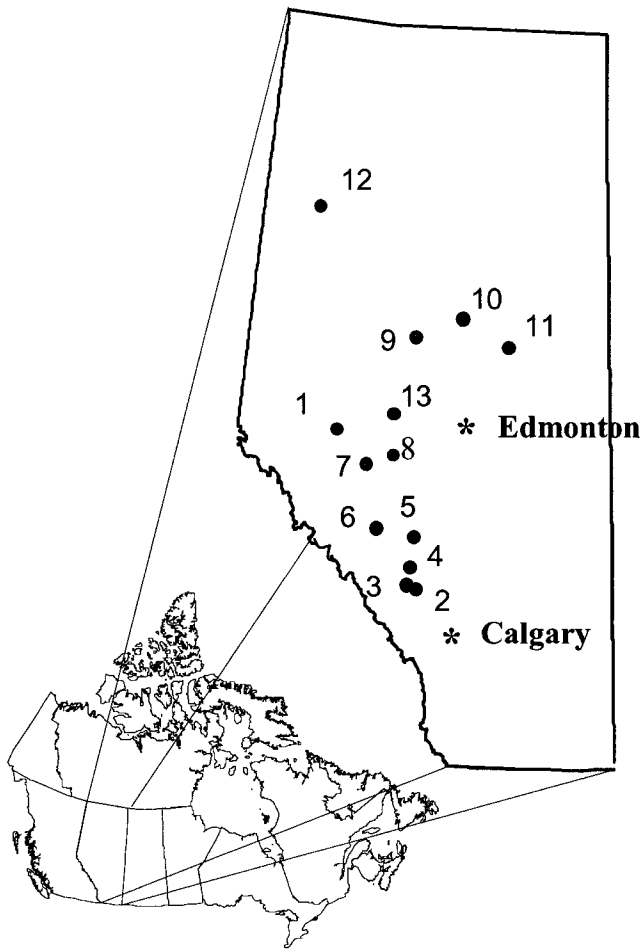


Table II- 2. Location and stand characteristics of feathermoss study sites

Site #	Location	Slope	Species, max DBH (cm) and Ht (m)	Moss cover	Soil
1	South Hinton	South East 4%	LP DBH19, Ht 20 WS undergrowth	Pl 70%, Hc 25%, Pt 5%	Moderately well drained Eluviated Dystric Brunisol
2	South Sundre	North steep 50%	WS DBH25, Ht 18 LP DBH26 Ht20	Pl 50%, Hc 45%, Pt 5%	Rapidly drained Eluviated Dystric Brunisol
3	Yara Creek Road	North East 3%	WS DBH10, Ht 12	Sparse Pl, Pt	Moderately well drained Eluviated Dystric Brunisol
4	Moose Creek	Level	LP DBH28, Ht 22 dense alder brush	Pl 50%, Hc 25%, Pt 25%	Moderately well drained Brunisolic Gray Luvisol
5	Rocky Mountain House	Level	LP DBH37, Ht 16 WS DBH28 Ht 13 WA DBH40 Ht 15	Pl 65%, Hc 25% Di 5%, Pt 5%	Well drained Orthic Gray Luvisol
6	Nordegg	East 30%	LP DBH26, Ht 25 WS DBH 9 Ht 10	Pl 10%, Hc 85%, Pt 5%	Well drained
7	Blackstone	West 20%	LP DBH19, Ht 20 WS undergrowth	Pl 80%, Hc 15%, Pt 5%	Well drained Orthic Gray Luvisol
8	Lodgepole	South West 5%	LP DBH30, Ht 23	Pl 90%, Hc 5%, Pt 5%	Well drained Orthic Gray Luvisol
9	Slave Lake	East 10%	LP DBH18, Ht 20	Pl 70%, Hc 25%, Pt 5%	Rapidly drained Orthic Regosol
10	Smith	North 25%	JP DBH19, Ht 20	Pl 65%, Hc 30%, Pt 5%	Rapidly drained Orthic Regosol
11	Boyle	Level	LP DBH16, Ht 17	Pl 80%, Hc 15%, Pt 5%	Moderately well drained Eluviated Dystric Brunisol
12	Hotchkiss River	South East 2%	LP DBH16, Ht 16	Pl 70%, Hc 25%, Pt 5%	Orthic Gray Luvisol to Gleyed Gray Luvisol
13	East Edson	Level	LP DBH12, Ht 14 WS undergrowth	Pl 70%, Hc 25%, Pt 5%	Moderately well drained Eluviated Dystric Brunisol

List of abbreviations

Tree species:	Moss species:	Stand parameters
LP - Lodgepole pine	Pl - *Pleurozium schreberi*	Ht – Height of the dominant trees
JP – Jack pine	Hc - *Hylocomium splendens*	DBH – Diameter at the breast height
WS – White spruce	Pt - *Ptilium crista-castrensis*	
WA – White aspen	Dc - *Dicranium polysetum*	

Figure II- 2. Peak release of N from rewetted feathermoss, calculated per dry weight of the moss, in relation to the percentage of total N in the dried moss

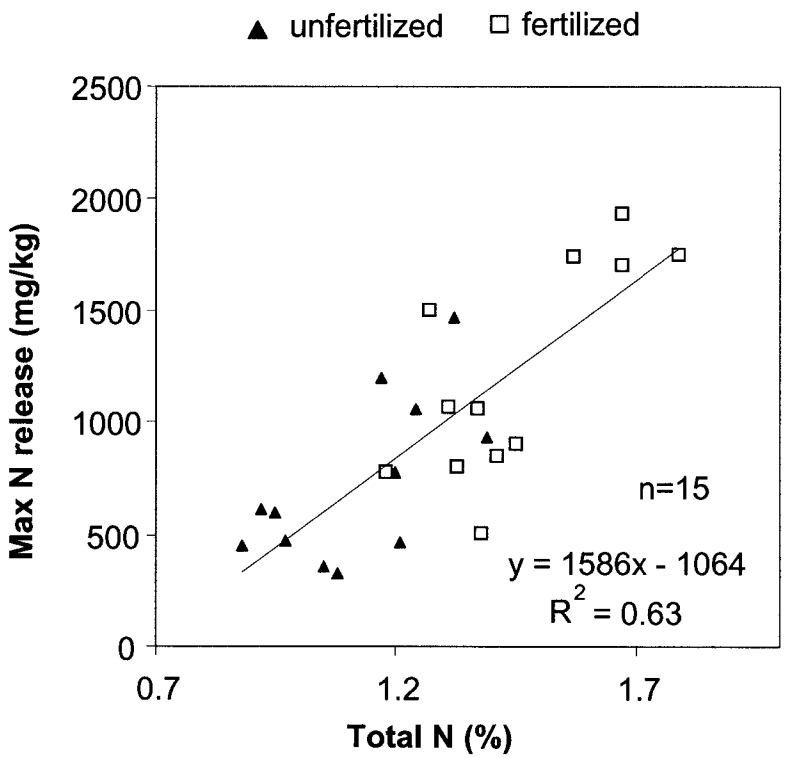
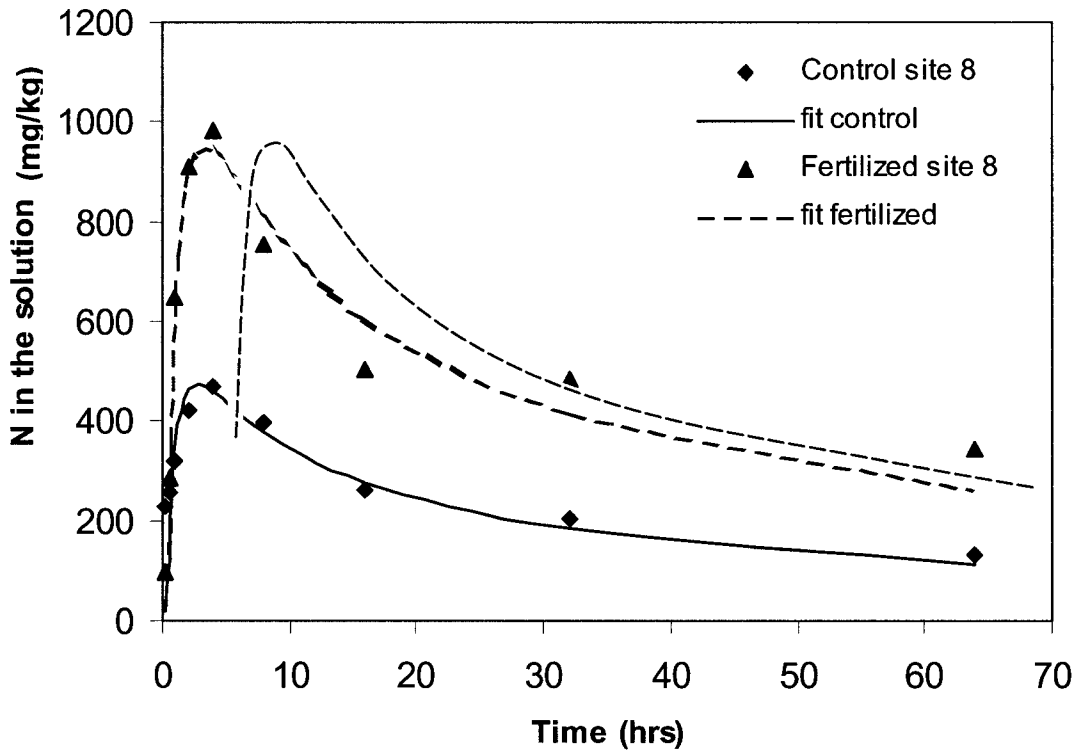
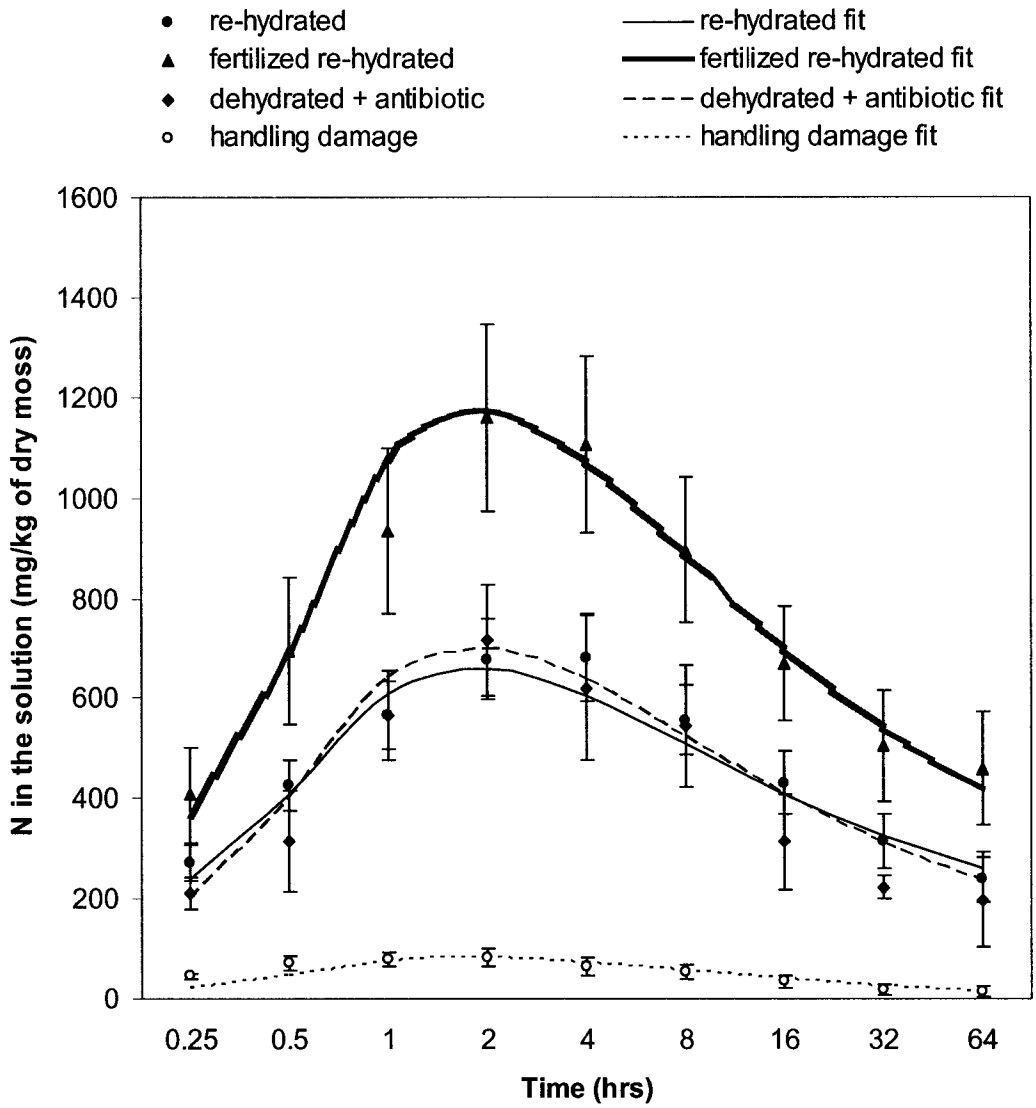


Figure II-3. Example curves of changes in mineral N concentrations in the solution surrounding previously de-hydrated moss tissues and the general fit for these example moss samples



Note: N values are expressed as the proportion of dry weight of the moss and time is expressed in hours since start of bathing in distilled water.

Figure II- 4. Mineral N in solution surrounding moss tissues, given different pre-wetting treatments



Note: Data shown in log scale with the proposed fit lines. Note that data for fully hydrated moss were not significantly different from 0 during the incubation and are therefore not shown on this graph. Each data point was related to 13 individual samples.

Table II- 2. Maximum release and subsequent re-absorption (after 64 hours of incubation) of mineral N from moss

	NH_4^+ release (SE)	NH_4^+ re-uptake (SE)	NO_3^- release (SE)	NO_3^- re-uptake (SE)
	---------- mg/kg of dry moss weight----------			
Control	0.2 (0.2) a	0.2 (0.2) a	0.0 (0.0) a	0.0 (0.0) a
Fertilized	0.2 (0.2) a	0.2 (0.2) a	0.0 (0.0) a	0.0 (0.0) a
Desiccated	738.6 (82.9) c	511.8 (50.7) c	41.0 (12.5) b	34.3 (12.0) b
Fert.+ Desic.	1173.6 (171.3) d	758.1 (146.9) d	74.1 (28.6) bc	42.3 (18.1) b
Desic.+ antit	699.6 (139.9) c	503.0 (83.2) c	10.3 (10.2) b	10.2 (6.8) b
Damaged by handling	13.5 (8.1) b	12.6 (6.8) b	64.5 (16.5) c	59.8 (12.5) c

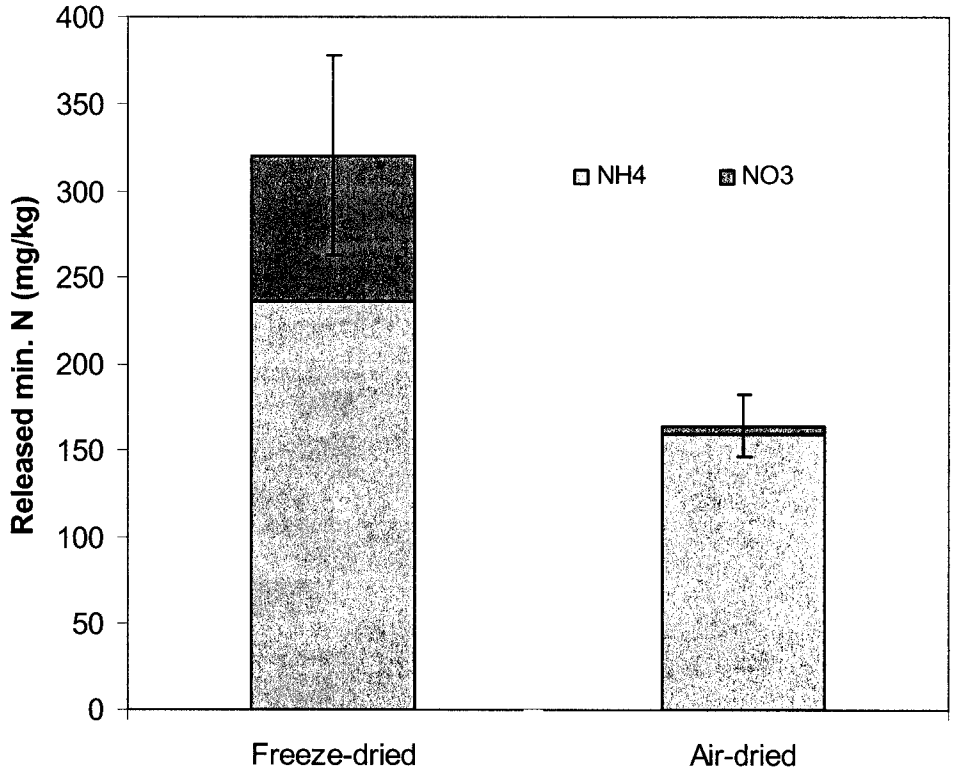
Note: Different letters in the same column, represent significantly different values (t-test, $\alpha = 0.05$).

