Nitrogen nutrition of lodgepole pine and Sitka spruce seedlings:
From whole-plant growth to individual-root ion flux

by

Heather L. Danforth
B.Sc. University of British Columbia, 2002

A Thesis Submitted in Partial Fulfilment of the
Requirements for the Degree of

MASTER OF SCIENCE

In the Department of Biology

Supervisor: Dr. Barbara Hawkins

ABSTRACT

This thesis investigates the nitrogen nutrition of lodgepole pine (*Pinus contorta* (Dougl.) ex. Loud.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings, two ecologically and economically important conifers of western North America. Sitka spruce is generally restricted to moist, nutrient rich sites, while lodgepole pine is able to tolerate soils of low fertility. The contrasting habitats of these two species beg the questions: Is one species more efficient at N uptake and N use? To what extent do growth characteristics such as growth rates, biomass allocation, morphology, and rates of photosynthesis and respiration differ between the two species? How plastic are these nutritional characteristics?

These questions were addressed by conducting growth analyses, allometric analyses, and measuring NH_4^+, NO_3^- and H^+ ion fluxes across the surface of individual roots of seedlings grown at high (free access to nutrients, FA) and low (4% relative addition rate, 4% RAR) levels of N supply. To enable quantitative comparisons between species, all seedlings were grown in Biotronic units using relative nutrient addition rate techniques.

Both lodgepole pine and Sitka spruce seedlings showed distinct growth responses to the nutrient environment. At FA, relative growth rates (RGR's) for both species were approximately 0.08 g g^{-1} day^{-1}, while at 4% RAR they were approximately 0.04 g g^{-1} day^{-1}. Seedlings of both species grown with free access to nutrients allocated a relatively greater proportion of biomass to leaves and shoots, while seedlings grown under conditions of 4% RAR nutrient stress allocated a relatively greater proportion of biomass to roots, regardless of whether comparisons were made at a common harvest or at a common plant size. RGR's were positively correlated with whole plant nitrogen concentrations (wpN), and the N concentrations of all plant components were lower in the 4% RAR nutrient than the FA nutrient treatment. A wpN target that maximizes nitrogen productivity (NP) for lodgepole pine and Sitka spruce seedling production is ~0.02 g N g^{-1} whole plant biomass.

Lodgepole pine seedlings with more fully extended secondary needles had a higher rate of increase in total plant biomass per unit leaf area (unit leaf rate, ULR) at a lower rate of photosynthesis. This suggests that secondary needles are more photosynthetically efficient. Both species had higher wpN's, photosynthetic rates and growth rates in the FA as opposed to the 4% RAR nutrient treatments.

RGR, wpN, NP, NUR (nutrient uptake rate), ULR (unit leaf rate) and photosynthetic rates were all positively correlated. When overall seedling carbon balance was represented as the balance between net photosynthesis and root respiration (R), expressed as a R:A ratio, RGR and R:A were not significantly correlated. RGR was also not significantly correlated with SLA nor specific root length (SRL).

Lodgepole pine and Sitka spruce seedlings showed distinct differences in parameters such as the plasticity of SRL, NUR and ULR in response to the nutrient environment, but minimal differences in biomass allocation. While lodgepole pine seedlings exhibited greater plasticity in SRL and had greater rates of net photosynthesis relative to root respiration in the 4% RAR treatment than Sitka spruce seedlings, the two species did not differ significantly in *overall* growth responses to high and low N environments.

The microelectrode ion flux measurement (MIFE) system showed a high degree of individual tree variability in the fluxes of NH_4^+, NO_3^- and H^+ simultaneously measured across the roots of lodgepole pine and Sitka spruce seedlings. NH_4^+ fluxes were the least variable, but neither species showed a significant preference for NH_4^+ over NO_3^-. Seedlings grown with high available N had higher NH_4^+ fluxes than seedlings grown under conditions of nutrient stress. Both lodgepole pine and Sitka spruce may have relatively plastic nutrient transport mechanisms, rendering previous N nutrition an important determinant of ion flux characteristics.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2.

Chapter 3.

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Net photosynthesis on a dry mass basis (μmol CO_2 g^{-1} leaf dry mass s^{-1})
A_{la}	Net photosynthesis on a leaf area basis (μmol CO_2 cm^{-2} leaf area s^{-1})
FA	Free access
LMF	Leaf mass fraction (g leaf dry mass g^{-1} total plant dry mass)
MIFE	Microelectrode ion flux measurement
NP	Nitrogen productivity (g total plant dry mass g^{-1} wpN day^{-1})
NUR	Nutrient uptake rate (g wpN g^{-1} root dry mass day^{-1})
R	Root respiration (μmol CO_2 g^{-1} root dry mass s^{-1})
R:A	Ratio of root respiration to net photosynthesis
RAR	Relative addition rate (g N added to Biotronic units g^{-1} N present in the whole plant day^{-1})
4% RAR	Four percent relative addition rate
RGR	Relative growth rate (g new total plant fresh mass g^{-1} pre-existing total plant fresh mass day^{-1})
RMF	Root mass fraction (g root dry mass g^{-1} total plant dry mass)
rN	Root nitrogen concentration (g N g^{-1} dry root mass, or %)
RUR	Relative uptake rate (g N taken up by plant g^{-1} N present in the whole plant day^{-1})
SLA	Specific leaf area (cm^2 leaf area g^{-1} leaf fresh mass)
SMF	Stem mass fraction (g stem dry mass g^{-1} total plant dry mass)
SRL	Specific root length (cm root length of the longest root g^{-1} root fresh mass)
ULR	Unit leaf rate (g total plant dry mass cm^{-2} leaf area day^{-1})
wpN	Whole plant nitrogen concentration (g N g^{-1} total plant dry mass, or %)

ACKNOWLEDGEMENTS

Many thanks to Dr. Barbara Hawkins for her continual guidance and support throughout the development of this thesis. This study would not have been possible without access to the Biotronic equipment and expertise of technicians and researchers from the Pacific Forestry Centre. I would specifically like to thank John Vallentgoed for his help with all aspects of the Biotronic units and each of my many harvests, and Dr. Al Mitchel and Tom Bown for their help with photosynthesis measurements and stimulating discussions on carbon balance and seedling growth. The stoicism of Becky Metcalf and Samantha Robbins is much appreciated. Their hard work made the grinding, processing and nitrogen analysis of all my samples possible. Thank you to my committee members Dr. Dorothy Paul, Dr. Patrick von Aderkas, and Dr. Rob Guy for taking the time to reading and critique the end product. Finally, I would like to thank James Miskelly for his daily insights and big-picture thoughts, and my partner Trevor Wurtele for his understanding, his relentless support in all my endeavours and his amazing ability to make me laugh.

GENERAL INTRODUCTION

The study species: Ecology and background information

Lodgepole pine

Lodgepole pine (*Pinus contorta* (Dougl.) ex. Loud.) is a two-needled pine of the Pinaceae family. This conifer has a wide ecological amplitude and is one of the most widely distributed pine species in western North America (Klinka *et al.* 1998; Lotan *et al.* 1983). It grows throughout the Rocky Mountain and Pacific coast regions, extending north to latitude 64° N in the Yukon Territory and south to latitude 31° N in Baja California, and from the Pacific Ocean to the Black Hills of South Dakota (Lotan *et al.* 1983) (Figure 1.1). Forests dominated by lodgepole pine cover 20 million ha in Canada and 6 million ha in the western United States (Lotan *et al.* 1983). As the lodgepole pine forest-type is the third most extensive commercial forest-type in the Rocky Mountains (Alexander *et al.* 1980), it is important to local communities throughout western North America. Lodgepole pine is an important timber species for pulp, lumber and specialty uses such as paneling, posts, corral poles, utility poles, and railroad ties (Fowells 1965). Not only is it an important timber species, it is also the major tree cover in many of British Columbia's water-sheds and recreational areas where it provides important wildlife habitat (Lotan *et al.* 1983).