Table II – 3. Analysis of variance of peak release and residual mineral N in the solution surrounding moss tissues as affected by fertilization and desiccation

Dependent Variable	Source	Degrees of freedom	Type III Sum of Squares	Mean Square	F Value	Pr > F
$NH_4^+{}_{max}$	Site	11	1666152	151468	1.60	0.1435
	Moisture regime	1	10897945	10897945	115.39	<.0001
	Fertility	1	569657	569657	6.03	0.0195
	M*F	1	565432	565432	5.99	0.0199
$NH_4^+{}_{res}$	Site	11	537604	48873	1.39	0.2216
	Moisture regime	1	1115226	1115226	31.82	<.0001
	Fertility	1	75129	75129	2.14	0.1526
	M*F	1	74099	74099	2.11	0.1554
$NO_3^-{}_{max}$	Site	11	35129	3194	1.13	0.3707
	Moisture regime	1	39329	39329	13.92	0.0007
	Fertility	1	3290	3290	1.16	0.2885
	M*F	1	3268	3268	1.16	0.2901
$NO_3^-{}_{res}$	Site	11	17726	1611	0.96	0.5022
	Moisture regime	1	4417	4417	2.62	0.1149
	Fertility	1	1897	1897	1.13	0.2963
	M*F	1	1888	1888	1.12	0.2974

Note: Dependant variables $NH_4^+{}_{max}$ and $NO_3^-{}_{max}$ represent maximum release of NH_4^+ and NO_3^-, N respectively, from moss tissues subjected to re-hydration and leaching into bathing the solution; Variables $NH_4^+{}_{res}$ and $NO_3^-{}_{res}$ represent amount of corresponding compounds remaining in the solution at the end of 64 hours experiment; all values are calculated to the weight of dry moss sample.

Figure II- 5. Water-extractable mineral N content in feathermoss tissues subjected to either a slow air-drying or an immediate freeze-drying



Note: The standard error bars refer to the total amount of extractable mineral N.

Chapter 3. Emission of N_2, N_2O and CO_2 upon rehydration of dry feathermosses

Summary

Three experiments were conducted to examine the apparent loss of N from feathermosses during dry-wet cycles that might occur repeatedly in natural moss beds a typical summer. Dry feathermosses were sealed in containers where N_2 atmosphere was replaced by He. After rewetting and incubating for 20 hours, gas in the containers had significant amounts of N_2, N_2O and elevated levels of CO_2 compared to blanks. In the second experiment, feathermosses went through five dry-wet cycles. By the fifth cycle there was a 9 % reduction in mass of the moss, but there was no accompanying increase in total N in the moss tissues. In contrast, the concentration of P increased and there was a significant decrease in N/P ratio, indicating an actual N loss. Scaling the results from the gas experiment up to the ecosystem level is difficult, but the data suggest that some N will be lost to the atmosphere in gaseous form in each dry-wet cycle. As moss occupies the lowest strata of the forest, a process minimizing available N levels would reduce the vigour of taller components of the forest, providing an advantage to the moss. In the third experiment, I observed that natural ^{15}N enrichment abundance was higher in the mineral soil than in the surface moss layers. The high ^{15}N at depth and advanced decomposition, was presumably due to discrimination against the heavier isotope in the processes leading to volatilization of N.

Introduction

In much of the boreal forests, a continuous layer of feathermosses covers most of the ground. This layer absorbs nutrients released from litterfall (Weber and Van Cleve, 1984). Nutrients are assumed to be partly released later to the mineral soil (Brown, 1990; Coxon, 1991) upon senescing and decay of older moss tissues. In past trials of forest fertilization, as much as 30% of the applied N could not be accounted for in a mass balance of N in the various parts of the ecosystem (Nömmik and Popovic, 1971). This apparent loss of N has been attributed to volatilization of ammonia in the process of hydration of urea

(Birch, 1959; Morrison and Foster, 1977; Foster et al., 1985), but even when NH_4NO_3 was used there were similar discrepancies (Nômik and Moller (1981). My overall objectives of this chapter were to measure gaseous emissions of N and C from the moss tissues subjected to fertilization and /or desiccation, and the effects that the desiccation has on the chemical composition of the moss tissues.

Feathermoss has little drought resistance and is often subjected to desiccation after several warm days without rain. In the first few hours after re-hydration of the moss there is an increased respiration of the moss relative to the normal hydrated condition (Dilks and Proctor, 1979), possibly resulting in measurable expenditure of carbohydrates. Acceleration of microbial activity after re-wetting (Klieft et al., 1987) could lead to consumption of carbohydrate contained in dead and senescing parts of the moss, contributing to the increased rates of respiration in the re-hydrated moss layer

In addition to carbohydrate depletion from the moss tissue, some of the N reserves can potentially be lost from the moss layer in desiccation/re-hydration cycle. There are indications that soluble N leaks from re-hydrated moss after re-wetting (Carlton and Read, 1990; Chapter II). Micro-organisms associated with the moss surface likely die during the desiccation stage and these also provide N-enriched substrate for surviving microflora. (Groffman and Tiedje, 1988; Kiefft et al.,1987). Such flush of nutrients into an organic media, in conjunction the relatively low pH of the moss substrate, could stimulate nitrifiers and denitrifiers present in the moss layer and increase emissions of nitrous oxides and N_2 (Groffman and Tiedje, 1988; Robertson and Tiedje, 1987). Some researchers consider loss of N from the moss layer through denitrification unlikely because of aerobic conditions and low NO_3^- content in moss (Wollum and Davey, 1975). However, numerous studies (O'Hara and Daniel, 1985; Goodroad and Keeney, 1984; Robertson and Tiedje, 1987) suggest the possibility of an alternative path of N transformations including simultaneous nitrification/denitrification (Helmer and Kunst, 1998) in aerobic conditions and chemo-denitrification in acidic organic substrates (Smith and Clark, 1960). Both processes result in production of both N_2 or N oxides, and subsequent loss of N from the system.

The objective of this experiment was to measure gas emissions (N_2, N_2O, and CO_2) following re-hydration of desiccated feathermosses. I also repeated these measurements

following re-hydration of fertilized mosses to determine if the amount of N in the moss tissue could contribute to the emission of nitrogenous gases and affect the respiration rate. Thirdly, I examined the loss of mass and N from feathermosses undergoing a succession of dry-wet cycles. And finally, I tested natural abundance of ^{15}N in the moss layer, organic matter layer and top of the mineral soil as indirect evidence of potential gaseous loss of N.

The null hypothesis tested in this study was as follows:

- There will be no differences in concentration of nitrous gases in the vials containing moss tissues and blank vials
- There will be no differences in nitrous gases and CO_2 production between treatments.
- Multiple desiccation and fertilization treatments will have no effect on total mass, N, P, starch and sugars concentration in the moss tissues.
- That there will be no difference in natural abundance of ^{15}N in the moss layer, the FH layer, and the mineral soil of natural stands.

Methods

Gas exchange experiment

Moss samples were collected from 13 natural lodgepole pine stands in western Alberta (Figure III-1), where 60-90% of moss cover was represented by *Pleurozium schreberi* with inclusions of *Hylocomium splendens*, *Ptilium crista-castrensis* and *Dicranium polysetum.*

Intact moss layers (21 x 50 cm rectangular mats, 1 from every collection location) were cut out, placed in flats and transported to a growth chamber. Photoperiod was set at 16 hours daylight and light intensity at the moss surface level was 50 μmol $m^{-2}s^{-1}$. Temperature was set to 20° C during day time and 16° C at night. Mosses were grown for 14 days and watered 6 times a day using a misting system. After this adaptation period, flats were divided in half and placed in 21 x 21cm trays. Half of the trays were fertilized once with 6 g of crystallized NH_4NO_3, which is equivalent to application of approximately 100 kg N/ha. Mosses were grown for another 3 weeks and then sub-sampled for analysis. During the period after fertilization the total amount of water passing through the moss layer was approximately 70 mm.

In order to measure gas production, including N_2, from living, photosynthesizing moss, a new procedure was developed. Three whole healthy *Pleurosium* plants (approximately 0.1 g dry weight) were carefully placed in the 10 ml glass Exetainer ™ tubes (Photo III-1). Twenty samples were taken from each of the 26 trays (13 control and 13 fertilized). Samples were kept moist and incubated in the greenhouse under light for three days to recover from the handling damage. Half of the tubes were opened and a 10 cm length of diagonally-cut drinking straw was inserted to improve ventilation; then vials were positioned under a ventilation fan in the greenhouse and dried to a constant weight.

Both fresh and dried samples were then sealed with a rubber septum. Air in the vials was replaced with an oxygen-helium mix using the following procedure: two needles were inserted through the septum of the sealed Exetainer ™. One needle was connected to a gas cylinder containing 22% oxygen and 78% helium with the pressure regulator adjusted to 0.32 MPa. The other needle was connected to a 2-meter long tubing to exhaust the gas into the air. Vials were flushed with the oxygen-helium mixture for 90 seconds, and then the needles were pulled out. After 30 minutes, the purging was repeated. After the third purging, additional gas mixture was injected into the vials with a syringe, to bring the internal pressure to 1.9 atm.

1 ml of de-gassed water was injected into each of the purged vials containing dry moss samples. Tubes were rolled gently to ensure complete re-hydration of the moss. All tubes were placed in the growth chamber for incubation. Twelve blank Exetainers without moss were purged with the He mix and 1 mL of degassed water was added and tubes were pressurized to 1.9 atm. Secondly, to test the completeness of N_2 evacuation from the dry moss tissues, dried mosses were purged as above (but no water was added) and incubated as described previously. As no measurable difference was found in N_2 between vials containing only the He/O_2 mixture and dry moss samples and vials after incubation, I assumed that the technique of N_2 evacuation from the dry tissues was satisfactory.

After 20 hours of incubation, a sample of gas in each Exetainers was extracted by syringe and analyzed on a gas chromatograph SRI 8610 C (Perkin-Elmer Company). N_2 and CO_2 contents were measured using a CT1 column and helium ionization detector. N_2O was measured using a MXT Q PIOT column (30 m) and electron capture detector. At the end of the experiment, the Exetainers were opened and moss was dried at 60° C, and

weighed. The mean reading for the blank Exetainers was subtracted from the reading for the samples, and results were calculated in mg of N per kg of dry weight of the moss sample.

Repeated dry/wet cycles experiment

Loss of mass and change in N concentration of feathermoss following repeated dry-wet cycles was determined on circular sections of moss cover ~5cm in diameter (2 per replication, per fertilization treatment). Dead parts were trimmed off and the green portions were placed in glass beakers. Beakers were randomly assigned to two groups; one group was immediately freeze-dried and the other left to air dry to constant weight in the same light and temperature conditions as during the pre-treatment. These samples were carefully re-moistened with distilled water just to their fully hydrated capacity, without any excessive water dripping from the stems and left to air-dry again. Dry-wet cycles were repeated 5 times, and the sample weight was recorded after each period of air-drying. After the last weighing, samples were freeze-dried. Then all samples, including the ones that were not subjected to repeated wetting and drying were ground and analyzed for total concentration of N, P, sugar and starch.

For carbohydrate analysis, samples were ground using a Wiley mill and passed through a 40-sieve. Each sample was treated three times with 80% ethanol at 95 °C. Ethanol extract was analyzed for total sugar using phenol-sulfuric acid (Dubois et al., 1956), and residue obtained after extraction was analyzed for starch by digestion using mixture of α-amylase and amyloglucosidase followed by colorimetric measurement of glucose hydrolysate using a peroxidase-glucose oxidase-o-dianisidine reagent (Rose et al., 1991). The results were calculated per air-dry weight of moss.

Natural abundance of ^{15}N in the moss/soil profile

One lodgepole pine/feathermoss site was selected approximately 30 km east of Edson, Alberta. Moss, organic layer samples and top 5 cm samples of mineral soil were collected from five random points for the analysis of the natural ^{15}N abundance. Moss mats, organic matter and the top 5 cm of the mineral soil were sampled for total N and ^{15}N

analysis. All samples were dried, weighed, and total N and ^{15}N content were determined in the respective layers.

Statistical Analyses

For the gas analysis experiment, data from the 13 collection sites were separately analyzed using 2x2 factorial design ANOVA (desiccation x fertilization, with 4 subsamples per site). A similar design was used for the repeated desiccation experiment, except there was only one replicate for each of the 13 sites (2 fertilization x 2water stress).

Results

Gas exchange experiment

The measurements of gas from the blanks indicated that there was relatively little leakage of N_2 into the vials and the low standard error in the blanks indicated that the seals were not compromised.

Most of the N emissions from the moss tissues occurred in the form of N_2 (Figure III-2). I have observed that N_2 concentration in the Exetainers was significantly higher after desiccation and re-wetting in both fertilized and unfertilized samples compared to the constantly hydrated mosses, but I was unable to find a significant difference between fertilized and unfertilized samples. N_2 levels varied with the site where moss samples were collected ($p<0.05$) (Table III-2).

After desiccation and rewetting, there were greater amounts of N_2O in the incubation tubes than those moss samples maintained continuously moist. N_2O production reached 0.2% of total N content in moss tissue or approximately $2.0x10^{-5}$ kg/kg of dry mass of moss (Figure III-2).

Fertilization increased N content in the moss by 15% (Table III-1). I have assumed that most of the applied N was incorporated into the living moss and microbial biomass or absorbed in dead organic matter, and any extra soluble N compounds were washed out of moss by abundant watering. There was not a significant increase in N_2O gas collected from the fertilized samples, either fresh or dried, compared to their respective unfertilized controls ($p>0.05$) (Table III - 2).

The largest and most consistent effect of the dry-wet cycle was observed as the levels of CO_2 in the overhead space. The amount of CO_2 emitted from re-moistened moss was greater compared to the continuously hydrated moss tissues, and reached 0.02 kg/kg of total moss mass (Figure III- 3). Oxygen content in overhead space of vials with re-wetted moss was reduced to approximately 3% by 20 hours of incubation.

Repeated dry/wet cycles experiment

Moss subjected to repeated dry-wet cycles lost mass in every drying event (Figure III-4). After the first drying, moss lost 2-4% of its mass. In subsequent dry-wet cycles the rate of mass loss decreased, eventually to 1% loss after the fifth desiccation. In total, however, there was nearly 10% loss of mass in the fertilized samples and 7% loss of mass in the unfertilized moss by the end of the fifth dry-wet cycle. The results for the total mass loss were significant ($P<0.05$) (Table III-2). Though the total mass of the moss declined after 5 dry-wet cycles, concentration of total N in the moss did not change, indicating loss of the total mass of N from the moss tissues (Table III-1).

There was no difference in P concentrations between fertilized and unfertilized mosses and while there was a trend for higher P concentration in moss tissues after 5 cycles of drying and rewetting, differences were not significant ($P =0.4$). There was, however, a significant increase in the N/P ratio after the drying and fertilization ($P<0.05$) (Table III-3).

Some of the loss of weight after the repeated desiccation experiment was accounted for in the decrease of the sugars and starches concentration in moss tissues. Starch concentration decreased significantly in all samples subjected to dry-wet cycles compared to the control, but decrease of sugar concentration was significant only for fertilized mosses subjected to the multiple dry-wet cycles (Figure III-5).

Natural abundance of ^{15}N

Natural abundance of ^{15}N in the control samples has increased significantly ($p<0.01$) down the profile (Figure III-6); ^{15}N was greatest in the mineral soil and least in the moss layer.

Discussion

Direct measurement of dinitrogen production from the plant tissues is complicated because of a high probability of contamination by atmospheric N_2 and the difficulty of providing light in the sealed containers to support photosynthesis. The proposed technique of incubating plant samples directly in the glass gas sampling vials (Exatainers ™) addressed both problems and permitted secure measurements of gas exchange of a large sample size. The portability of the Exatainers ™ eliminated a stage of sub-sampling of the overhead gas, thus decreasing a chance of contamination. Samples with inhibited photosynthetic ability remained aerobic for 20 hours of incubation in the sealed glass vials. The method of isolating shoots of mosses in Exatainers and replacing the N_2 with He was successful in excluding exogenous N_2 from the experimental system. Low concentrations of N in the blanks with added de-gassed water shows that the system was reliable at controlled N_2 leakage and contamination.

I have observed that the moss tissues produced considerable amounts of N gases during a 20-hrs incubation. The greatest emission of N-gases took place after re-wetting of the dry moss (on average 300 mg/kg of dry sample weight). Most gas emissions occurred in the form of N_2, though some N_2O production from re-wetted moss was also recorded. On average N_2 emissions exceeded production of N_2O by at least an order of magnitude, corresponding to 1% and 0.1% of the total N in the moss, respectively (Figure III-2). Evidently recovery of the moss tissues after a desiccation stress was associated with a significant loss of N.

Apparently some N_2 was produced from fully hydrated moss tissues. However, it is likely that some N_2 was dissolved in the living protoplasm of moss tissues (1 ml of water can dissolve 0.016ml of N_2 or approximately 1.3% of the total volume of the vial) and gradually emanated during the incubation. Difficulty of evacuation of dissolved N_2 from the hydrated moss tissues may have resulted in overestimation of N_2 production in the control samples.

Data collected in multiple dry-wet cycle experiment supports the hypothesis that nitrogenous gases are emitting from re-hydrated moss tissues. The fact that N concentration in the moss tissues did not change after repeated desiccation, despite a considerable loss of mass can only be explained by the decline of N content in moss

simultaneously with the other components of biomass (Figures III-2, III-3 and III-4). However, as both N and C are lost from the fertilized moss tissues, concentration of P in the remaining biomass increased (Table III-1), providing a reference for the rate of volatilization. Assuming that P content was not directly affected by repeated dry-wet cycles, decline of N/P ratio indicates that N content was reduced during the experiment.

The process leading to conversion of organic N into N_2 or N-oxides is not immediately obvious. When discussing gaseous losses of N from ecosystems, most researchers (Smith and Zimmerman, 1981; Keeney, 1980) assume that the main source of N-gases production is a dissimilative denitrification in anaerobic conditions. Aerated moss layers, with inherently low concentration of NO_3^-, therefore, appear to be an unlikely media for denitrification losses of N. However, several other studies have demonstrated production of N oxides from aerobic soils (Johansson and Galbally, 1984). Aerobic nitrate reduction can occur in neutral or acidic conditions (Stevenson et al., 1970) provided that there is organic matter present and there are elevated levels of soluble N. N_2, N_2O, NO and NO_2 can also be produced in aerated acidic media in the presence of NO_2 and NH_4^+ (Smith and Clark, 1960; Allison et al., 1952) as a result of an abiotic reaction. A second reason why possibility of biological nitrate reduction is discounted in these types of systems is the relatively low concentrations of NO_3^- in the boreal forests. However, it is well-demonstrated that moss tissues that become dehydrated will readily release inorganic N to the surrounding solutions upon rewetting (Carleton and Read, 1991; Gupta, 1981), some of it in the form of NO_3^-. Another effect of desiccation is death of microbial population during dehydration which creates an additional source of N (Klieft et al., 1986). During a short period following re-hydration there is an increase of available N, in the acidic environment of the moss layer (Chapter II), with easily available organic matter; this creates all of the necessary conditions for aerobic denitrification (Stevenson et al., 1970; Davidson, 1992) and chemical nitrate reduction (Smith and Clark, 1960) resulting in emission of nitrogenous gases as discussed above.

I found that respiration rate has increased after re-moistening of the dry moss; total production of CO_2 reached 2% of the dry weight of the moss samples during the first 20 hours of incubation (Figure III-3). Both the gas emission and repeated desiccation experiments indicated that C was converted to CO_2 after rehydration.

Increase in respiration after desiccation of moss has been attributed to an increased consumption of energy required for the repair of tissues (Dilks and Proctor, 1979). Marked depletion of starch content (Figure III-5) is in agreement with the studies by Noailles (1978), who has described disappearance of the starch grains from the chloroplasts of re-hydrated moss cells.

The decrease in starch concentration (0.12 %, Figure III-5), however, was not sufficient to account for the loss in total mass of the moss (Figure III-4). Presumably a portion of the CO_2 release was related to a boost of microbial activity in the presence of elevated levels of nutrients. Klieft and his co-authors (1986) demonstrated that up to 60% of total bacterial biomass is turned over in re-wetting of the substrate, resulting in rapid increase in bacterial respiration associated with the bacterial consumption of the dead cells.

High levels of CO_2 in the overhead space of the vials of fresh moss samples can be partially explained by the stress during the air replacement and pressurizing the vials. When vials with healthy moss shoots were incubated under the light for longer periods of time (72 hours) the CO_2 concentration decreased, indicating moss recovered sufficiently for photosynthesis to surpass respiration.

A continuous moss layer in boreal forests intercepts most of the N input in the ecosystem (Brown, 1990). The feathermoss layer is capable of sustaining photosynthetic activity comparable with assimilation by the tree foliage on the same site (Martin and Adamson, 2001). However, the feathermoss layer is frequently subjected to desiccation during the summer period. Complex biological processes associated with nutrient turnover during the period of re-hydration are likely to result in considerable gaseous loss of N, presenting a drain of already limiting resource. Although emission of N_2O is comparatively small, suggesting that conversion of organic N to N_2O is of lesser importance as a pathway for the gaseous loss of N, it might be considered as an important source of a greenhouse gas in the atmosphere.

An indirect evidence of the increase in natural ^{15}N enrichment with depth also points to the likelihood of gaseous losses of N in the moss layer. Studies by Nômmik with co-authors (Nômmik et al, 1994) and Högberg (1991) have indicated that the process of N transformation discriminates against heavier isotopes and that forests with active gaseous N loss usually have increased concentration of ^{15}N (Högberg, 1991). Plant debris deposited

on the moss surface has the lowest ^{15}N proportion. As the litter decomposes and passes through the moss layer, we can reasonably assume that part of the N volatilizes and the remaining N becomes enriched with ^{15}N.

As feathermosses occupy the lowest strata in the forest, depleting the available N in the system may be a strategy to weaken the vigour of and reduce shading components of the trees and shrubs of the system.

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Figure III- 1. Locations of the moss collection sites.

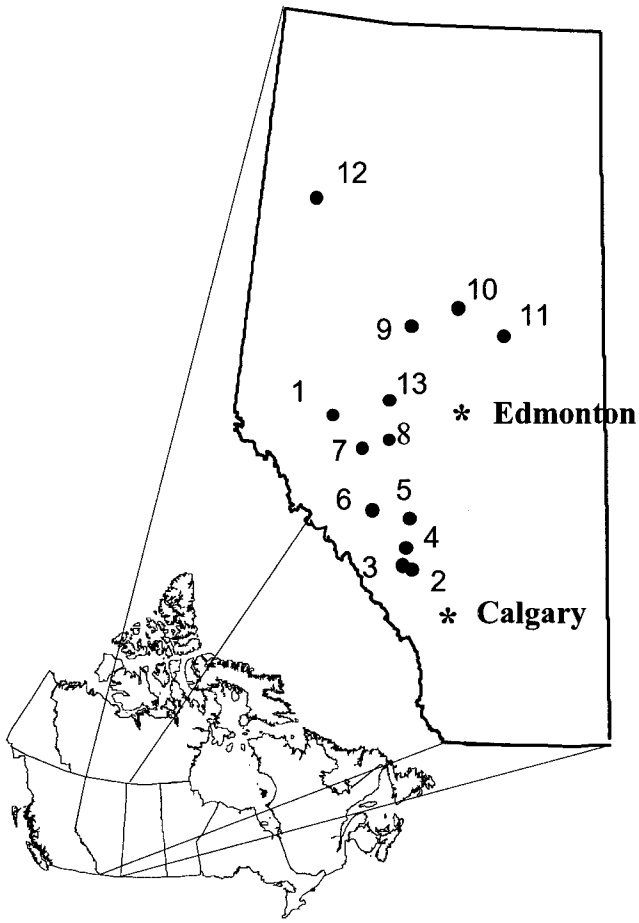
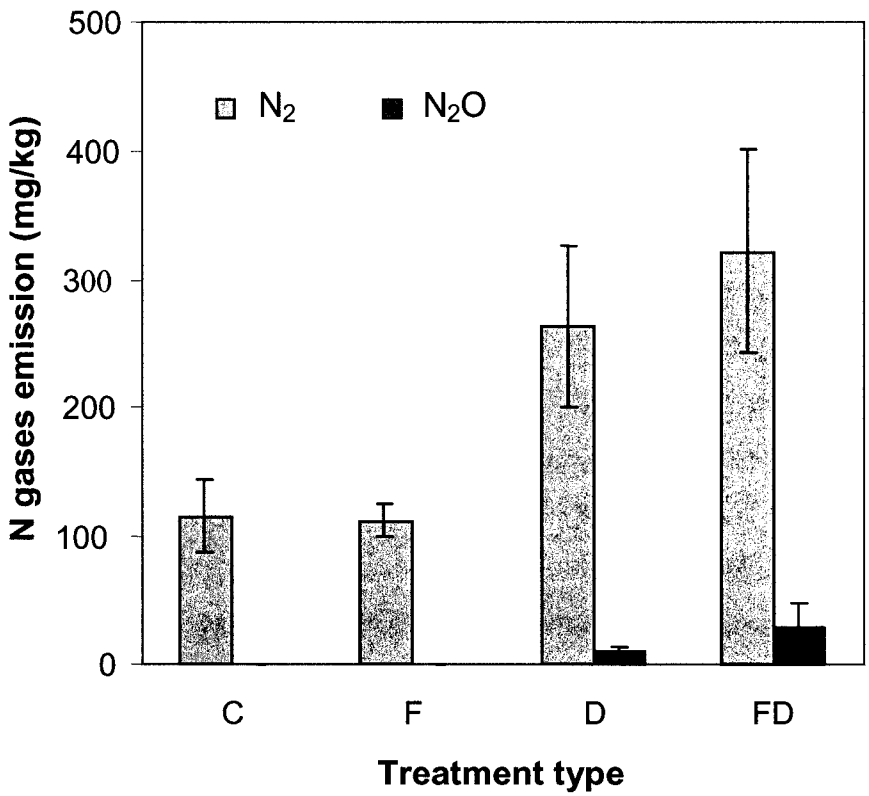


Figure III- 2. Emission of N_2 and N_2O from the moss tissue that remained continuously moist or from mosses that were dried and rewetted (D) (expressed in kg N per kg of the dry moss tissue).



Note: Treatments are: C – control, F – fertilized moss, D – moss dried before the experiment, FD – moss fertilized and dried before the experiment (n=12).

Figure III- 3. Emission of CO_2 from moss tissue subjected to fertilization and/or a single drying/re-wetting cycle (expressed in kg/kg of the dry moss tissue, after 20 hours of incubation). The treatments are the same as in Figure III - 2.

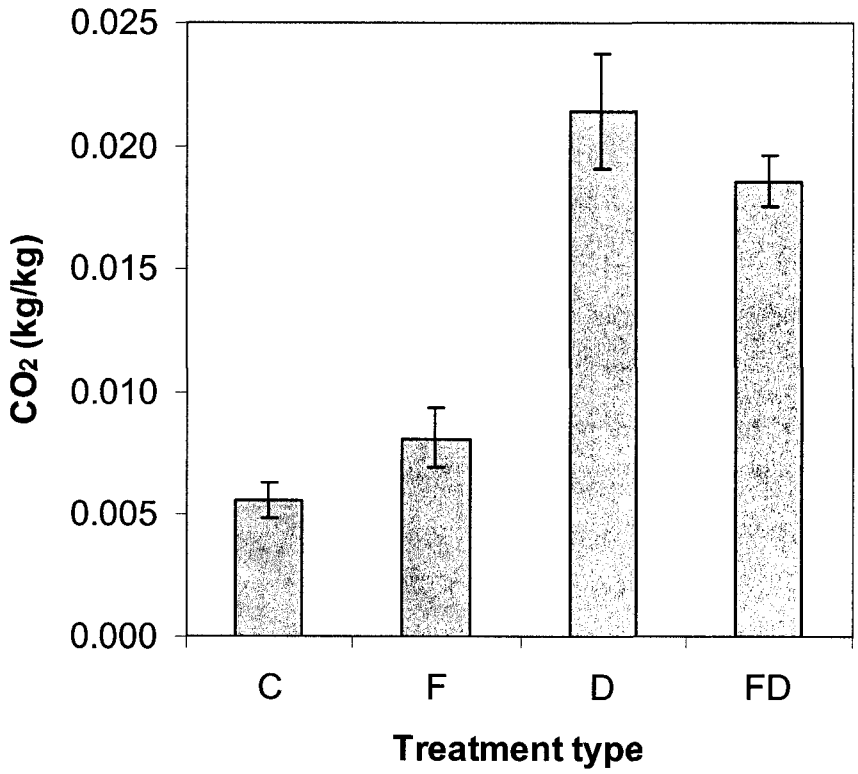
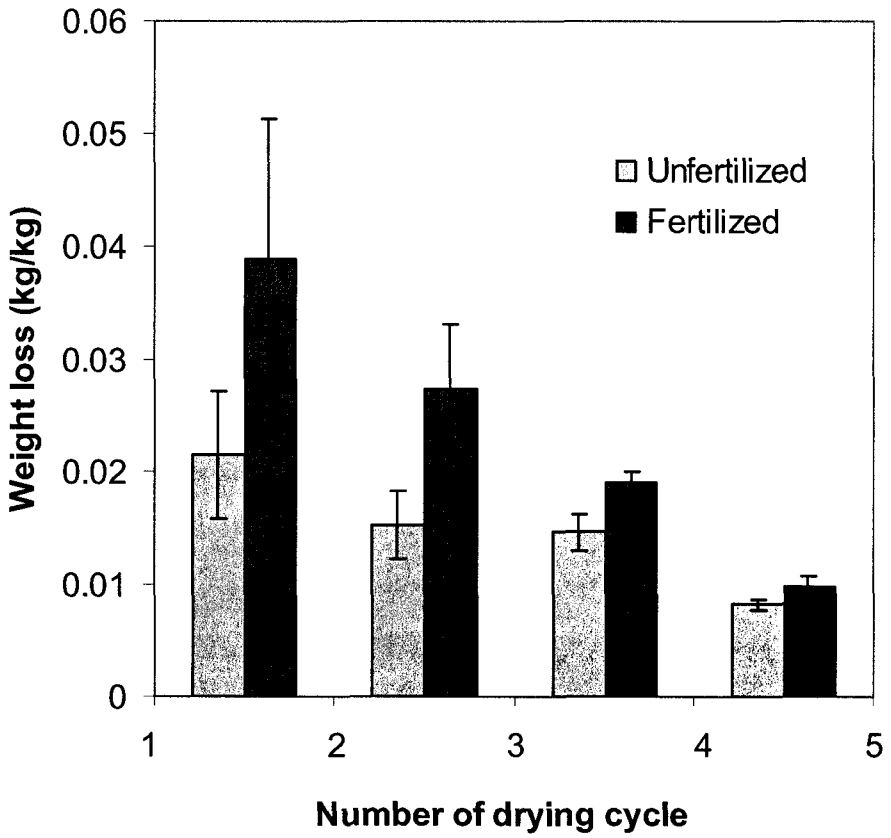


Figure III- 4. Loss of mass of fertilized and unfertilized feathermoss over repeated dry/wet cycles.