Lodgepole pine is divided geographically into four varieties: *P. contorta* var. *contorta,* the coastal form known as shore pine, coast pine, or beach pine; *P. contorta* var. *latifolia,* the inland form often referred to as Rocky Mountain lodgepole pine, black pine or, in this province, interior pine; *P. contorta* var. *bolanderi,* a Mendocino County White Plains form in California called Bolander pine; *P. contorta* var. *murrayana* in the Sierra Nevada, called Sierra lodgepole pine or tamarack pine (Lotan *et al.* 1983). The seedlings used in this study are *P. contorta* var. *latifolia* from provenance 29172, which originates from interior British Columbia at 53 ° 20' latitude, 123 ° 25' longitude, and 1100 m elevation.

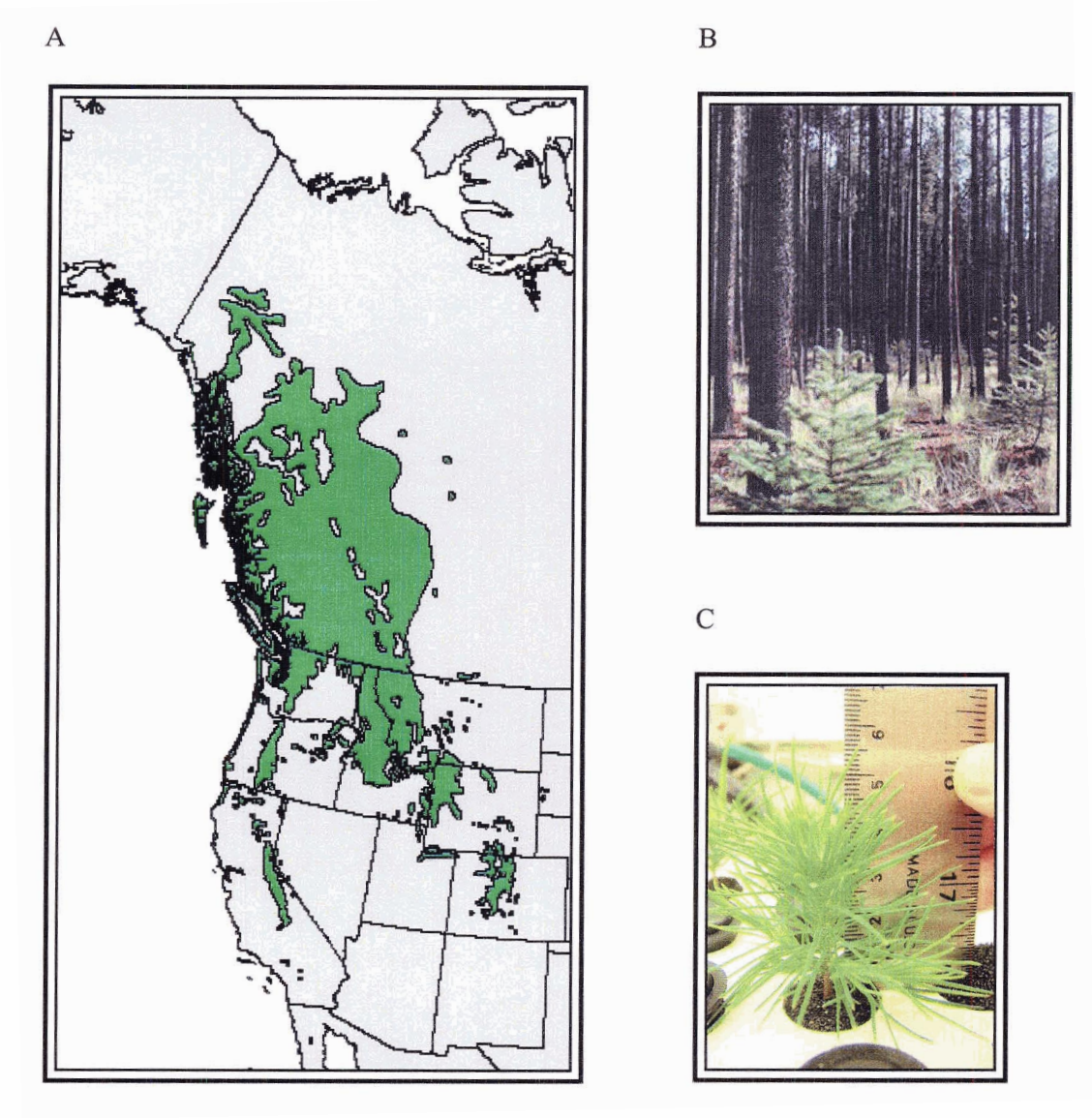


Figure 1.1. A. The native range of lodgepole pine (from Lotan *et al.* 1983), **B.** a typical, densely stocked 50 yr old lodgepole pine stand **C.** a lodgepole pine seedling used in this experiment (the picture was taken 32 days after germination in the FA treatment; seedlings were this size at harvest number two as described in the first chapter of this thesis).

Lodgepole pine probably has the greatest environmental tolerance of any conifer in North America. It grows under a wide variety of climatic conditions (Satterlund 1975). In Canada, extensive stands of the interior form of lodgepole pine (var. *latifolia*) occur on calcareous glacial tills (Smithers 1961). Glacial drift provides a balance of moisture and porosity on which the species seems to thrive. At low elevations in the interior, lodgepole pine grows in areas receiving only 250 mm of mean annual precipitation, whereas it receives more than 500 mm along the northern coast. Many interior sites are low in summer rainfall; here snowmelt supplies most of the soil water used for rapid growth in early summer. The coastal form of lodgepole pine (var. *contorta)* is often found on peat bogs or muskegs in southeastern Alaska, British Columbia, and western Washington (Lotan *et al.* 1983).

Low soil nutrient concentrations, extremes in soil moisture, and frost often favour lodgepole pine locally over other species (Pfister and Daubenmire 1975). On infertile, N-deficient soils, lodgepole pine is often the only tree species that will grow. Nevertheless, experiments have demonstrated significant growth increases from fertilization, particularly nitrogen (Cochran 1975). It appears that the growth of lodgepole pine is better on acidic soils than on basic soils (Klinka *et al.* 1998). Typically, low pH, low temperature, accumulation of phenolic-based allelopathic compounds, and poor oxygen supply result in higher rates of net ammonification than net nitrification, due to the inhibition of nitrifying microorganisms (Vitousek *et al.* 1982; Gosz and White 1986; Olff *et al.* 1993; Eviner and Chapin 1997; Stark and Hart, 1997). Accordingly, ammonium compounds are probably a more important source of N for lodgepole pine than nitrates (Krajina *et al.* 1973). However, Min *et al.* (2000) found the NO_3^- uptake capacity of lodgepole pine (via the low affinity transport system) to be substantial, and they suggest that this may represent an important adaptation for colonizing sites with high soil NO_3^- after fire disturbance.

Lodgepole pine's successional role depends upon environmental conditions and extent of competition from associated species (Lotan *et al.* 1983). Lodgepole pine is a minor seral species in warm, moist habitats and a dominant seral species in cool dry habitats. It can, however, attain edaphic climax at relatively high elevations on nutrient poor sites (Pfister and Daubenmire 1975).

Lodgepole pine forests have long been regarded as fire-maintained subclimax forests, and fire regimes have played a role in the successional continuum of this species (Lotan 1976). Lodgepole pine is a prolific seed producer, and repeated fires can eliminate the seed source for other species (Fowells 1965). Its serotinous cones do not open at maturity because of a resinous bond between the cone scales. The bonds break when temperatures reach 45 - 60 °C, and cone scales are then free to open hygroscopically (Perry and Lotan 1977). Large quantities of seeds are thus available for regenerating a stand following fire. Closed cones at or near the soil surface (less than 30 cm) are also subjected to temperatures from insolation sufficient to open them. This may provide seed in harvested areas (Cochran 1969). From a forestry perspective, a common problem of regenerating lodgepole pine stands is overstocking, which results in stagnation at early ages. Many sites are stocked with tens of thousands or even hundreds of thousands of trees per hectare causing severe reductions in growth and yield (Johnstone 1975).