Note: Weight of the moss samples after each drying was compared to the initial air-dry weight (i.e., the first drying time) and the weight differences calculated as a fraction of the initial weight.

Figure III- 5. Sugar and starch concentrations in the moss tissue after repeated dry/wet cycles. The treatments are the same as in Figure III – 4.

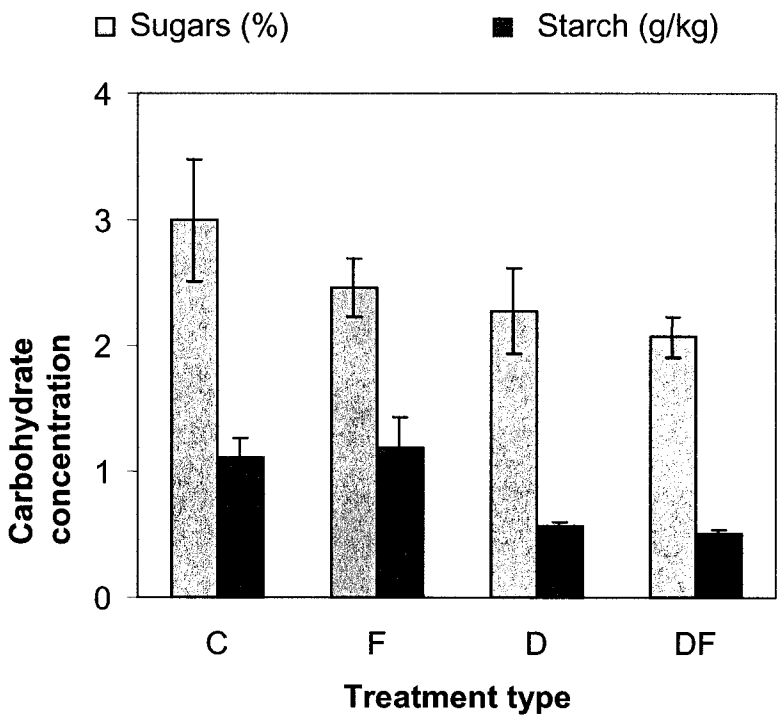
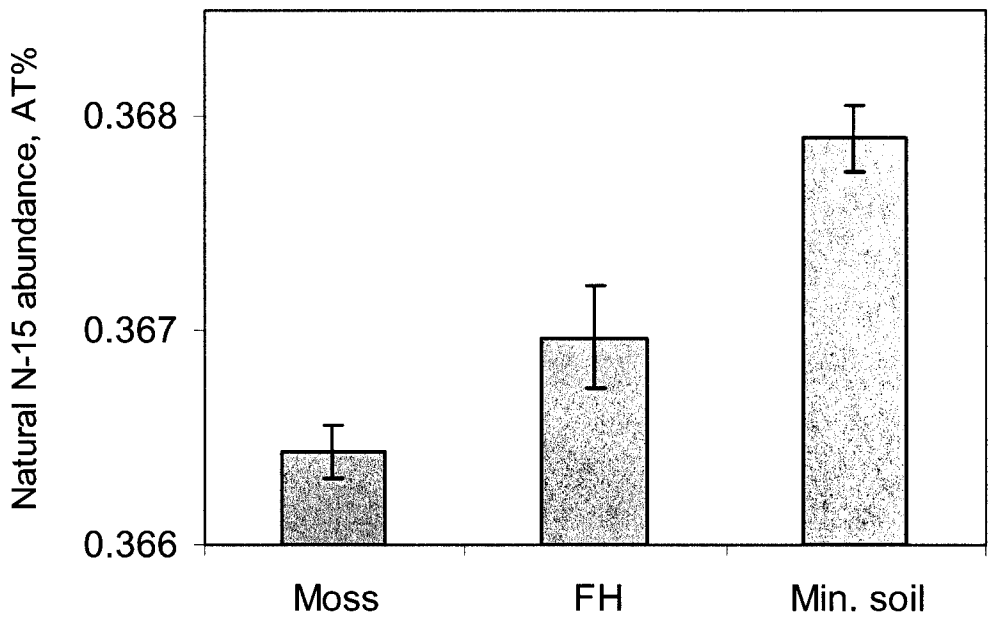


Table III- 1. Effect of 5 dry/wet cycles on total nitrogen and phosphorus content and mass decline of the moss samples (n=13).

Treatment	Total N concentration	Total P concentration	N/P ratio	Total weight loss
	kg/kg $*10^{-2}$	kg/kg$*10^{-3}$		kg/kg
Control	1.21 (0.08)a	1.24 (0.05)a	9.8 (0.3) b	N/A
Fertilized	1.41 (0.05)b	1.21 (0.05)a	11.8 (0.5)d	N/A
Desiccated	1.22 (0.06)a	1.29 (0.04)ab	9.3 (0.3) a	0.071 (0.008)a
Fertilized + Desiccated	1.41 (0.05)b	1.30 (0.04)ab	10.9 (0.2)c	0.103 (0.015)b

Note: Means in same column with similar letter are not significantly different ($\alpha = 0.05$).

Figure III-6. Natural abundance of ^{15}N (no ^{15}N added) for the various strata in the moss/soil columns.



Note: Apparent increase in abundance indicates discrimination against heavier isotope in decomposition and gaseous loss of N.

Photo III- 1. Incubation of the moss tissues in an Exatainer™ (shown in real size).

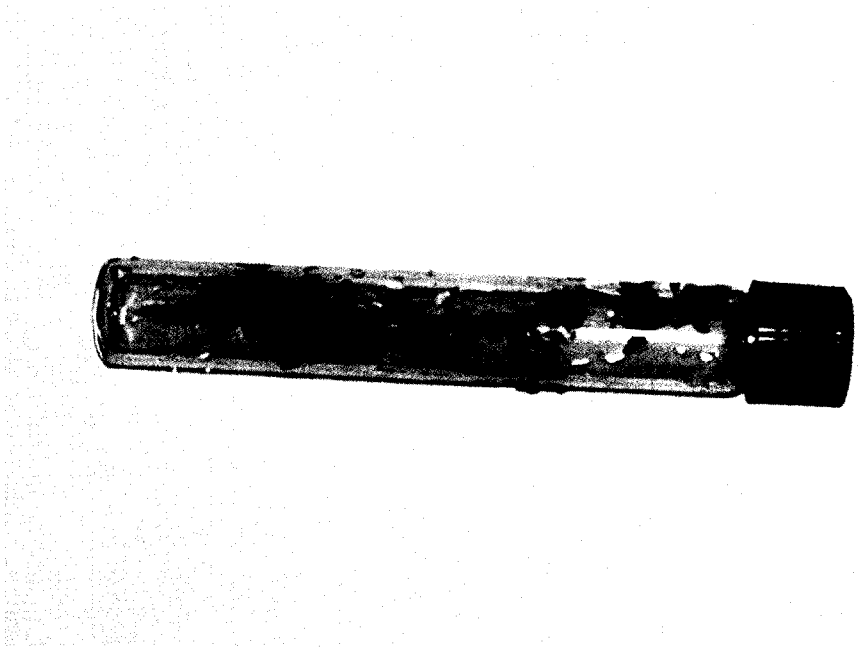


Table III – 2. Analysis of variance of gas emissions from moss tissues as affected by re-hydration and fertilization.

Dependent Variable	Source	Degrees of freedom	Type III Sum of Squares	Mean Square	F Value	Pr > F
N_2O	Site	12	8.150E-09	1.358E-09	1.14	0.3555
	Moisture	1	5.112E-09	5.112E-09	4.28	0.0441
	Fertility	1	1.358E-09	1.358E-09	1.14	0.2916
	M*F	1	1.341E-09	1.341E-09	1.12	0.2947
N_2	Site	12	4.763E-07	7.939E-08	2.31	0.0497
	Moisture	1	4.513E-07	4.513E-07	13.11	0.0007
	Fertility	1	1.039E-08	1.039E-08	0.30	0.5854
	M*F	1	1.319E-08	1.319E-08	0.38	0.5389
CO_2	Site	12	1.267E-04	2.111E-05	0.7	0.6530
	Moisture	1	2.413E-03	2.413E-03	79.72	<.0001
	Fertility	1	2.800E-07	2.800E-07	0.01	0.9235
	M*F	1	1.010E-04	1.010E-04	3.34	0.0742

Table III – 3. Analysis of variance of N, P, sugars and starch concentration in the moss tissues as affected by fertilization and repeated desiccation/rewetting.

Dependent Variable	Source	Degrees of freedom	Type III Sum of Squares	Mean Square	F Value	Pr > F
Total N in moss tissues	Site	12	1.608	0.134	7.07	<.0001
	Moisture	1	0.000	0.000	0.02	0.8997
	Fertility	1	0.503	0.503	26.51	<.0001
	M*F	1	0.000	0.000	0.00	0.9952
Total P moss tissues	Site	12	0.000	0.001	2.61	0.0130
	Moisture	1	0.000	0.000	0.70	0.4070
	Fertility	1	0.000	0.000	0.53	0.4718
	M*F	1	0.000	0.000	0.93	0.3401
Sugars content	Site	12	20.857	1.738	1.55	0.1506
	Moisture	1	4.050	4.050	3.62	0.0651
	Fertility	1	1.727	1.727	1.54	0.2221
	M*F	1	0.338	0.338	0.30	0.586
Starch content	Site	12	0.057	0.005	2.39	0.0219
	Moisture	1	0.048	0.048	24.08	<.0001
	Fertility	1	0.000	0.000	0.01	0.9203
	M*F	1	0.001	0.001	0.33	0.5688
Mass loss	Site	12	14.75	0.314	3.32	0.1386
	Moisture	1	2.518	2.522	8.21	0.0062
	Fertility	1	1.971	1.968	6.43	0.0150
	M*F	1	0.514	0.509	1.62	0.2100

Chapter 4. Effects of moss removal, thinning and fertilization on lodgepole pine growth, soil microclimate and N availability

Summary

The influence of a continuous feathermoss layer on the soil microclimate and tree growth was assessed in a five year study. The feathermoss layer was manually removed from twelve 900 m^2 plots in lodgepole pine stands in North-western Alberta. Changes in soil temperature and water content were monitored using thermocouples and dataloggers. In order to compare the ability of the moss layer to compete for the water and nutrients within the trees, the moss removal treatment was compared with undisturbed plots and tree thinning treatment. The role of the moss layer in the interception and retention of nutrients was estimated in N fertilization trials with and without moss removal.

Removal of the moss layer increased summer soil temperatures as well as the length of the period when soil was not frozen, but it also increased the depth of the frost penetration during winters with below average snow cover. Thinning had little effect on the rate of tree growth during the first 3 years after application, but produced a significant increase in growth during the fourth year. Fertilization had a consistently positive effect on the radial stem increments and N content in the foliage regardless of the presence of the moss layer on the trial plots.

Introduction

Feathermosses are widely distributed across the boreal regions of the world. A blanket of feathermosses often covers most of the ground surface and in many forests retains as much or more photosynthesizing biomass than the trees on the same sites (Martin and Adamson, 2001). Continuous layers of feathermoss are thought to play a significant role in the nutrient cycling of these forests (Brown, 1990; Oechel and Van Cleve, 1986), capturing a significant part of the N input from rainwater and litterfall (Brown, 1990). Only a small fraction of N deposition on the surface of the moss layer passes through to the

mineral soil (Weber and Van Cleve, 1984). It is thought that these forest ecosystems depend on N release from the moss layer as a major source of this nutrient (Carleton and Read, 1991; Weber and Van Cleve, 1984). The general assumption is that mosses release the captured N after a period of time, likely through the death and decomposition of older portions of the moss stems at the base of the moss layer (Schulze et al., 1995).

A decline in growth of lodgepole pine in stands older than 50 years old (Oechel and Van Cleve, 1986) is accompanied by the establishment of a uniform moss layer. A thick carpet of moss insulates the mineral soil, reducing both seasonal and daily amplitudes of temperature fluctuations (Viereck et al., 1993) and intercepts the flow of nutrients (Weber and Van Cleve, 1984). I hypothesize that stands with thick continuous moss layer might have reduced tree productivity due to colder soil and decreased nutrient supply.

Accepted forestry practices, such as fertilization and thinning often have limited effects in mature stands. Presence of a continuous moss layer might contribute to the decrease of the treatment efficiency. Moss intercepts most of the surface-applied fertilizer (Brown, 1990), and competes for resources with the trees. Thus, pine growth is affected not only by intraspecific competition with other trees, but also interspecific competition for water and nutrients with the moss layer. The extent of the effect of continuous moss layer on nutrient supply and tree growth can be assessed by manipulations of the moss layer such as killing it (Norberg et al., 1998) or physically removing it from the surface (Viereck et al., 1993). The objective of this study was to compare the effects of moss removal, thinning or fertilization on stand growth and N supply.

The null hypothesis tested in this study was that moss removal, thinning and fertilization will have no effect on soil microclimate, N availability and pine growth

Methods

This study was established in 1998 in a 90-years-old lodgepole pine stand (Photo IV-1) located in North Western Alberta (latitude 57° 02' and longitude 117° 30'). Mean annual precipitation is 410 mm, average January temperature is - 17 °C and July temperature is 16 °C. The stand had an average density of 1750 stems per hectare and basal

area of 38 m^2/Ha, had a continuous moss cover, 8 cm thick, and dominated by *Pleurozium schreberi* (80%). Other species ranked in declining importance were *Hylocomium splendens*, *Ptilium crista-castrensis* and *Dicranium polysetum* (Photo IV-2). The research site was located on a 2% south-east slope. The soils were an Orthic Gray Luvisol on the upper part of the slope and Gleyed Gray Luvisols on the lower part (Photo IV-3).

To assess the site and stand conditions, four 900 m^2 plots were selected. Within these plots, all trees were numbered, their status recorded, and height and DBH were measured. To determine the amount of moss and organic matter at the site, bulk samples of moss and FH layer were collected from each plot using a 17 cm x 43 cm metal frame. Each sample was divided into three fractions containing live moss, debris and FH material. The samples then were dried at 70°C, and the dry weight of each fraction was determined per area. Three experimental treatments, with four replications each, were imposed on 12 similar 900 m^2 plots: control (C), moss removal (R) and forest thinning (T) (Figure IV-1). The control was undisturbed. Moss and most of the organic layer were removed manually using manure forks, with minimal mechanical disturbance to the mineral soil. A small amount of H layer (depth less than 1 cm) was occasionally left on the surface of the mineral soil. Thinning was performed on the same plots that were used for the stand survey. Thinning was carried out from below, to remove 35% of the basal area, reducing the stem density from 1750 to 850 stems per hectare, and leaving residuals well spaced (Photos IV-4 and IV-5). An untouched buffer of at least 5 m surrounded each plot.

Eight additional 200 m^2 plots were established at the same time to measure the effect of fertilization on pine productivity: fertilized only (F) or fertilized combined with moss removal (FR). Fertilizer was granulated, agricultural grade urea and was manually applied at the rate of 200 kg/ha of N, in April 2000.

Monitoring of the forest stand

To monitor microclimate effects of the treatments, sensors were installed in two locations: in the middle of a control plot and in the middle of a plot with moss removed. Thermocouples were placed inside white, screened shelters at 1.4m height to record air temperature, and soil temperature and water potential were assessed directly under the moss layer at the interface with the mineral soil (if moss was present), and at 5, 10 and 30

cm depths, using thermocouples and gypsum blocks. Snow depth on the control plot was monitored during the winters in 2000, 2001, and 2002 using a snow sensor.

Changes in the tree growth were assessed by measurement of ring increments and sapwood area in the cores collected from five dominant trees in every plot in 2001 and again in 2003. Relative annual tree ring increments for each year were calculated as a ratio between post-treatment radial increments to the average of 4 pre-treatment increments for each tree. The foliage biomass on the site was estimated using correlation between sapwood area and the total foliage biomass (Dean and Long, 1986).

Light intensity was measured simultaneously under the canopy and in a nearby open area using an AccuPAR ™ PAR ceptometer. Measurements were conducted between 12 and 1 PM, on a clear day, at 10 random points in each location. At each point, light was measured in four directions and an average reading recorded. The control measurements were taken before and after taking readings at each plot, on a clearing adjacent the north side of the research site. Average light levels were expressed as a percentage of the control readings.

Effect of moss cover on N cycling and on the fate of applied nutrients

Site nutrient status was assessed twice, in September of 2000 and 2001, by collecting samples of litter, mineral soil and moss using the following techniques: At every plot, 10 sampling points were selected: one in the centre of the plot and nine on the perimeter of a 2m radius circle around the centre, roughly equally spaced from each other. Bulk samples of organic layer and mineral soil at the depth 0-2.5, 2.5-5, 5-7, 7.5-12.5 and 12.5-25 cm were collected at each point and samples from the same depth were combined for each plot. Composite soil samples were brought to the lab, extracted with 2M KCl (Carter, 1993) and analyzed for NO_3^- and NH_4^+ concentration and results were expressed in mg/kg of available N.

In order to measure bulk density of the mineral soil, four 132.5 cm^3 cores were collected in 1999 from each sampling depth at one representative location next to the research site. Cores were dried overnight at 105°C and weighed to determine soil bulk density. The average bulk density for each depth was used to calculate the nutrient pools per area basis.

Samples of the foliage were collected in September 2000, 2001 and 2003 by shooting at least 3 branches from the upper part of the crown of 3 dominant trees in each plot. The needles were separated by age class, dried at 58°C, and the weight of 100 needles determined. Dry foliage samples were ground and digested using the peroxide method (Carter, 1993) and analyzed for the total N and P content. N pool in the foliage per area basis was calculated using average N concentrations for each plot and estimated needle biomass (see above).

Vector Diagnosis

Growth and N status (i.e., concentration and content) of the pine trees grown in control plots and plots subjected to thinning, fertilization and/or moss removal treatments were evaluated by the vector diagnosis technique (Timmer, 1991; Haase and Rose, 1995). This technique has been applied previously to studies on the effect of silvicultural treatments on the early growth of white spruce seedlings (Munson et al., 1993). The vector diagrams (Figure IV-7) compare the relative responsiveness of the lodgepole pine foliage in terms of N content (x-axis) and N concentration (y-axis) using the foliage collected from the trees grown in the control plots as the reference, which is normalized to 1. Traditionally, vector diagrams are used for the assessment of change in nutrient supplies in the individual plants before and after fertilization (Timmer, 1991; Haase and Rose, 1995); however, use of vector analysis as applied to the averaged results of the randomized plots (treated vs. control) was successfully applied by Hangs and co-authors (Hangs et al., 2003).

Fertilization and Feathermoss Growth

In order to assess effects of thinning and fertilization on annual moss growth, samples of *Hylocomium splendens* were collected from the centers of the control and fertilized plots in 2001 (n = 4 for each treatment). In each plot with moss cover, ca. 100 *Hylocomium* shoots were collected and annual growth segments were clipped from the moss stems, sorted by age, pooled in each of 5 age classes and dried at 58°C. Changes in the growth rate were assessed by comparing dry weight each of the pooled annual segments with the average dry weight of annual increments of all 5 age classes. Segments were ground and total N content was analyzed using the same technique as for the pine

needles. N and P concentration and relative content were subjected to the vector analyses as described above.

Statistical analysis

The effects of the moss removal and thinning on tree growth, soil nutrient content and microclimate were compared to control plots were estimated using t-tests. The effects of moss removal and fertilization on nutrient availability and pine growth were compared using ANOVA in a 2x2 randomized factorial design. For this purpose foliar and soil samples collected from the center of 0.02 ha fertilized plots were compared to the samples collected from the center of 900m^2 unfertilized plots with and without moss removal.

Results

Effects of moss removal

Removal of the moss cover resulted in significant changes in the soil microclimate, including an increase in the fluctuations of the soil temperature and the extent and length of soil freezing (Figure IV-2). In December of 2002 the soil freezing reached the depth of at least 0.3 m on the plots where moss was removed and soil temperature in these plots dropped below –13°C at 5 cm depth. Annual degree-days (base t = 0°C) in the mineral soil at a depth of 5 cm increased by 21% (from 1215 ° to 1473°), and degree-days (base t = 5°C) increased by 72%. Removal of the moss had little effect on soil water potential during the warm months (Figure IV-3). Abrupt changes in water potential at the time of thawing (Spaans and Baker, 1996) occurred 2-3 weeks later in the moss plots as compared with plots where moss cover was removed (Figure IV-3)

Moss removal had little or no effect on the amount of available N in the mineral soil (Figure IV-4). Foliar concentration of N was lower one year after the moss removal than in control trees (Figure IV-5), though this was not reflected in the needle weight (Figures IV-6, IV-7, and; Table IV-1; also see Figure IV-8). It is noteworthy that the direction of the vectors immediately following N fertilization tended to move in the direction of deficiency but the moss removal had little influence on the vector (Figure IV – 7). By 2003, however, the moss removal plots tended to move in the direction of 'toxicity' relative to the control (Figure IV – 8).

Effect of moss removal on the radial growth was not consistent from year to year. In 1999 some positive changes in the stem growth were observed (Figure IV-9), but during the years 2001 and 2003 radial increments of the trees on the plots subjected to the moss removal tended to be lower than in control plots.

Effects of fertilization

Fertilization increased total N content in all age classes of needles (Figure IV-5, Table IV-1), including the cohorts that have formed after fertilization. However, increased size of the needles occurred only in post-fertilization cohorts (Figures IV-6, IV-7 and IV-8). A significant increase in radial increments was noted within 2 years following application of the fertilizer (Table IV-2). Differences in available N content between fertilized and unfertilized plots decreased by the second year after application of fertilizer (Figure IV-4), and was followed by decrease in total N concentration in the needles 2 years later (Figure IV-5). Annual radial increase by 2001 was greater on the plots with moss cover removed.

The moss layer responded to fertilization by increase in annual growth (Figure IV-10) and N content in tissues (Figure IV-11), and accelerated decomposition of the dead parts.

Effect of forest thinning

Thinning increased light intensity under the crowns from 12% prior to thinning to 40 % after thinning, apparently decreased crown retention of precipitation, especially interception of snow (see Photo IV-9). No differences in radial increments were recorded for the first two years after thinning but by years 3 and 4 I observed an increase of radial growth in the thinned plot relative to the control (Figure IV-9). Needle size and weight were significantly greater on the thinned plots than under any other treatment. Total moss biomass in the thinned plots has decreased by approximately 15% in the thinned plots.

Discussion

Effect of treatments on soil microclimate

The most noticeable effect of moss removal was associated with an increase in soil temperatures during the summer, earlier spring thawing of the mineral soil and a wider range of soil temperatures throughout the year (Figure IV-2). Overall there was an average of 70% increase in degree-days (base t = 5°C) over 3 years following moss removal, which signifies a considerable change in the soil microclimate. Insulating properties of moss and thick layer of litter were observed in earlier studies (Bonan, 1990, 1991), indicating that the moss layer is a significant factor controlling soil microclimate in boreal forests. In my study, moss removal had little effect on soil water potential. Apparently, increased surface evaporation was counteracted by greater amount of precipitation that reached the mineral soil instead of being retained by the moss layer.

An increase in soil temperature was expected to produce a favorable growing environment for the tree roots, to accelerate decomposition of organic matter and, consequently, to increase the rate of nutrient mineralization. Early spring warming of the exposed mineral soil (Figure IV-2) decreased the risk of physiological drought, when the water uptake is restricted by low soil temperatures (Coates et al., 1991). However, these positive effects were overridden by greater winter freezing of exposed mineral soil, especially when the snow cover was below average (Figure IV-2). For example, during the winter 2002 soil temperature on the plots where moss was removed fell to –13°C, well below the ability of the pine roots to resist the cold (Coleman et al., 1992; Vapaavuori et al., 1992).

Although no direct measurements of soil microclimate was conducted in the thinned plots, the indirect evidence of increased solar irradiation at the forest floor indicates a possibility of warmer soils under this treatment. Reduced crown retention after thinning possibly permitted more moisture to reach the ground (Photo IV-1).

Effect of treatments on N availability

As expected, the greatest changes in the nutrient content occurred after fertilization. Increase in N content was registered in the mineral soil, foliage and moss (Figures IV-4, IV-5 and IV-11). N content increased in all needles, including the cohorts that developed

before the application of the fertilizer. However, the duration of this effect was relatively short. By the second year after application of the fertilizer, the amount of N in the soil decreased considerably (Figure IV-4). In fact, the presence of the applied N steadily decreases in the fertilized plots, and by the end of the experiment the amount of the measured N in fertilized plots was not significantly different from control plots.

The effect of the moss removal on the nutrient status of the sites was not obvious. The nutrients incorporated in the moss layer were irreversibly lost from the plots where moss was removed. On the other hand, fresh nutrient input from the precipitation and litterfall was deposited directly onto the surface of the mineral soil and was not intercepted by the moss for undetermined period of time. In addition, bacterial activity and nutrient mineralization were likely accelerated due to warmer soil in the growing season, boosting the flow of nutrients to the pine roots.

Why the moss layer removal did not affect the efficiency of fertilization is unclear. As the fertilizer was applied directly onto the mineral soil in the plots without moss, I have expected a greater amount of available N in these treatments. However, by one year after fertilization, the amount of N in the exposed mineral soil did not exceed the N under the moss, which suggests that the rate of N uptake/loss from the exposed mineral soil was greater than at the sites with the moss was left intact (Figure IV-4). N could have either been assimilated by the roots or lost in gaseous form. As five out of eight fertilized plots were located on poorly to imperfectly drained soils and were susceptible to prolonged water-logging during the spring months, the loss of N due to anaerobic denitrification is a possibility. Elevated temperature of the soil in the plots without moss could actually accelerate the denitrification rate (McKenney et al., 1984). Finally, damage to the roots of the trees during moss removal may have reduced the efficiency of nutrient uptake by the trees.

Thinning generally improved the N availability in the plots, mostly due to decomposition of the roots remaining in the soil and the slash left on the plots after the thinning. It is also possible that the increased light intensity and moisture recharge on the thinned plots inhibited moss growth (Busby et al., 1978) and accelerated decomposition rates, thus releasing more nutrients to the mineral soil.

Vector Diagnosis

Use of vector diagrams (Figures IV-7 and IV-8) provides additional information on the effects of treatments on needle growth and N content. After the first growing season, vector diagnosis revealed enhanced N uptake, N concentration, and biomass production (i.e., moving toward the right across diagonal lines) in conifer needles from the trees grown in fertilized plots with and without moss layer. Compared with the control plots, fertilization resulted in 40% increase in N concentration and nearly doubled N content in the needles. This vector represents a typical deficiency response because of improved N availability (Timmer, 1991). The response to the thinning or moss removal alone was not significant one year after the fertilization (Figure IV-7). However, three years after the treatment (Figure IV-8) the length and direction of vectors change. In 2003, for the fertilized treatment, the surplus of N in the fertilized needles decreased to only 10% compared to control. At the same time, the effects of thinning and moss removal were more noticeable. Thinning has increased the needle biomass production, without significant alteration to the N concentration. The apparent negative effect of the moss removal on the needle biomass production in both fertilized and unfertilized plots might be attributed to the aftereffects of the winter root freezing in the December of 2002, when in the absence of the snow cover soil temperature in the top 5 cm of the mineral soil dropped to -13°C (Figure IV-2).

Moss condition

The moss layer is a sensitive indicator of the forest condition (Richardson, 1981). The treatments affected moss conditions and growth perceptibly. For example, fertilization increased N content in the moss, altering the rate of moss growth and decomposition (Figure IV-10, IV-11). A considerable amount of applied N was retained within the moss layer, which stimulated both growth of moss shoots and accelerated decay of the dead material within the moss stratum (Figure IV-10). It can be reasonably assumed that increased decomposition probably led to improvement in the transfer of N from moss to the root zone.

Thinning tripled the amount of solar radiation reaching the forest floor and exacerbated the amplitude and frequency of desiccation in the moss layer. As a result,

decomposition of the moss layer was accelerated and total biomass of the moss decreased. Although a continuous moss layer was still present in thinned plots, we can reasonably assume that the flow of nutrients to the roots has improved because there were fewer trees to compete for the resources.

Within three years after the moss removal, rudimental patches of feathermoss re-appeared over the soil surface despite the fact that the nearest source of spores could be as far as 15m. It can be expected that after several more years a continuous moss cover will be restored over the entire area of the plots where moss was removed.

Tree growth

Fertilization had increased foliage and radial growth within one year after fertilization, with and without moss removal. Although the amount of available N in the mineral soil declined significantly by the second year after fertilization, trees retained enough added N to sustain accelerated growth for another four years. However, a reduction in foliar N in 2003 may indicate that the effect of fertilization was declining.

Effect of thinning on the radial tree growth was not apparent during the first two years after treatment, but it became significant by the fourth year. For the first 3 years the only effect of thinning was increased needle weight, but this did not correspond with an increase in radial increments. During the last 2 years of the experiment, radial increments of the trees on the thinned plots were significantly greater than on the controls and moss removed plots. A lag in the time of increase in stem growth following thinning of a mature lodgepole pine stands has also been reported by Yang (1998).

The eventual increase in productivity on thinned plots might be caused by factors other than the reduction of the competition alone. After the thinning, excessive light inhibited the growth of the moss but did not cause damage to the roots, and thus reduced the ability of the moss layer to compete for the resources with the trees. In addition, the slash was left on the site, and provided an additional supply of nutrients to the trees, especially under conditions of the increased illumination of the forest floor (Figure IV-10) and subsequently elevated decomposition rates (Vesterdal et al., 1995). If the increase in productivity was indeed caused by elevated supply of nutrients from slash rather than

reduced competition, it would be a temporary fertilization phenomenon and bring little long-term change to the overall tree productivity.

Moss removal was associated with a variety of factors that could have contributed to both benefit or inhibition of the tree growth. The mechanical removal of the moss was likely to tear and damage fine tree roots that are usually concentrated at the interface between the mineral soil and organic layer. Increased soil moisture and heating in the plots with moss removal would promote recovery of the roots during the summer; but extremely low winter temperatures (Figure IV-2) likely added to the root damage (Vapaavuori et al., 1992).

In my study, there was no apparent improvement in tree productivity in plots with moss removal relative to the controls. However, this result might be limited to the particular situation of the study site and results might differ in different locations or years with more snow cover in winter. Data interpretation was necessarily limited, however, because the study was based on a single research site and any wider extrapolation of the results should be done cautiously.

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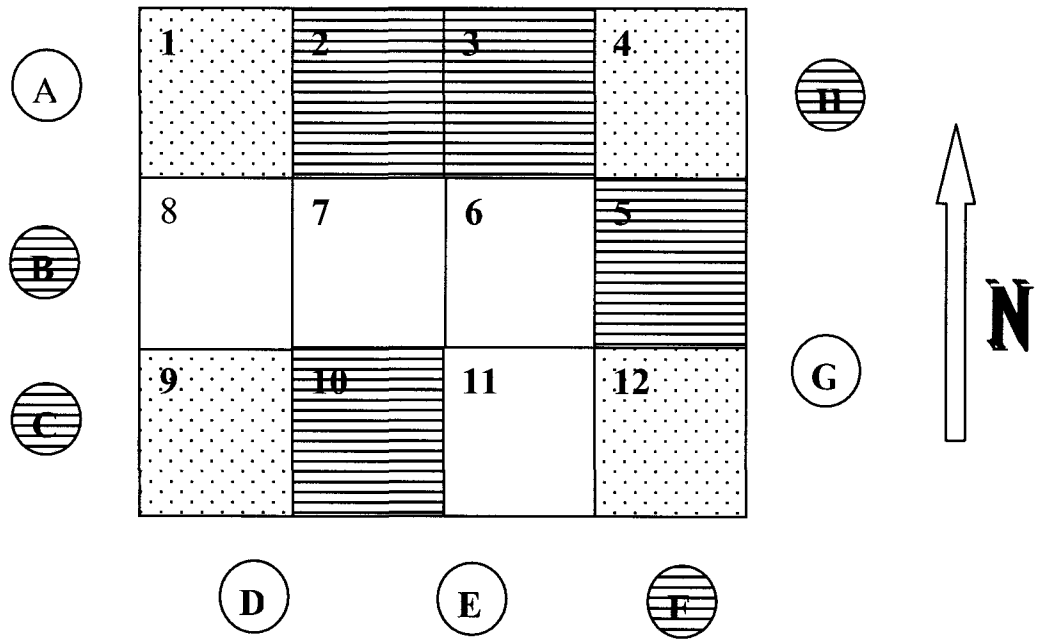
Figure IV- 1. Study site experimental design.