Sitka spruce

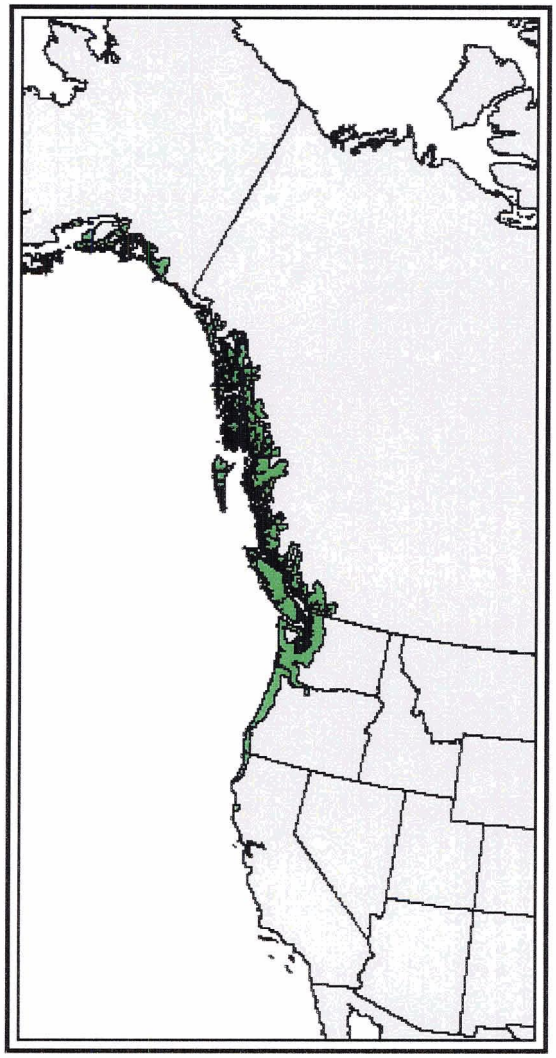
Sitka spruce (*Picea sitchensis* (Bong.) Carr) belongs to the Pinaceae family. It is also known as tideland spruce, coast spruce, and yellow spruce. This species is the largest spruce in the world, reaching heights of >100 m in the Carmanah River Valley (Klinka *et al.* 1998). As such, it is a prominent forest tree in stands along the northwest coast of North America. Sitka spruce grows from latitude 61° N Alaska to 39° N in northern California (Figure 1.2). The most extensive portion of the range in both width and elevation is in southeast Alaska and northern British Columbia, where the east-west range extends for about 210 km to include a narrow mainland strip and the many islands of the Alexander Archipelago in Alaska and the Queen Charlotte Islands in British Columbia (Ruth 1965). In southern British Columbia, the range includes a narrow mainland strip and offshore islands with the best development occurring on the northern tip and west side of Vancouver Island. On the mainland south to Washington, the range tends to be restricted to sea-facing slopes and valley bottoms but may extend inland for several kilometers along the major rivers. In northern California, the range becomes discontinuous, and a disjunct population in Mendocino County, CA, marks the southern

limit of the range (Harris 1990). The seed used in this study originated from provenance 09043 found at 52 ° 14' latitude, 127 ° 14' longitude and 31 m elevation.

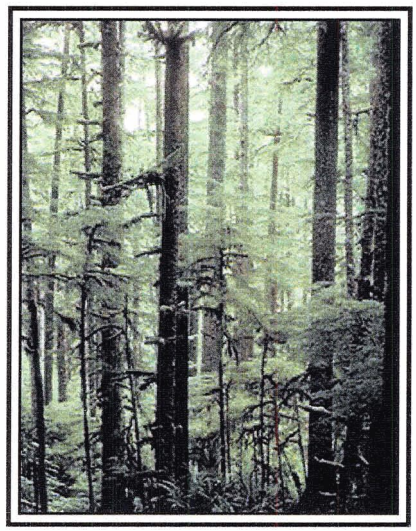
Throughout most of its relatively narrow coastal range from northern California to Alaska, Sitka spruce grows in dense stands (most commonly associated with western hemlock (*Tsuga heterophylla*)) where growth rates are among the highest in North America (Eyre 1980). It is a valuable commercial timber species for lumber, pulp and the high strength-to-weight ratio and resonant qualities of clear Sitka spruce lumber are attributes that have traditionally made its wood valuable for specialty uses (Harris 1990). Examples include sounding boards for high-quality pianos, guitar faces, spars for custom-made or traditional boats, and turbine blades for wind energy conversion systems (Harris 1970; 1978; 1990). As well as providing wildlife habitat, stabilizing soil, filtering water etc., the great size attained by Sitka spruce and its presence in old growth coastal forests has made it a culturally and aesthetically valuable species. It is a component of many national, provincial and state parks, and protected areas. Sitka spruce has also been introduced into Britain and Northern Europe where its high growth rates and climatic adaptations have made it a successful plantation species.

In contrast to the wide ecological amplitude of lodgepole pine, Sitka spruce is restricted to an area of maritime climate with abundant moisture throughout the year, relatively mild winters, and cool summers. It does not tolerate the extremes found in more continental locations (Harris 1990). The most productive growth of Sitka spruce occurs on moist, nutrient rich, hypermaritime sites where soils are derived from rocks rich in calcium and magnesium. Sitka spruce is absent from poor, nitrogen deficient sites (Krajina 1969). This species appears to require relatively high amounts of available nitrogen, calcium, magnesium, and phosphorus (Klinka *et al.* 1998). Along the Pacific coast where ocean spray has a strong influence on vegetation, pure stands of Sitka spruce develop because other species do not tolerate high sodium inputs. Ocean spray is also a good source of external phosphorous (Klinka *et al.* 1998), thus its prominence on the outer coast may also be tied to nutrition.

A



B



C

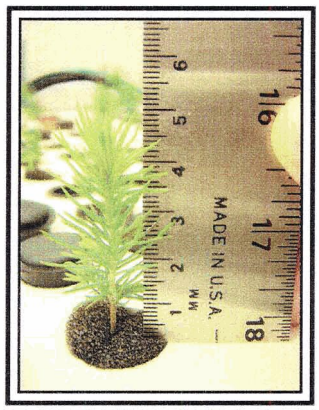


Figure 1.2. **A.** The native range of Sitka spruce (From Harris 1990), **B.** a typical uneven-aged Sitka spruce stand, **C.** a Sitka spruce seedling used in this experiment (the picture was taken 32 days after germination in the FA treatment; seedlings were this size at harvest number two as described in the first chapter of this thesis).

Sitka spruce development is best in deep, moist, well-aerated soils. Drainage is an important factor, and growth is poor on swampy sites (Harris 1990). Soils are usually acidic (pH values of 4.0 to 5.7 are typical), resulting in an abundance of ammonium compounds. However, calcium-rich soils can provide a relatively favourable medium for nitrification (Klinka *et al.* 1998).

Sitka spruce can be found in a wide range of successional stages, depending on environmental conditions. Pure stands usually occur in early successional situations and when stands are influenced by salt spray. This species can be an early pioneer on immature soils recently exposed by glacial retreat or uplift from the sea (Harris 1990). In Oregon and Washington, spruce follows lodgepole pine in succession on coastal sand dunes as they become stabilized by vegetation. In later successional forests, Sitka spruce is generally found in mixed stands, associated most commonly with hemlock (*Tsuga heterophylla*) but also with other conifers such as red-cedar (*Thuja plicata*), redwood *(Sequoia sempervirens),* yellow-cedar *(Chamaecyparis nootkatensis)* and white spruce (*Picea glauca*). Sitka spruce maintains height growth and lives longer than hemlock (few hemlock live more than 500 years; Sitka spruce may live to 700 or 800 years), thus very old Sitka spruce eventually assume a dominant position in old-growth hemlock-spruce stands (Ruth *et al.* 1979).