2 **900 m^2 plot, moss removed**

4 **900 m^2 plot, thinned**

8 **900 m^2 plot, control**

A **200 m^2 plot, fertilized**

H **200 m^2 plot, moss removed, fertilized**

Figure IV- 2. Soil temperature at 5 cm depth for the years 2000-2003.

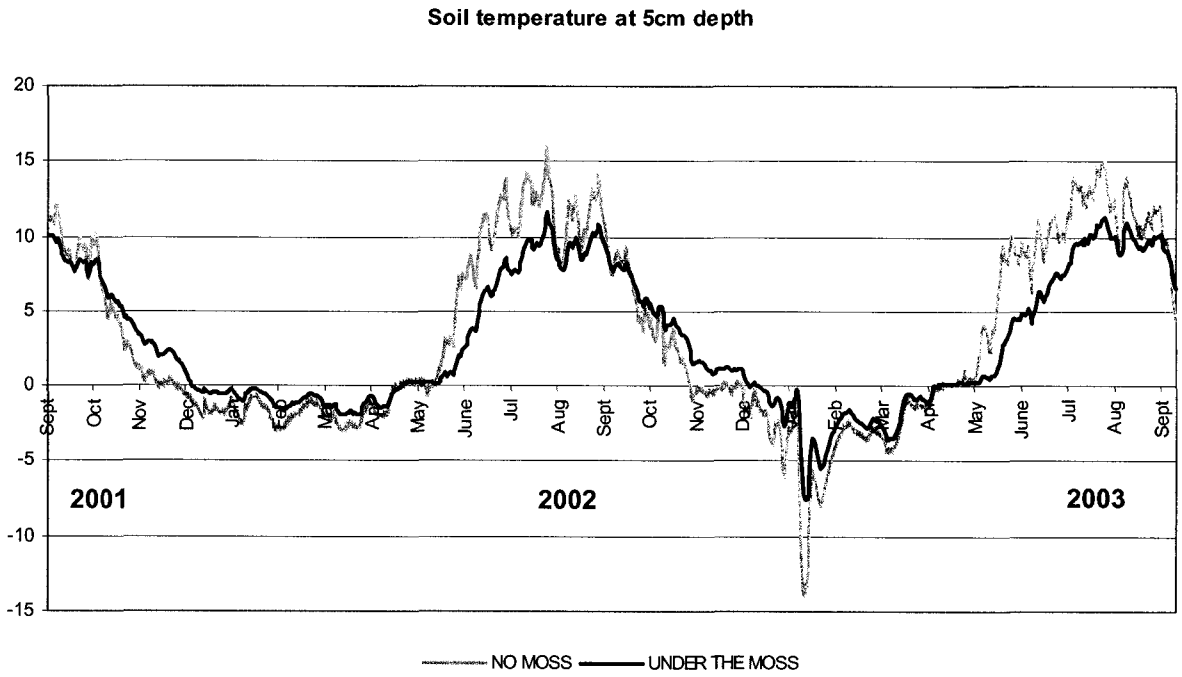


Figure IV- 3. Soil water potential at the depth of 5 cm in the mineral soil, for the years 2000-2001, for control and moss removal plots.

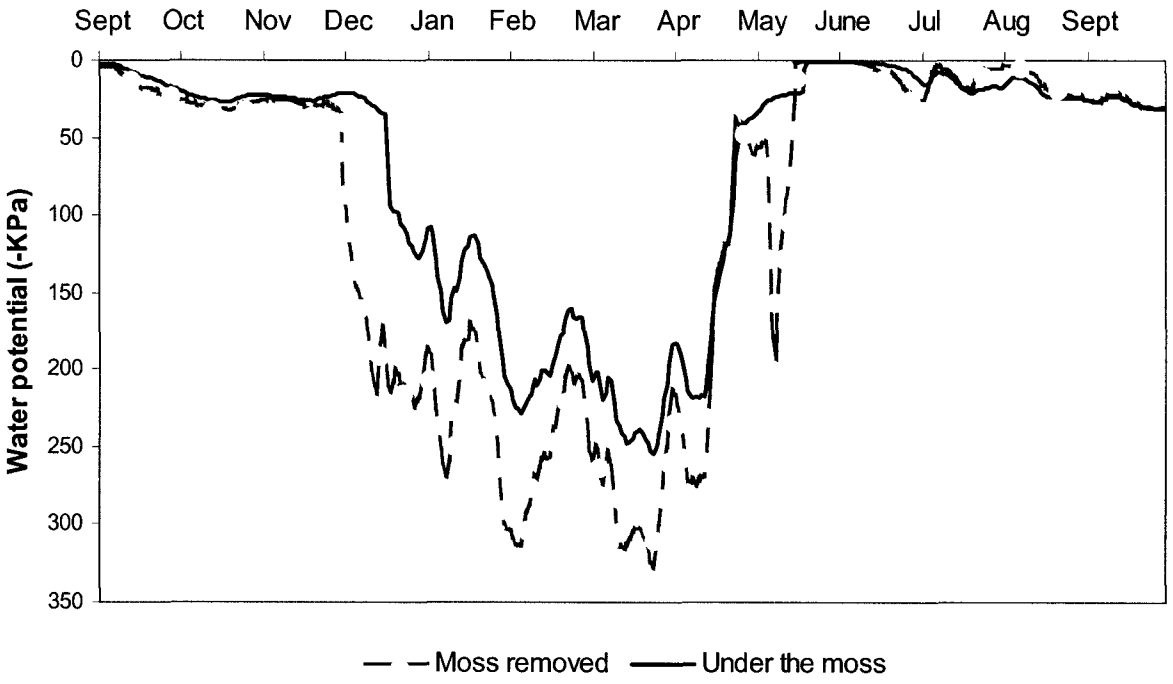
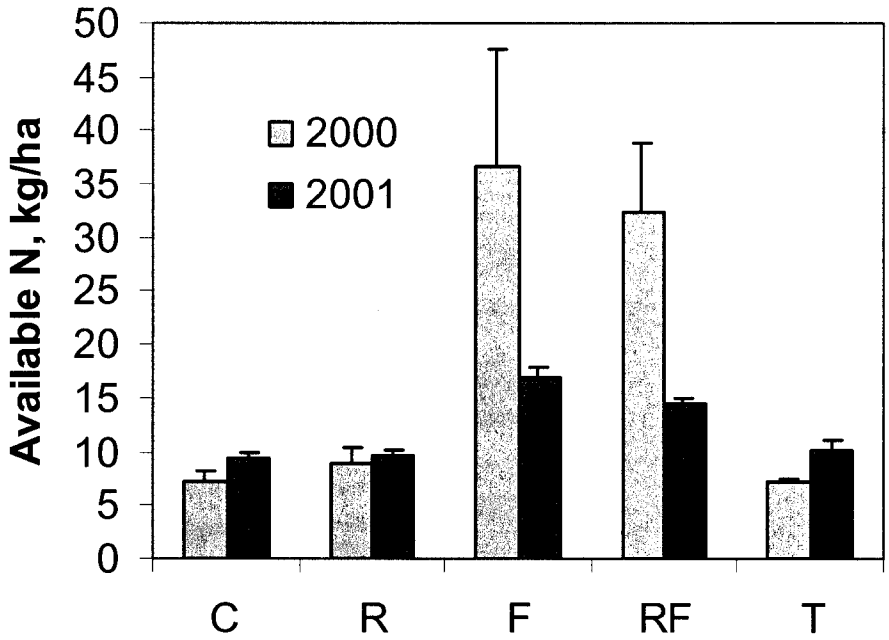


Figure IV- 4. Available N content in the mineral soil (0 to 30cm layer).



Note: Treatments are: C-control; R – moss removed treatment; F – fertilized with 200 kg/ha of N in form of urea; RF – moss removed and fertilization applied on top of mineral soil.

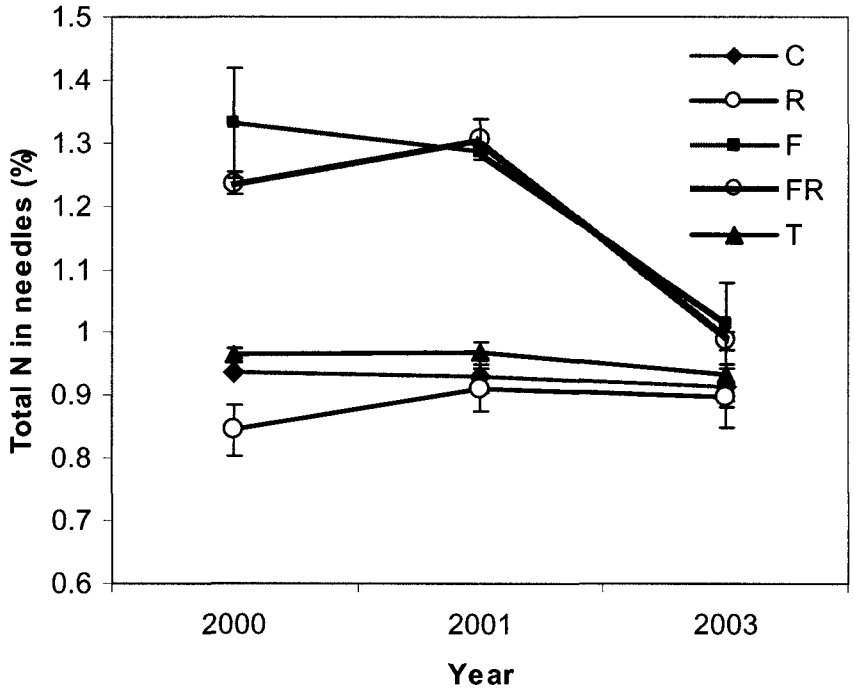
Table IV-1. Distribution of N in the ecosystem during the first two years after establishment of the moss removal, fertilization and thinning treatments.

	Year	Soil N content*	Moss N content	Foliage	Total
		kg/ha			
Control	2000	7.31 (0.83)	11.49 (1.07)	140.48 (4.07)	159.27 (5.97)
	2001	9.45 (0.47)	10.69 (1.35)	139.73 (4.12)	159.87 (5.94)
Moss removal	2000	9.00 (1.27)	N/A	127.80 (5.16)	136.80 (6.43)
	2001	9.78 (0.48)		136.43 (5.06)	146.20 (5.54)
Fertilized	2000	36.65 (9.97)	22.60 (3.00)	199.73 (0.64)	258.98 (13.61)
	2001	16.86 (1.10)	16.69 (2.47)	193.05 (1.32)	226.59 (4.89)
Fertilized + moss removal	2000	32.39 (6.46)	N/A	195.83 (4.72)	228.22 (11.17)
	2001	14.49 (0.41)		185.33 (2.81)	199.81 (3.22)
Thinned	2000	7.17 (0.40)	11.87 (0.31)	86.99 (1.66)	106.03 (2.37)
	2001	10.14 (1.06)	11.13 (0.79)	87.12 (1.95)	108.39 (3.80)

Note: Values indicate arithmetic means (n=4). Standard errors are shown in parenthesis.

* Soil available N as calculated for the 0-30 cm layer.

Figure IV- 5. Changes in the N concentration in the most recent cohort of needles.



Note: The treatments were imposed in the year 1998. Treatment codes are the same as in Figure IV- 4.

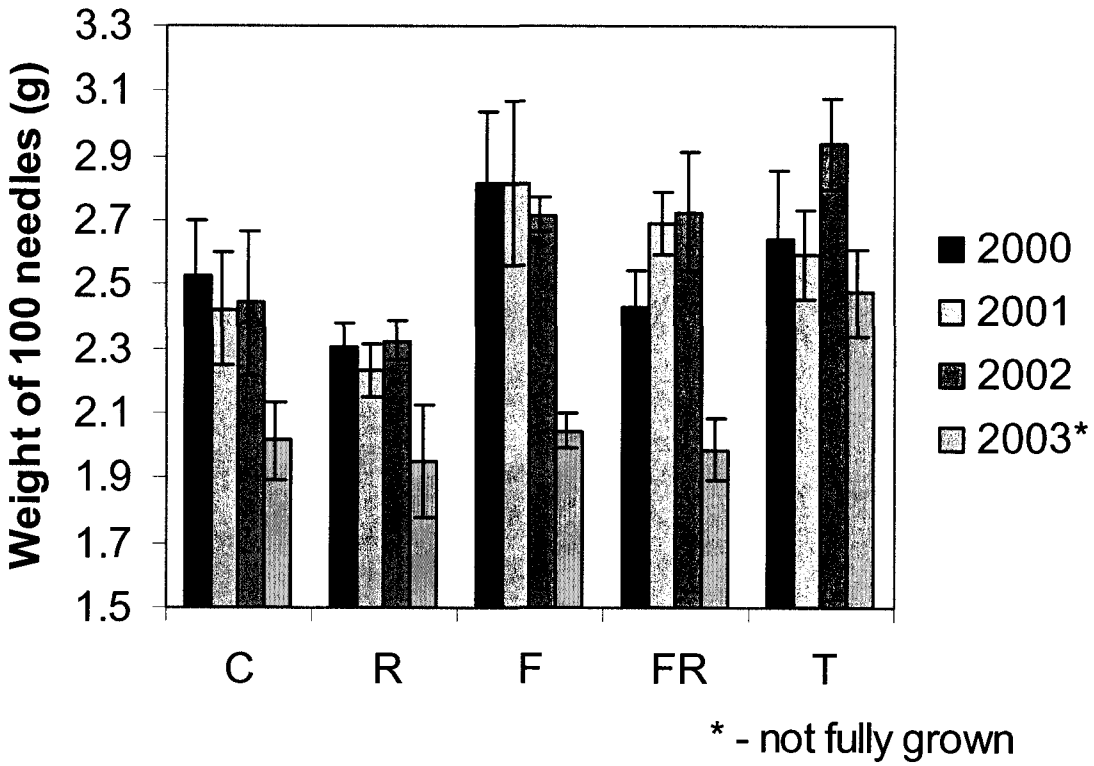
RF

Table IV-2. Weight of 100 needles (g) and foliar N concentration one, two and four years after the establishment of treatments.

	Weight of 100 needles			Foliar N		
	2000	2001	2003	2000	2001	2003
	----------g----------			----------%----------		
Control	1.69 (0.31)	1.71 (0.13)	2.33(0.33)	0.94(0.00)	0.93(0.03)	0.91(0.03)
Moss Removed	1.86 (0.06)	1.72 (0.23)	1.95(0.17)	0.85(0.04)	0.91(0.03)	0.90(0.05)
Fert.	2.28 (0.31)	2.36 (0.15)	2.26(0.22)	1.33(0.09)	1.29(0.00)	1.02(0.07)
Fert. + Removed	2.26 (0.29)	1.94 (0.07)	1.99(0.09)	1.24(0.02)	1.31(0.03)	0.99(0.01)
Thinned	2.20 (0.01)	1.80 (0.15)	2.47(0.13)	0.97(0.01)	0.97(0.02)	0.93(0.04)

Note: standard errors are shown in parenthesis.

Figure IV- 6. Average weight of 100 needles of four annual cohorts in relation to treatments.



Note: All needles were collected in 2003. Treatments codes are same as in Figure IV - 4.

Figure IV- 7. Vector diagram of effect of the treatments on relative N content and concentration in recent pine needles one year after the application of fertilizer.

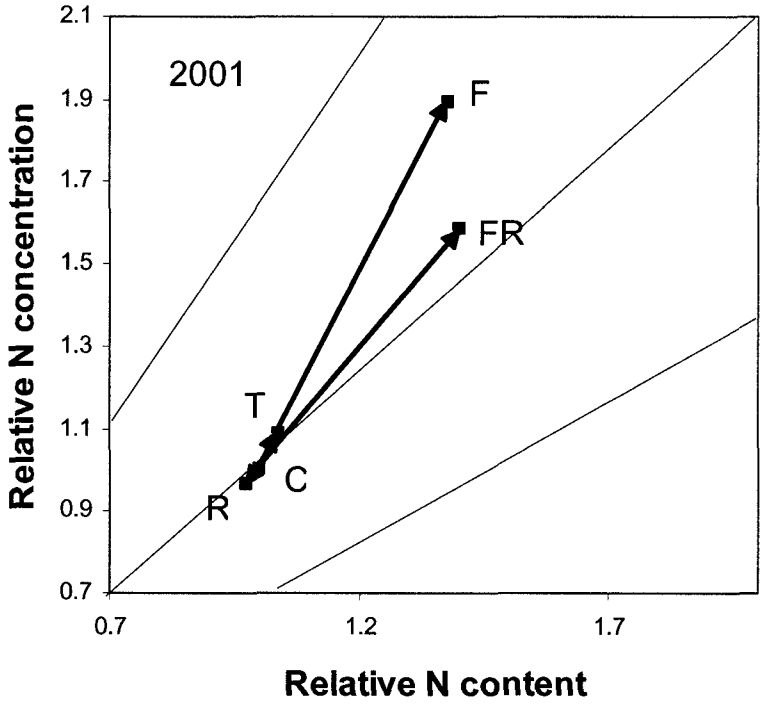


Figure IV- 8. Vector diagram of effect of the treatments on relative N content and concentration in recent pine needles three years after the application of fertilizer.

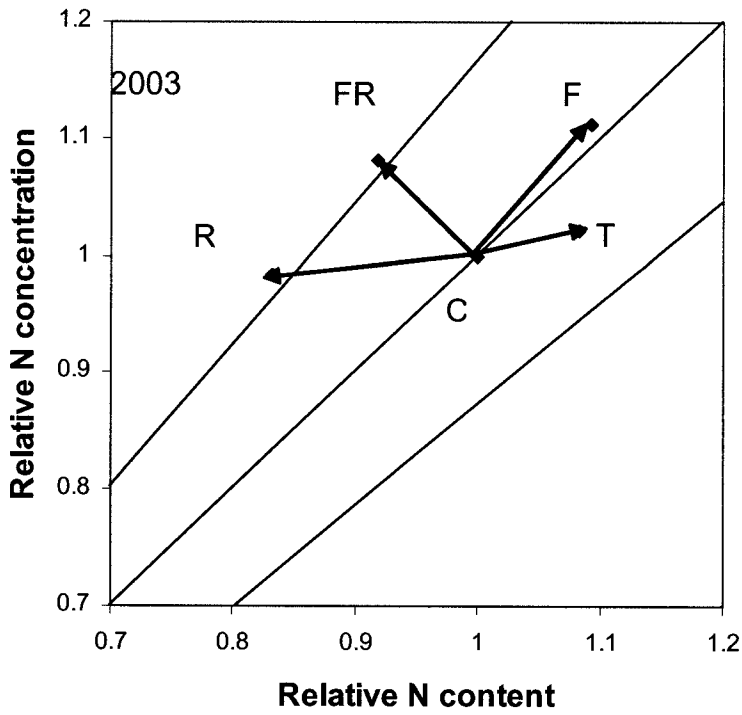
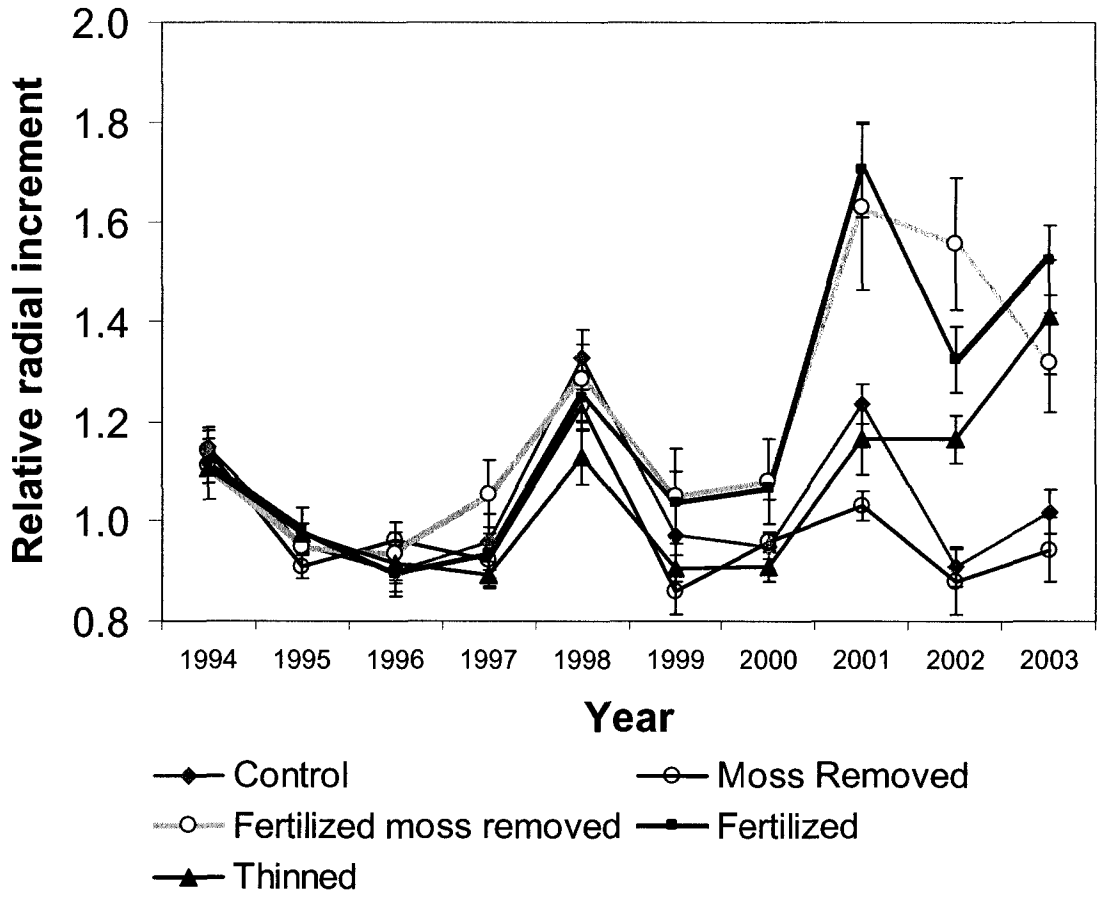


Figure IV- 9. Relative annual tree ring increments for dominant pine trees after the treatments were imposed in the year 1998.



Note: Individual values for each tree were calculated as a ratio the measured increment to the average annual increment for the same tree core for 4 years before imposing the treatments.

Table IV – 3. Analysis of variance of the weight of 100 needles and needle N content of the pine trees as affected by presence of moss cover and fertilization.

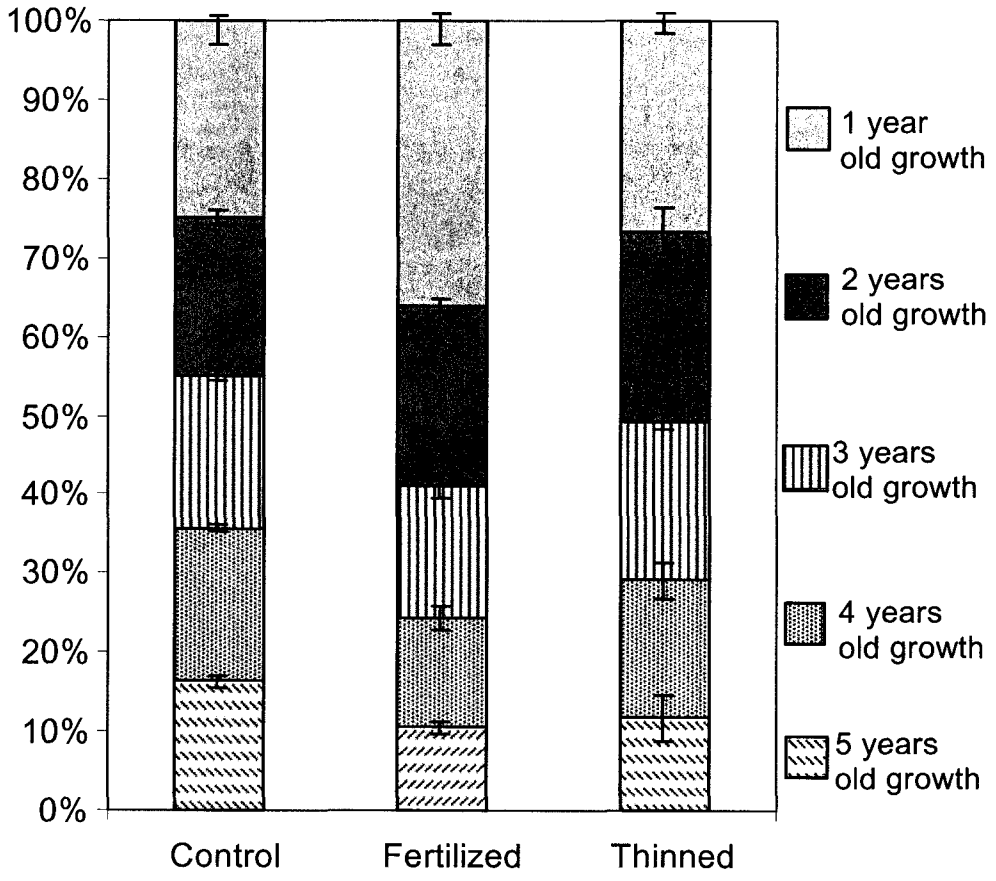
Dependent Variable	Source	Degrees of freedom	Type III Sum of Squares	Mean Square	F Value	Pr > F
Weight of 100 needles	fertilized	1	0.757	0.757	7.74	0.0166
	moss presence	1	0.168	0.168	1.72	0.2144
	fertilized*moss	1	0.181	0.181	1.85	0.1992
N concentration in the needles	fertilized	1	0.565	0.565	192.86	<.0001
	moss presence	1	1.225E-05	1.225E-05	0.00	0.9495
	fertilized*moss	1	0.002	0.002	0.56	0.4686

Note: the data refer to the most recent needles collected in the year 2003.

Table IV – 4. _Analysis of variance of relative radial increments of the pine trees as affected by presence of moss cover and fertilization.

Dependent Variable	Source	Degrees of freedom	Type III Sum of Squares	Mean Square	F Value	Pr > F
2003 relative radial increments	fertilized	1	1.429	1.429	24.24	0.0004
	moss presence	1	0.060	0.060	1.01	0.3339
	fertilized*moss	1	0.008	0.008	0.14	0.7175
2002 relative radial increment	fertilized	1	1.076	1.076	37.02	<.0001
	moss presence	1	0.069	0.069	2.38	0.1491
	fertilized*moss	1	0.038	0.038	1.32	0.2734
2001 relative radial increments	fertilized	1	1.166	1.166	15.20	0.0021
	moss presence	1	0.008	0.008	0.10	0.7575
	fertilized*moss	1	0.045	0.045	0.58	0.4593
Repeated Measures Analysis for relative radial increments						
Relative radial increments	Year	2	0.667	0.334	6.47	0.0035
	fertilized	1	3.657	3.657	70.96	<.0001
	Moss presence	1	0.002	0.002	0.03	0.8612
	fertilized*moss	1	0.034	0.034	0.18	0.7000

Figure IV- 10. Relative dry weight of annual segments of *Hylocomium splendens* as affected by fertilization and thinning, arranged by the age of segments, one growing season after fertilization.



Note: Years 1-5 refer to the average contribution of each annual segment to the dry weight of the moss shoot.

Figure IV- 11. Changes in the total N concentration in annuals segments of *Hylocomium splendens* 1 year after treatment.

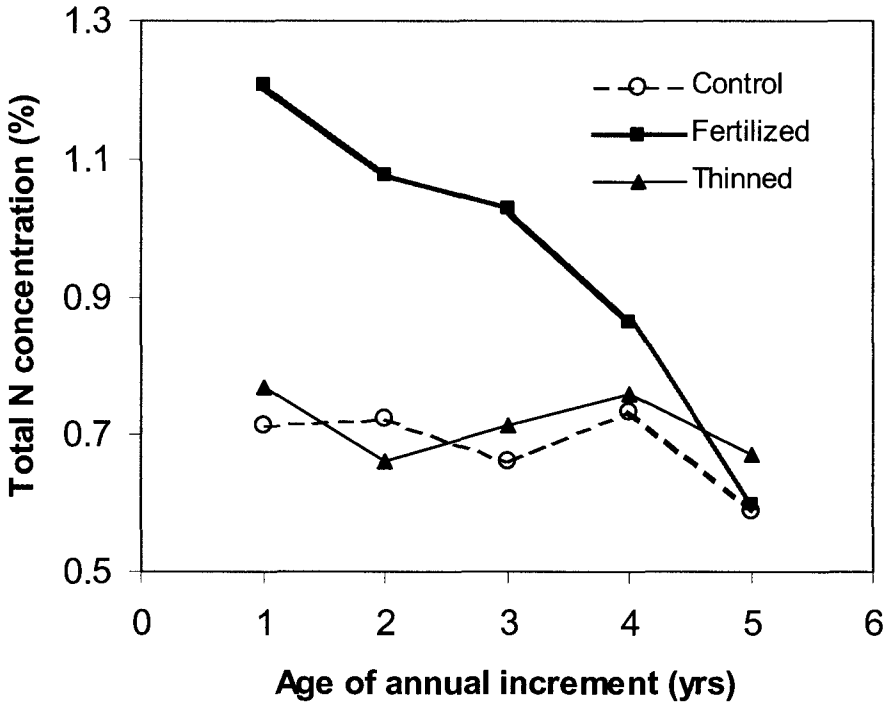


Photo IV- 1. Research site.

Photo also illustrates crown retention of the snowfall in control plot and after thinning. To the right, thinned plot, in the spring of 2000 snowfall left ~3cm snow on the ground. To the left –control plot, as more of the snow was retained in the crowns there is little snow on the ground.

Photo IV- 2. Undisturbed ground cover at the research site.

Photo IV- 3. Soil profile of the research site.