Ammonium and nitrate concentrations in northern forest soils

In surveys of boreal and temperate forest ecosystems it has been shown that forest floor solution ammonium concentrations ($[NH_4^+]$) range from approximately 0.1-2.1 mM and nitrate concentrations ($[NO_3^-]$) range from 0.7-6.5 mM (Kamminga-van wijk and Prins 1993, Vitousek *et al.* 1982; George *et al.* 1999; Bijlsma *et al.* 2000). The relative abundance of NH_4^+ compared to NO_3^- is determined by a number of soil factors. Most important are pH, temperature, accumulation of organic matter, soil oxygen status (Rice and Pancholy 1972; Haynes and Goh 1978; Lodhi 1978), and the presence of allelopathic chemicals (Dijk and Eck 1995). Typically, low pH, low temperature, accumulation of phenolic-based allelopathic compounds, and poor oxygen supply result in higher rates of net ammonification than net nitrification, due to the inhibition of nitrifying

microorganisms (Vitousek *et al.* 1982; Gosz and White 1986; Olff *et al.* 1993; Eviner and Chapin 1997; Stark and Hart 1997). Soils exhibiting these conditions tend to be late successional (Rice and Pancholy 1972; Britto and Kronzucker 2002), while NO_3^- -rich soils tend to be early successional. Forest disturbances such as fire, avalanche, windthrow or clearcut harvesting can drastically alter soil nutrient profiles, converting a greater proportion of soil nitrogen to nitrate (Likens *et al.* 1969; Vitousek *et al.* 1982; Kronzucker *et al.* 1995a, b, 1997).

Biotronic units and steady-state nutrition theory

Examining the effects of nutrient stress on seedling growth requires large numbers of plants to be grown in highly controlled environments where they are exposed to defined levels of nutrient supply. To interpret results from nutrition experiments, it is necessary to know the variation over time of either the nutrient uptake rate or the amount of nutrient in the plant (Agren 1985; Ingestad and Lund 1986). As well, to compare properties of plants, especially under different environmental conditions, their nutrient status must be stable (Tamm 1964,1968; Linder and Rook 1984; Ingestad and Kähr 1985). Most laboratory or greenhouse experiments control external parameters such as day length, relative humidity and temperature. Experimental plants are generally provided with nutrient media of constant chemical composition, often in the form of hydroponic nutrient solutions with defined initial concentrations of a specific set of inorganic salts. However, the value of such controls is limited if set parameters are changed by plant activity (i.e. if the processes of uptake and growth alter nutrient solution concentrations), of if they do not translate into a controlled physiological state of the experimental plants.

Few experiments have attempted to control the relative growth rate and internal nutrient concentration of experimental plants. However, plant growth characteristics are likely to respond to differences in relative growth rate and internal nutrient concentrations, as well as to the experimental variables of interest. Standard nutrient regimes involving periodic applications of fixed amounts of fertilizer will result in declining growth rates and fluctuating N concentrations. In the early years of a plant's life, the amount of nutrients required per unit time increases with increasing plant

biomass, therefore the amount of nutrients supplied must increase correspondingly. This dynamic increase in plant requirements is not contained in classical concentration-driven nutritional concepts and needs to be expressed by means of a time dependent variable (Ingestad and Lund 1986).

The concept of relative addition rate (RAR), and steady-state nutrition was introduced for this purpose by Ingestad and Lund in 1979. RAR is analgous to the relative growth rate of the plant (RGR) and is expressed as the amount of nutrient to be added per unit time in relation to the amount of nutrient present in the plant (Ingestad and Agren 1988). In this model, nutrient flux, instead of medium concentration, is the variable driving plant growth.

Using relative nutrient addition rate techniques, steady-state plant growth and nutrition can be maintained and experimental conditions can be controlled in a meaningful sense (Ingestad and Lund 1979; Ingestad 1982; Jia and Ingestad 1984; Ågren 1985; Ingestad and Kähr 1985). Growing plants at constant relative growth rates within Biotronic units eliminates the possibility that differences in biomass allocation within a nutrient treatment are only due to differences in growth rate. By adding nutrients exponentially, changes in relative growth rate and internal nutrient concentration during an experiment are minimized, making it possible to directly examine the effects of environmental variables on plant morphology and physiology (e.g. Ingestad and McDonald 1989; Pettersson and McDonald 1994).

Optimal partitioning and ontogenetic drift

For decades, plant ecologists have been studying plant response to variation in the availability of resources. Optimal partitioning models and theories suggest that plants respond to variable environmental resources by partitioning biomass and internal resources among plant organs to optimize the capture of nutrients, light, water and CO_2 in a manner that maximizes plant growth rate (Brouwer 1962; Davidson 1969; Thornley 1969,1972; Mooney 1972; Bloom *et al.* 1985; Szaniawski 1987; Levin *et al.* 1989; Hilbert 1990; Dewar 1993; Mooney and Winner 1991; Reynolds and D'Antonio 1996). According to optimal partitioning theories, plants exposed to a limited below-ground resource, such as nutrients, are predicted to respond by shifting carbohydrates to

processes associated with nutrient capture in lieu of carbon acquisition (Bloom *et al.* 1985). Conversely, plants exposed to a limited above-ground resource, such as light, are predicted to shift resources towards stem and leaf growth in lieu of directing carbohydrates to nutrient uptake (Bloom *et al.* 1985).

There is extensive evidence in support of the predictions of optimal partitioning models (reviews Mooney 1972; Bloom *et al.* 1985; Szaniawski 1987; Mooney and Winner 1991; Reynolds and D'Antonio 1996). Several studies have even investigated the mechanisms underlying the observed partitioning responses (e.g. Hirose 1987; Chu *et al.* 1992; Dewar 1993; Luo *et al.* 1994). However, some authors question the validity of optimal partitioning theory. Coleman *et al.* (1994) suggested that adjustments in biomass allocation cited as support for optimal partitioning theories may be a natural consequence of plant growth. When a trait changes in a predictable way as a function of plant growth or development it is defined as 'ontogenetic drift' (Evans 1972). Coleman *et al.* (1994), point out that few plant studies account for the morphological and physiological patterns that occur during the normal course of growth and development before they examine adjustments in biomass allocation in response to fluctuating resource levels.

Studies that explicitly distinguish between allocational changes due to ontogenetic drift and those that occur in response to the environment have shown that biomass partitioning seems to be partially dependent on the species of plant and on the resources altered. For instance, after three to four growing seasons in elevated CO_2, Norby *et al.* (1992, 1993) showed that red oak (*Quercus alba*) saplings partitioned more biomass to roots relative to shoots, whereas tulip tree (*Liriodendron tulipifera*) showed no such response. Murray *et al.* (1996) found no shifts in biomass partitioning in Sitka spruce after three years of exposure to elevated CO_2. Gebauer *et al.* (1996) found that elevated CO_2 had no direct effect on biomass partitioning in *Pinus taeda* seedlings. King *et al.* (1996) showed that neither *Pinus ponderosa* nor *Pinus taeda* altered biomass partitioning in response to elevated CO_2; however, *Pinus ponderosa* seedlings partitioned less biomass to secondary roots (lateral) relative to primary and taproot fractions at elevated temperature. *Pinus taeda* increased partitioning to lateral roots in response to both increased temperature and nitrogen supply (King *et al.* 1996), and preferentially

allocated biomass to lateral roots when grown in a N solution concentration of 0.5 mM NH_4NO_3 (Gebauer *et al.* 1996).

In general, studies show that adjustments in biomass allocation, beyond those due to ontogenetic drift, occur in response to nutrient limitation (Cromer and Jarvis 1990; Li *et al.* 1991; Coleman *et al.* 1993; Van de Vijver *et al.* 1993; Hartvigsen and McNaughton 1995; Gebauer *et al.* 1996; Gedroc *et al.* 1996; King *et al.* 1996, 1999; McConnaughay and Coleman 1999). Fewer studies have reported biomass allocation responses due to water (Ledig *et al.* 1970; McConnaughay and Coleman 1999; King *et al.* 1999), CO_2 (Coleman *et al.* 1993; Farnsworth *et al.* 1996; Gebauer *et al.* 1996), or light availability (Hughes and Evans 1962; Ledig and Perry 1965; Terry 1968; Ledig *et al.* 1970; Evans 1972; Corre 1983; Rice and Bazzaz 1989; McConnaughay and Coleman 1999).

To test optimal partitioning theories it would be useful to compare genotypes that only differed in their allocation pattern under sub-optimal conditions. However, there are no such mutants or varieties whose biomass partitioning is constant throughout ontogeny. Thus, to distinguish between allocational changes that occur as a consequence of ontogenetic drift and those that occur in response to the environment, researchers have corrected for plant size by conducting allometric analysis. In an experiment involving two nutrient treatments, a typical allometric analysis would involve plotting the natural logarithm of one biomass parameter (e.g. leaf mass) against the natural logarithm of another biomass parameter (e.g. root mass) and testing to determine whether the lines that fit the data points of the two treatments are statistically different from one another. This transformation allows an assessment of treatment effects with linear regression techniques by testing for significant differences between slopes. By conducting classic growth analyses, as well as allometric analyses, my aim is to better understand the degree and direction of phenotypic changes involved in lodgepole pine and Sitka spruce seedling responses to nutrient availability.

Component mass fractions verses S:R ratios

Throughout the last century, plant biomass allocation has generally been analysed in terms of above- and below-ground compartments. Such a dichotomy is easy to apply, but

from a functional point of view it is unsatisfactory (Körner 1994; King *et al.* 1999; Poorter and Nagel 2000). The combination of leaves and stems into one compartment does not acknowledge the very different functions of these organs. By combining their own literature survey with the work of Körner (1994), who compared the biomass allocation of full-grown evergreen and coniferous trees, Poorter and Nagel (2000) illustrate this point convincingly. Using a two compartment breakdown, shoot to root ratio (S:R) values were higher for deciduous than coniferous species, and S:R values for both types of species did not deviate greatly from those compiled for tree seedlings and herbaceous plants (S:R for deciduous trees = 4.1, coniferous = 5.2, tree seedlings = 2.1, herbaceous plants = 2.3). However, when the same data were broken down into a three compartment model, it appeared that allocation of biomass to leaves was three fold higher for adult coniferous trees than deciduous (leaf mass fractions of 0.04 vs. 0.01 g g^{-1}, respectively), and large differences in allocation between full-grown trees and herbaceous plants were obvious (leaf mass fraction of the latter being 0.40 g g^{-1}).

As well as combining functionally different plant parts, S:R ratios combine perennial tissues (stems, branches, coarse roots) with ephemeral tissues (fine roots, foliage), which further confounds our understanding of the functional response of biomass partitioning and does not account for the seasonal dynamics of tissues (King *et al.* 1999). The controlled environment, young age of the seedlings, and short duration of this study makes seasonal dynamics unimportant. In the field, however, conifer foliage biomass can vary substantially over the course of the growing season (Kinerson *et al.* 1974). Time of year may have a large impact on the allometric relationships between foliage and other plant parts.

By analysing biomass allocation using at least three compartments (leaves, stems and roots), and expressing the biomass of each organ relative to that of the total plant (leaf mass fraction, LMF; stem mass fraction, SMF; root mass fraction, RMF) one gains considerably more insight into plant growth than from S:R ratios, where the relative contribution of leaves and stems to the numerator is unknown. Biomass fractions are also less sensitive to small changes in allocation than S:R ratios, especially when one component forms a small percentage of the total plant biomass (Poorter and Nagel 2000). Further, LMF and RMF variables form important components of growth analysis and

carbon economy models (Garnier 1991; Poorter and Pothman 1992; Poorter and Nagel 2000). Quantifying allocation in such a way will aid those interested in testing such models. Finally, presenting biomass allocation data as component mass fractions would make more of the literature accessible for comparative purposes. LMF, SMF and RMF data can easily be converted into S:R values, as they bear the same information, but the reverse is not possible.

Mycorrhizal associations

Given that ectomycorrhizal associations are ubiquitously found in the roots of field-grown coniferous trees (Wilcox 1991; Marschner 1995), the validity of tree growth studies where these associations are lacking has frequently been questioned. A common response to such arguments is that we first need to understand the growth and physiology of tree roots independently. Without baseline information on species-specific allocation, morphological, and physiological responses to the nutrient environment, we cannot understand how these are affected by the presence of mycorrhizae in the field. Given that root infection with mycorrhizas is enhanced by a pre-existing network in the soil, and that severe soil disturbances (e.g. clear cutting, ground fires or rigorous soil mixing) can severely depress and delay mycorrhizal infection (Jasper *et al.* 1989; Miller and McGonigle 1992; Marschner 1995), it may be particularly relevant to understand the growth and N nutrition of young seedlings in the absence of mycorrhizal infection.

Mycorrhizae can greatly extend the absorptive surface of the symbiotic root system making a significant contribution to phosphate acquisition (Read 1991). Plassard *et al.* (1991), however, found that in pine, there are no major differences in N uptake and assimilation between mycorrhizal and non-mycorrhizal parts of the root system. Substantial recent evidence further indicates that ectomycorrhizal fungi do not contribute significantly to the acquisition of NH_4^+ by tree seedlings (Eltrop and Marschner 1996; Plassard *et al.* 2000; Constable *et al.* 2001) and that a similar situation may exist for other N sources (Scheromm and Plassard 1988; Chalot and Brun 1988; Persson and Nasholm 2001; Gobert and Plassard 2002).

Climate change and conifer nutrition

Ecosystem response to climate change is being feverishly explored in the scientific community. Increases in atmospheric carbon dioxide concentration are linked to increases in temperature. Both of these factors affect how trees respond to soil nutrient availability.

In this century, greenhouse gas emissions are predicted to cause a 3-6 °C increase in mean land surface temperature at high and temperate latitudes (Houghton *et al.* 1996, Kattenburg *et al.* 1996). Growth rate and ultimate carbon gain of many woody perennials, both coniferous and broad-leaved species, have been shown to increase in response to increasing CO_2 concentration and temperature (see reviews by Eamus and Jarvis 1989; Ceulemans and Mousseau 1994; Idso and Idso 1994; Curtis and Wang 1998; Saxe *et al.* 2001). Growth increases are indirect results of the direct effects of elevated atmospheric CO_2 concentration and temperature on photosynthesis, photorespiration, respiration and transpiration. Warmer temperatures increase rates of virtually all chemical and biological processes in plants and soils, if substrates are available, up to a point where enzymes denature (Jackson *et al.* 1994). Since current levels of atmospheric CO_2 limit photosynthesis, increases in CO_2 should theoretically enhance net photosynthetic rates and hence biomass accumulation (Murray *et al.* 2000). However, if Leibig's law of the minimum, which states that "the environmental resource present in the least amount will determine growth" applies, then increased plant growth in future temperature and CO_2 regimes may not occur to the extent predicted by many researchers (Kirschbaum *et al.* 1994).