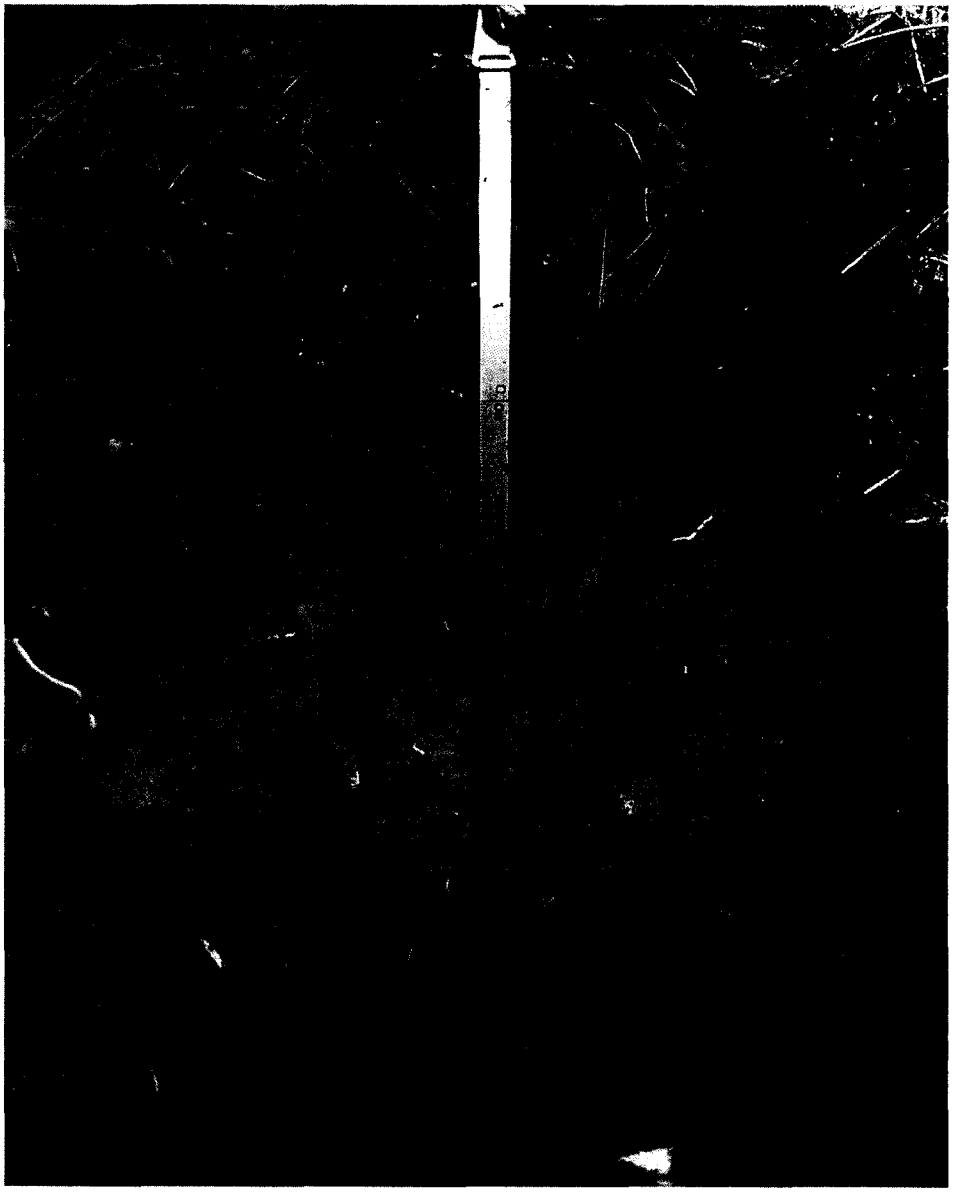
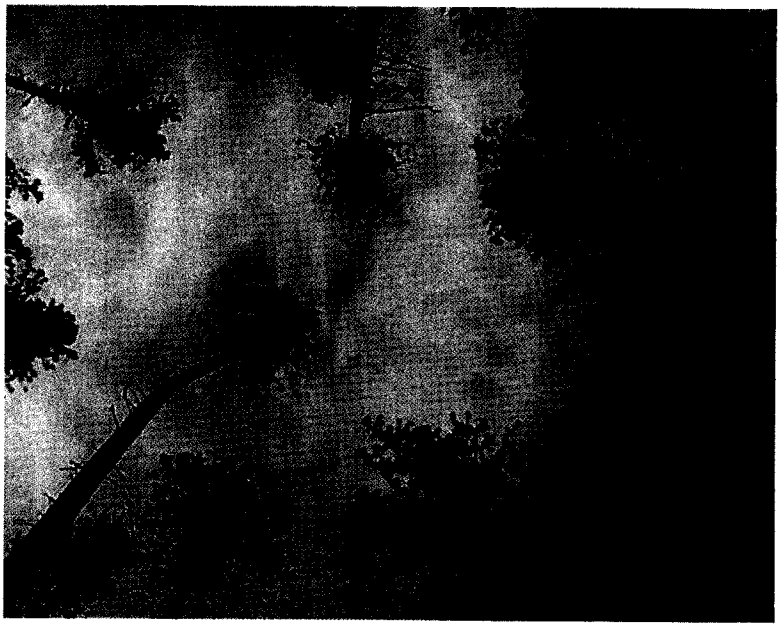


Photo IV- 4. Crown projection in the control plot.

Photo IV- 5. Crown projection in the thinned plot.



Chapter 5. General discussion and conclusions

This study was undertaken with the goal to determine the role of feathermoss layers in N cycle and productivity of lodgepole pine forests. The focus was on the effects of moss on N transformation, transfer and volatilization associated with subjecting feathermoss to cyclic wetting-drying, which commonly occurs in boreal forests.

My most important findings

- I found that the feathermoss tissues not only release N to the solution after the re-hydration but are also capable of re-assimilating it after several hours of recovery. This indicates that the previous assumption that the released N becomes available to the tree roots and microflora is overestimated. I have established a time frame for the moss recovery after desiccation that allows protection of the cell content and recovery of the lost mineral N.
- N_2 and N_2O are emitted from mosses that were dried and re-wetted. This therefore, removes N from the soil-plant ecosystem with every dry/wet cycle.
- 15N decline with depth suggesting that N is partially lost from the moss layer to its transition to mineral soil.
- Evidence was found that that in order to recover from a dry/wet cycle, moss tissues increased respiration by depleting carbohydrate resources stored in the cells.
- Loss of total moss biomass was demonstrated in each dry/wet cycle imposed on the feathermoss layer.
- Indications were found that suggests that gradually drying feathermoss invokes mechanisms to protect internal N from excessive leakage after re-hydration; in particular, the moss removes NO_3^- from the protoplasm.
- It was confirmed that the removal of the moss layer increases soil temperatures during the warm months and shortens the freezing period in the mineral soil; however, it also exposes the tree roots to the winter freezing during cold winters with below-average snow cover. Overall, moss layer removal did not affect the tree growth significantly.

Ecological Implications

In the boreal forests with a continuous feathermoss cover, the moss layer forms a complex system with many mutually dependant components. Because of the insulating properties of the moss layer (Chapter 4, Figure IV-2) the mineral soil is much colder than without moss in the same climatic zone, and therefore it is likely that much of the bacterial activity of these soils is found in the moss layer. Decomposition and nitrification /denitrification gaseous loss happen in the moss layer, while most of the tree roots are located in the top layer of the mineral soil and at the interface between the organic layer and the mineral soil.

Feathermoss layers intercept virtually all N input moving towards the soil including N from decomposition, precipitation and N-fixation. As with any other plants in the ecological community, moss competes for resources such as nutrients and water. A continuous moss layer interrupts the flow of nutrients from tree crowns and roots and thus affects the tree growth. Considerable portions of N accumulated the moss (Chapter 3, Figure III-2) can be volatilized in a single wet-dry cycle and is lost from the ecosystem to the atmosphere.

N transfer from feathermoss layer to the tree roots primarily occurs as a result desiccation and following flushing with large amount (more then 25 mm) of rain water sufficient to saturate the moss layer and wash nutrients down to the mineral soil; such rainstorms are relatively rare in Alberta boreal forests. If the amount of precipitation is light to moderate, N-enriched solution will be retained within the moss layer and will not reach the tree roots. However, N transformations and possibly subsequent volatilization (Chapter 3, Figure III-2) can take place in each wet/dry cycle, regardless of the amount of precipitations.

Physiological conditions of the moss plants and associated organisms determine the amount and form of N in the tissues and in the immediate environment surrounding the moss plants. Moss layer can release some of the accumulated nutrients from the tissues in a number of ways. Senescing parts of the moss die out and decompose, releasing the nutrients for the uptake by microflora. However, the amount of nutrients in the senescing tissues is reduced as moss is capable of internal recycling of resources from the dying cells.

Nutrients can also be released from the mechanically damaged moss tissues. The damage can be caused by hail, raindrops, animals, or falling heavy debris. Although the moss tissues recover quickly from mechanical damage (Chapter 2, Figure II-3), part of the resources contained in the lacerated cells can be carried down the soil profile by precipitation. And lastly, desiccation of the moss layer can damage moss membranes and allow nutrients to leak out of the moss tissues after re-hydration.

In the conifer forest feather moss layer plays a complex role in N cycling. Most commonly the moss layer is viewed as an N capacitor that accumulates and processes N input from debris and precipitation and eventually passes it down to the root zone. The indications of the positive effect of the moss layer on the ecosystem include:

- Observations *in situ* of N from the fertilizer being filtered out of solutions by the moss layer (Weber and Van Cleve, 1984).
- Data on the capacity of the feathermoss layer to assimilate N (Turetsky, 2003)
- Observations of the eventual delivery of applied N to the mineral soil (Chapter IV).
- Evidence that desiccation and following re-wetting of the moss layer results in release of N to the solutions (Carleton and Read, 1991; Gupta, 1977).
- Observations that despite the moss' ability to re-assimilate N from the solutions upon recovery from desiccation, about 1/3 of released N can remain in the solution and be washed out to the mineral soil (Chapter II).
- Indications of feathermoss association with N-fixing micro-organisms (DeLuca et al., 2002) that imply that the moss layer actually increases the amount of N in the ecosystem.
- Evidence that the winter freezing is reduced under the insulating blanket of the moss layer.

However I have also found indications that the transfer of N through the moss layer might be less efficient that was expected; some N can be converted to the gaseous forms and escape from the ecosystem. The facts that indicate toward possibility that the moss layer can have a negative impact on the ecosystem include:

- Direct measurements of N_2 and N_2O emissions from the moss layer after re-hydration (Chapter III).

- Deficit in the recovery of the applied N in the forest ecosystem (Nômmik and Popovic, 1971).
- Increase in the natural ^{15}N abundance with the depth of moss and organic layer (Chapter V), which is an accepted indication of a gaseous loss of N from the ecosystem (Högberg, 1991).
- Decrease of N/P ratio in the moss tissues with repeated desiccations (Chapter III).
- Indications that repeated desiccations result in a weight loss of the moss, but without change in C/N ratio, indicating that C and N are lost at the same rate during recovery after the desiccation (Chapter III).
- Decrease of the soil temperatures during the growing season and of the sum of the annual positive temperatures in the soil that points toward reduced root activity under the moss layer.

Based upon the findings of this study, I have developed a new conceptual model of N cycling in conifer-feathermoss ecosystem, which takes into account frequent wet/dry cycles in the feathermoss layer and their role in the C and N balance in the boreal forests (Figure VI-1).

De-hydration stage. Desiccation has a profound effect on both the moss plants themselves and associated microorganisms. The effect of desiccation largely depends on the rate of the water loss and the length of time the moss layer spends under desiccation. Usually tree crowns partially protect the surface of the moss from excessive drying, and the onset of desiccation is relatively slow. Moss tissues have adapted to the intermittent desiccation and have developed defensive mechanism to control the damage (Tucker et al., 1975). Under the slowly increasing desiccation stress, moss cells convert NO_3^- to organic form and reduce the total amount of mineral N stored in protoplasm. As the protoplasm shrinks, osmotic pressure and concentration of solutes in the cell increases. Water stress causes cessation of photosynthesis while moss tissues protect the cell contents, probably at the cost of loosing senescing parts. At this point, some microflora may still be active in the drying moss and able to process released N, while increasing the probability of N volatilization.

Dry stage. As moss reaches full desiccation, it enters a period of virtual inactivity. In this state, moss plants can be heated to 105 degrees C without suffering any apparent damage (Nörr, 1974, as cited in Richardson, 1981). Presumably, the microbial activity is minimal or absent at this stage.

Re-wetting. The first rain after a period of desiccation first saturates the moss layer. Moss regains its water content in seconds, but it takes the cell up to 24 hours to recover from the previous damage. Intracellular content leaks freely into the surrounding solution through coagulated membranes and soluble nutrients can be carried away from the living moss tissues with the flow of water.

Recovery stage. Eventually cells enter recovery stage, associated with deformation of internal cell structures (Chapter 1, Figure I-3), expenditure of carbohydrates and increased respiration. Surviving microflora regains its activity and flourishes in the presence of elevated levels of soluble N. Within several hours, moss tissues recover and become able to re-assimilate the lost nutrients and resume normal functions (Dilks and Proctor, 1976).

Nutrients released from the moss remain in the solution saturating moss layer for several hours. By the end of the moss recovery period the nutrients may follow different pathways. The released nutrients can be partly re-captured by the moss upon restoration of the moss tissues while the rest of nutrients are likely to be consumed by the recovering microflora or absorbed on the active surfaces of the organic layer. Mineral N in the acidic well-aerated and rich in microflora moss layer is susceptible to fast nitrification, denitrification and potentially can be converted to gaseous forms. And finally, some nutrients are washed down to the root zone, if the water input rate is sufficient to carry solutes down into the mineral soil.

Growth stage. As a fully hydrated moss recovers, it enters the period of optimal assimilation and growth. It accumulates carbohydrates and absorbs N, which is supplied with precipitation, from decomposing organic matter and through symbiotic links with microflora. The moss can also be a substrate for N fixing algae in the moss layer (DeLuca et al., 2002).

Methodological advances

In order to resolve the technical problems that arose during the study, several new laboratory techniques were developed. The most challenging task was associated with measuring the emissions of dinitrogen, from live photosynthesizing moss tissues. Dinitrogen measurements are complicated because of the danger of contamination from the atmospheric N_2. Existing techniques either employ indirect measurements of N_2 or include the use of air-tight chambers with N_2 replaced with inert gases. Both versions are expensive and have limited accuracy. The overhead gas must be extracted by syringe, placed in the evacuated sampling vial, then transported to the gas spectrometer and re-sampled. Each step has a possibility of contamination.

For the first time, I employed a simplified method that excludes the necessity of labor intensive and expensive chamber –syringe manipulation. My technique reduced the risk of contamination with atmospheric gases through the open syringe needle because one step of transferring a gas sample with a syringe was eliminated. This technique has the potential for wider application for short-term gas exchange incubation of small living plants (including water vegetation), germinants, small seedlings, bryophytes, algae, or separate leaves with or without soil. This procedure permits inexpensive and accurate measurement of most gases, which is usually complicated by potential contamination of samples with atmospheric air.

The technique I have proposed does not require complex chambers. Small plants of leaves are placed directly in the glass Exatainer™ purged with He and incubated in the light. This eliminates one step as the experimental chambers are at the same time sampling vials and gas can be transferred directly from the Exatainer™ to the gas spectrometer. Exatainers prevent contamination reliably, are inexpensive and can be re-used.

The second technique developed in the course of this study was repeated analysis of N exchange between solution and plant tissues using shakers and flasks and sample extraction for injection into an autoanalyser. This method allows repeated sampling of the solution for determination of the rate of mineral nutrient transfer between plant tissues and solution.

Another innovative technique employed in this study was assessment of moss adaptation mechanism to the gradual desiccation and the role of this gradual adaptation to minimize nutrient exchange after rewetting. The slow-dried mosses were compared to rapidly dried (freeze-dried) samples. There was much greater leakage of nutrients, particularly NO_3^-, from the freeze-dried mosses.

Recommendations for future research

Further physiological studies of moss desiccation and recovery present will be of value. I have found that N undergoes chemical transformations, such as reducing the concentration of NO_3^- in the tissues. These changes result in decreased leakage of mineral N from the damaged moss tissues in moss cells subjected to desiccation-rewetting. However, the products of such transformations are unknown. Is there a possibility that N escapes from the tissues in organic form? What is the mechanism that restores the presence of NO_3^- inside the recovered feathermoss tissues?

Moss tissues contain significant amounts of highly unsaturated fatty acids (Prins, 1981). In the presence of oxidizers such as oxygen, fatty acids can be transformed into super-oxides and oxidize N compounds (Esterbauer and Cheeseman, 1990). After re-hydration of the dry feathermoss increased production of superoxide (Mayaba et al., 2002) can cause internal N oxidation and gaseous loss of N directly from the moss tissues. Effects of oxidative boost on N transformation and possibly partial conversion to gaseous form, during the post-rehydration period of the moss, deserve future research.

Hydrological studies of the water and nutrients movement through the moss layer in situ would further improve the understanding of nutrient cycling in the conifer forest.

Similarly, the amount of N loss due to volatilization after rewetting of dried feathermoss in field conditions needs be determined.

The role of fungae in the capturing N released from the moss layer after re-hydration and movement to the tree roots must be studied in order to understand the mechanism of nutrient flux from the moss to the trees.

Rate of N release from the moss layer to the root zone as a result of moss desiccation-rewetting in situ would provide valuable information for evaluation of nutrient regime and status of conifer forests. The amount of N moved to lower levels following rewetting of moss needs to be addressed.

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Figure V- 2. Schematic representation of the wet/dry cycle and nutrient transfer in the moss layer