Environmental resources that commonly limit plant growth are soil nutrients. In many temperate forest ecosystems, it is possible that nutrient limitations could ameliorate the effect of CO_2 fertilization on net sequestration of atmospheric CO_2 into organic life forms (Murray *et al.* 2000). The extent to which nutrients become limiting to forest ecosystems depends on a multitude of factors, not least of which are tree species' nutritional adaptations and nutrient demands. A preference for ammonium over nitrate as an inorganic nitrogen source has been observed in many coniferous tree species (Marschner *et al.* 1991; Peuke and Tischner 1991; Kronzucker *et al.* 1997; Malagoli *et al.* 2000). This can be attributed to a lower capacity of the NO_3^- transport system due to the

adaptation of these species to soil with low pH and higher NH_4^+ availability (Stadler and Gebauer 1992). Bassirirad *et al.* (1997) observed that when loblolly and ponderosa pine trees were exposed to high CO_2 concentrations, NH_4^+ uptake was repressed and NO_3^- uptake was enhanced. The inability of many late successional coniferous trees species to efficiently utilize NO_3^- (see Figure 1.3 and accompanying discussion) may be deleterious in disturbed, CO_2 rich environments.

In their study of the photosynthetic responses of Sitka spruce to elevated CO_2 and nutrition, Murray *et al.* (2000) showed that N did indeed become limiting to growth in enhanced CO_2 environments. Elevated CO_2 concentration increased seedling dry mass by 37 % in their high N treatment but had no effect in their low N treatment. Seedlings receiving low N supply had a 33 % lower light-saturated rate of photosynthesis than seedlings receiving the high N supply rate. This agrees with several observations from a range of C_3 species, showing that assimilation rate was generally more strongly stimulated by elevated CO_2 when plants received high nutrient supply rates (Tissue *et al.* 1993; Ceulemans and Mosseau 1994; Idso and Idso 1994; Petterson and McDonald 1994).

While soil nutrients have the potential to limit growth in a CO_2-enhanced atmosphere, it is also possible that increased CO_2 may positively affect root growth, rhizosphere conditions, and plant nutrient use efficiency. Adjustments in the nutrient pool within the plant and/or adjustments in metabolic requirements could lower nutrient demand and increase nutrient use efficiency. Less nitrogen would be needed per unit dry matter increment if the efficiency of ribulose 1,5 bisphosphate carboxylase-oxygenase (RubisCO) was higher in elevated CO_2 (see Appendix I). Murray *et al.* (2000) reported an increase in nitrogen use efficiency (NUE) in Sitka spruce seedlings as a result of growth in elevated CO_2. Photosynthetic rates of seedlings grown and measured at elevated CO_2 concentrations were higher than those of seedlings grown and measured in ambient CO_2 concentration at similar foliar nitrogen concentration. This suggests that the photosynthetic system optimized N distribution by moving it from RubisCO to the more limiting proteins involved in the light reactions.

Soil nutrient supply may increase by stimulating biological activity in the rhizosphere as a result of soil mineralization brought about by enhanced exudation of

carbon (van Veen *et al.* 1989). Zak *et al.* (1993) suggest that elevated CO_2 concentrations will lead to increased mineralization rates as a direct result of increased root activity. If fine root growth is also stimulated, nutrient acquisition rates could increase.

Plants growing under nutrient poor conditions respond less to elevated CO_2 than well-nourished plants. This idea is often taken as axiomatic. It has been argued, however, that the considerable emphasis in the potential role of nitrogen limitations in constraining ecosystem responses to increasing CO_2 is misplaced. Although plants in natural ecosystems show growth increases when given N fertilizer "it is by no means a corollary that relative growth enhancements at higher C_a [atmospheric carbon] will be reduced where nitrogen fertilization is not optimal" (Lloyd and Farquhar 1996). Plants growing under conditions of low N nutrition can exhibit greater relative growth enhancements to increased CO_2 concentrations than those growing with high N supply. Lloyd and Farquhar (1996) suggest that plants with high respiratory costs and/or plants which have a high rate of nutrient uptake relative to their rate of carbon assimilation will show the greatest increases in photosynthetic rates as a result of increases in atmospheric carbon (see model Appendix 2, p. 29, Lloyd and Farquhar 1996). Many models showing nitrogen limitations when CO_2 is increased assume that terrestrial plant C/N ratios are constant (Rastetter *et al.* 1991; Melillo *et al.* 1993; Hudson *et al.* 1994; Comins and McMurtrie 1993); however, tree C/N ratios vary with plant size independent of effects of nutrient availability (Gifford 1994; Kirschbaum 1994; Lloyd and Farquhar 1996). Allometric carbon and nitrogen interrelationships need to be taken into account in models attempting to link carbon and nitrogen cycles (see 'optimal partitioning and ontgenetic drift' section above).

Clearly, the ways in which nutrient availability interacts with enhanced [CO_2], temperature and other variables associated with climate change are complex. Factors that need to be considered include the effects of plant nitrogen content on photosynthesis (Field and Mooney 1986; Evans 1989), how root carbon density and photoassimilate availability influence the enzymes of nitrogen metabolism and nitrogen uptake (Asiam *et al.* 1979; Pace *et al.* 1990; Vincentz *et al.* 1993), the effects of enhanced plant growth under high atmospheric carbon on rates of nitrogen mineralization (Zak *et al.* 1993) and how root exudates influence rates of soil N mineralization (van Veen *et al.* 1989; Billes *et*

al. 1993). It is possible that global warming may disrupt mechanisms regulating nutrient uptake to the extent that changes in species distributions may occur. However, greater understanding of the physiology of plant carbon and nutrient allocation, along with factors that influence nutrient uptake rates in natural ecosystems, is needed before we can address the crucial question of whether global warming will cause ecosystem conditions to shift outside the range in which trees can behave optimally. This study will further our understanding of tree nutritional physiology and improve our ability to answer these types of questions.

Nitrogen nutrition and patterns in forest succession

A recent paper by Kronzucker *et al.* (2003) hypothesizes that root ammonium transport efficiency can determine forest colonization patterns. These authors suggest that the specialized N adaptations of trees may lead to reduced competitive ability in soils with altered nitrogen profiles. Forest disturbances such as clearcut logging, fire, windthrow, and avalanche can lead to substantial increases in soil nitrate (Bormann *et al.* 1968; Likens *et al.* 1969; Vitousek *et al.* 1982; Kronzucker *et al.* 1995a, b, 1997). Conversely, undisturbed forest soils in temperate and boreal forest zones are generally dominated by ammonium, to the point of its exclusive presence as an inorganic N source on many sites (Robertson 1982; Blew and Parkinson 1993). Kronzucker *et al.* (2003) explain that white spruce is excluded from disturbed, NO_3^--rich soils because of an atrophic utilization capacity for NO_3^- at the level of uptake, metabolism, and intracellular storage. White spruce is a conifer with wide distribution and dominance in late successional stages of temperate and boreal forests (Farrar 1995). The poor adaptation of early successional tree species such as Douglas-fir and aspen to NH_4^+ as an N source results from a situation quite distinct from the exceptionally poor uptake of NO_3^- in white spruce. These species do not lack sufficient uptake capacity for NH_4^+, rather, unidirectional flux studies reveal that uptake is excessive and insufficiently regulated (Kronzucker *et al.* 2003). At external NH_4^+ concentrations of 1.5 mM, the efflux of NH_4^+ from the cytosol of trembling aspen and Douglas-fir root cells was substantially higher than from white spruce. Analysis of subcellular partitioning of radio-labelled N revealed

that the large efflux in the early successional species was due to an unusually high unidirectional influx across the plasma membranes of root cells, while only a small fraction of this incoming N was channelled to N metabolism, to the shoot, or to root cell vacuoles (Figure 1.3) (Kronzucker *et al.* 2003).

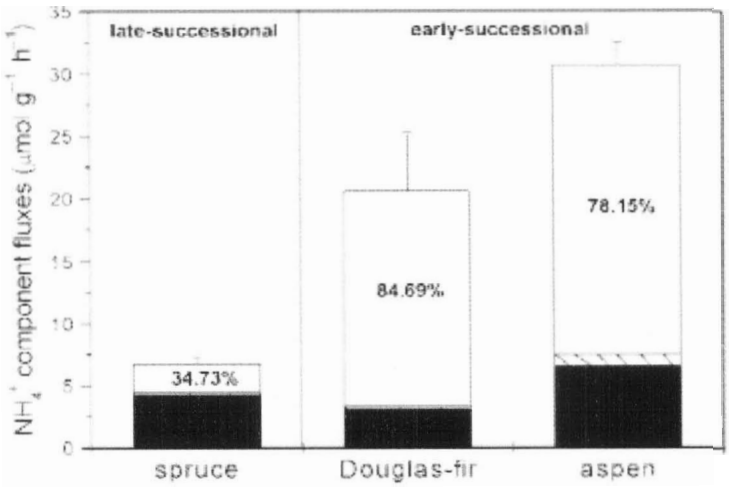


Figure 1.3. Subcellular component fluxes of NH_4^+ in root cells of white spruce, Douglas-fir and trembling aspen exposed to 1.5 mM external NH_4^+. Black segments represent combined NH_4^+ flux to metabolism and to the root-cell vacuoles, cross-hatched segments represent N flux to the shoot, and open segments represent NH_4^+ efflux from the roots. The sum of these component fluxes equals influx of NH_4^+ into root cells and the standard error bars pertain to these values (n = 8). Note the high efflux percentage in early-successional species. Data are from Kronzucker *et al.* (1995a,b) and Min *et al.* (1999). Figure was adapted from Kronzucker *et al.* (2003).

The futile cycling of NH_4^+ at the plasma membrane in the early successional species (efflux equalling 78-85 % of influx, compared to 35 % in spruce (Figure 1.3)) explains the low efficiency of acquisition for this N source: NH_4^+ appears to be lost almost as rapidly as it is gained. Kronzucker *et al.* (2003) argue that this cellular malfunction in NH_4^+ transport may provide a physiological basis for the elimination of colonizing tree species such as trembling aspen and Douglas-fir as NO_3^- is depleted and NH_4^+ starts to dominate in forest soils during the course of ecological succession. Further

support for this hypothesis comes from de Graff *et al.* (1998) who have shown that sensitivity to excess N can lead to extirpation of heathland herbaceous species.

These studies provide valuable context for my thesis research. Cellular level physiological adaptations to N nutrition are predicted to significantly affect forest tree species colonization patterns. By investigating NH_4^+ and NO_3^- ion uptake of Sitka spruce, a species that is generally restricted to sites with nutrient rich soils, and lodgepole pine, a species that is able to tolerate soils of low fertility, I will test some physiological mechanisms underlying the diverse nutritional adaptations of coniferous trees.

Chapter 2. Biomass allocation, morphology and metabolism of lodgepole pine and Sitka spruce seedlings grown at high and low levels of nitrogen supply

INTRODUCTION

In order to meet the increasing demand for forest products on a declining land base, silvicultural treatments such as fertilization can be used to increase the availability of site resources and, in turn, productivity of forest plantations. The concept that site quality is a fixed property has been replaced with the understanding that productivity is largely dependent on resource availability and that site resources, especially nutrients, can be manipulated (Albaugh *et al.* 1998). Increases in worldwide plant production over the last century have been proportional to the increased use of fertilizers (Evans 1980). However, the success of this venture and the immense production of cheap fertilizers, has often led to excessive and careless use of fertilizers (Ingestad 1991).

While most coniferous trees show positive responses to fertilization (Klinka *et al.* 1998), the practice of applying fertilizer in a few large doses has little to do with the continuous natural processes (i.e. weather and season-dependent mineralization rates, biological decomposition, plant nutrient uptake rates etc.) that take place in the soil and determine its fertility. The concentration levels required for optimum nutrient uptake rates are extremely low (Olsen 1953; Ingestad and Agren 1988), and leaching of excessive fertilizer from soils is of widespread environmental concern. Despite years of plant physiology research and recent advancements in molecular biology, fundamental relationships between photosynthesis, nutrition and total plant growth remain poorly understood (Linder and Rook 1984; Garnier 1991; Ingestad 1991; Burns *et al.* 1997; Shinano 2001).

Nitrogen is the nutrient most often limiting in northern forest soils (Millard 1996; Reich *et al.* 1998b). The design of appropriate N fertilization programs for coniferous seedlings requires an understanding of biomass allocation patterns, and how seedling growth is influenced by tissue N concentrations. It has often been reported that trees respond to low nitrogen supply by increasing their root to shoot ratios (Ingestad and Lund 1979; Linder and Rook 1984; Ingestad and Ågren 1991; Proe and Millard 1994) and by

altering a range of leaf characteristics such as number, size and specific leaf area (Heilman and Xie 1994; Ibrahim *et al.* 1997). In addition to changes in dry matter partitioning, nitrogen can affect rates of gas exchange per unit tissue in different plant components (Bowman and Conant 1994; Ibrahim *et al.* 1997). Overall plant response to limited nitrogen arises from complex interactions between rates of gas exchange per unit tissue, dry matter and N allocation between tree components. To resolve these interactions, growth response and carbon allocation must be studied at the whole plant level.

Unfortunately, few definite patterns have been indentified that relate plasticity in biomass allocation and tissue N concentrations to whole seedling growth. It is difficult to evaluate species-specific growth responses of coniferous seedlings from the literature. Conifers may be less responsive to nutrients than deciduous hardwood trees (Linder and Rook 1984), and increased nutrient availability may have greater effects on the growth of plant species from fertile habitats than on species from chronically infertile habitats (Chapin 1980). However, growth responses of a given species can vary considerably among different studies (Rook 1991). This is influenced by factors such as size variation in seedlings, self shading, disequilibrium between N supply and plant demands (Linder and Rook 1984), and deficiencies of other nutrients (Reich and Schoettle 1988).

To be able to interpret results from nutrition experiments, it is necessary to know the variation over time of either the nutrient uptake rate or the amount of nutrient in the plant (Agren 1985; Ingestad and Lund 1986). If we are to compare properties of plants under different environmental conditions, the nutrient status of the plants must also be stable (Tamm 1964,1968; Linder and Rook 1984; Ingestad and Kähr 1985). While most laboratory or greenhouse experiments control external parameters such as day length, temperature, and concentration of nutrient solutions, few control the relative growth rate and internal nutrient concentration of the experimental plants. Plant growth characteristics, however, are likely to respond to differences in relative growth rate and internal nutrient concentrations, as well as to the experimental variables of interest. Standard nutrient regimes involving periodic applications of fixed fertilizer amounts will result in declining growth rates and fluctuating N concentrations. However, by using relative nutrient addition rate techniques, steady-state plant growth and nutrition can be

maintained (Ingestad and Lund 1979; Ingestad 1982; Jia and Ingestad 1984; Ågren 1985; Ingestad and Kähr 1985). To achieve steady-state nutrition, the exponentially increasing nutrient demand that occurs in the early stages of seedling growth is supplied by exponential increases in solution concentration. By adding nutrients in a rate-correlated manner, changes in relative growth rate and internal nutrient concentration are minimized. It then becomes possible to examine the effects of environmental variables on plant morphology and physiology (e.g. Ingestad and McDonald 1989; Pettersson and McDonald 1994).

Growth rates, biomass allocation patterns among plant parts, tissue nutrient concentrations, morphological characteristics such as specific root length (SRL) and specific leaf area (SLA) and physiological parameters such a photosynthesis vary according to species, to the environment, as well as during ontogeny. Studies published as early as 1916 have shown that biomass allocation to roots increases with decreasing nutrient availability (Brenchley 1916). Since then it has been established that plants are capable of adjusting the relative sizes and distributions of organ systems, such as leaf canopies and root networks, in response to changes in the external supply of nutrients (Johnson 1985; Robinson 1986; Johnson and Thornley 1987; Van der Werf *et al.* 1993). Plants often distribute a relatively high proportion of biomass to leaves in nutrient-rich environments (e.g.Tilman 1988), while they distribute a relatively high proportion of biomass to roots in nutrient-poor environments (e.g. Brouwer 1962; Chapin *et al.* 1987; Crick and Grime 1987). These patterns have led to the formulation of functional equilibrium or optimal partitioning theories (Brouwer 1962; Davidson 1969; Thornley 1969,1972; reviewed by Mooney 1972, Bloom *et al.* 1985; Szaniawski 1987; Mooney and Winner 1991; Reynolds and D'Antonio 1996). According to these theories, plants shift their allocation to towards shoots if a low level of above-ground resources, such as light and CO_2, impairs the carbon gain of the shoot, while plants shift their allocation to roots if below-ground resources such as nutrients or water are limiting. These adjustments then optimize growth as they enable the plant to capture more of the limiting resource (Bloom *et al.* 1985).

As plants increase in size, many phenotypic traits change, such as the relative partitioning of biomass between organs. This phenomenon is referred to as ontogenetic

drift (Evans 1972). Comparisons of plants grown at high and low levels of resource supply have generally been made at common points in time or at common plant ages, and this can lead to serious complications in interpreting the data (Ledig *et al.* 1970; Evans 1972; Coleman *et al.* 1993; Coleman *et al.* 1994; Gebauer *et al.* 1996; Gedroc *et al.* 1996; McConnaughay and Coleman 1999). Obvington (1957) showed that root:shoot ratios of *Pinus sylvestrus* L. reached a maximum of 0.82 at age seven and then declined to 0.29 by age 55. Bazzaz *et al.* (1989) observed that in several herbaceous plants the ratio of root:shoot biomass is initially very high because of early root growth and establishment in the soil, but then drops rapidly over the course of the first few weeks of growth. In addition, experimental treatments that accelerate growth, such as fertilization, may alter allometry simply because they increase plant size during the experimental period. Thus, plants subject to different experimental treatments that are sampled at common points in time or at common ages will frequently be ontogenetically dissimilar regarding the relative distribution of biomass to different plant organs.

When examining shifts in biomass, morphology and tissue nitrogen concentrations, it is necessary to use statistical methods that allow the separation of direct nutrient treatment effects from ontogenetic effects. Allometric analyses have been employed to correct allocation patterns for possible size differences between plants of different treatments (Erikson and Michelini 1957; Ledig and Perry 1966; Ledig *et al.* 1970; Evans 1972; Packard and Boardman 1988; Coleman *et al.* 1994; Jasienski and Bazzaz 1999). After applying allometric analyses, differences in biomass allocation frequently disappear. This indicates that allocation differences are due to plant size rather than treatment *per se* (Doley 1975; Coleman *et al.* 1993, Coleman *et al.* 1994 and references therein; Gedroc *et al.* 1996; Gunn and Farrar 1999). It is necessary to account for phenotypic changes that occur in the normal course of growth and development before claiming that adjustments in morphology and physiology are plant responses to altered levels of environmental resources.

In this study, lodgepole pine (*Pinus contorta* (Dougl.) ex. Loud.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings were grown in Biotronic units (BIOTRONIC AB, Upsala, Sweden) using relative nutrient addition rate techniques. Because Sitka spruce is generally restricted to moist, nutrient rich sites (Harris 1990; Klinka et al.

1998), while lodgepole pine is able to tolerate soils of low fertility (Pfister and Daubenmire 1975; Lotan and Perry 1983; Klinka et al. 1998), these two species may demonstrate unique patterns of biomass allocation, tissue N concentrations, and show different morphological and physiological responses to environmental N supply. Growing plants with stable whole-plant N concentrations at constant relative growth rates within Biotronic units allows us to make quantitative comparisons between species. Further, controlled relative growth rates eliminate the possibility that differences in biomass allocation within a nutrient treatment are solely due to differences in growth rate.

In addition to conducting classical growth analyses, this study addresses whether lodgepole pine and Sitka spruce seedlings alter biomass, SLA and SRL to compensate for limitations of below ground resources in a manner consistent with optimal partitioning theory, or whether these parameters are strongly controlled by ontogenetic drift. Allometric analyses were conducted to determine if observed differences in allocation patterns were independent of seedling size, or if they were brought about indirectly by nutrient treatment- and/or species-induced size differences.

MATERIALS AND METHODS

Plant Culture

Seedlings of lodgepole pine (provenance 29172, 53 °20' latitude, 123 °25' longitude, and 1100 m elevation) and Sitka spruce (provenance 09043, 52 ° 14' latitude, 127 ° 14' longitude and 31 m elevation) were imbibed for 24 h, surface sterilized with 3 % hydrogen peroxide for 5 min and then rinsed thoroughly in de-ionized water. Washed seeds were then placed onto moistened Kimpack in the base of petri dishes. The dishes were covered, sealed with Parafilm and placed in the refrigerator at (4 °C) for 21 d. At the end of the stratification period seeds were evenly spaced in germination trays and placed in a germination chamber set for 8 h days at 30 °C and 16 h nights at 20 °C. When the roots of the germinants were approximately 3 cm long they were inserted into closed-cell foam collars (two seedlings per collar) and placed into the Biotronic units containing 5 L of nutrient solution that was continuously circulated and sprayed aeroponically on the roots. Table 2.1 lists the contents of the pre-treatment nutrient solution. Once the seedlings reached approximately 0.05 g (2 - 3 weeks) they were weighed and each individual was assigned to a Biotronic treatment unit. Seedlings were subject to two nutrient treatments, free access to nutrients (FA), and 4 % relative addition rate (4 % RAR) nutrient stress (see Table 2.2 for nutrient solutions). Due to equipment limitations (there were only 4 Biotronic units), 4% RAR nutrient stress and free access treatments occurred at different times (Table 2.3). For each treatment, two Biotronic units contained lodgepole pine seedlings and two contained Sitka spruce. Sixty seedlings were inserted singly into foam collars and distributed randomly within each Biotronic unit containing 5 L of nutrient solution. The four units were arranged randomly within a Conviron growth chamber set for 16 h days with 500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 8 h nights with 0 PPFD. A relative humidity of 50 % and air temperature of 20 °C were maintained throughout.

solutions and plant roots were kept in the dark, and the foam plugs holding the seedlings in the lids of the units were changed regularly.

The theoretical background for the nutrient addition technique used in the units has been reviewed by Ingestad (1982, 1986), and Ingestad and Lund (1986). The aim of this technique is to maintain a constant internal nutrient concentration or steady-state nutrition, i.e.: $d(n/\mathrm{W})/dt = 0$ (n is the amount of nutrient in the plant, W is plant biomass, and t is time). It follows from the steady-state condition that:

$$(1/n)(dn/dt) = (1/\mathrm{W})(d\mathrm{W}/dt) \qquad (1)$$

For steady-state nutrition to be achieved the relative nutrient uptake rate (RUR) must equal the relative growth rate (RGR) and must be matched by an equal relative addition rate (RAR) (RAR = RUR = RGR). Relative growth rate (RGR) was measured by weighing the plants at each harvest:

$$\mathrm{RGR} = (1/\mathrm{W})(d\mathrm{W}/dt) = d(\ln \mathrm{W})/dt \approx (\ln \mathrm{W}_2 - \ln \mathrm{W}_1)/\, t_2 - t_1\,, \qquad (2)$$

where W is plant biomass, t is time, and W_1 and W_2 are plant biomass at time t_1 and t_2, respectively. According to Ingestad (1982) and Ingestad and Lund (1986) plant mass must increase by at least 7 times during the experimental period to fulfill the theoretical requirement for assessment of steady-state, and experimental accuracy is acceptable if there is no systematic change in RGR over time.

Nutrients were automatically titrated, hourly, into the growth units (see Figure 2.1). For the FA treatment, nutrient additions were made by means of conductivity titrations, which restored the culture solution to its original nutrient contents. For the 4% RAR treatment, the amount of nutrients (n_t) that needed to be added to provide for one hour of uptake at any time t hours from the beginning of the experiment was calculated from the following equation:

$$n_t = n_0(e^{\mathrm{RAR}/24} - 1)(e^{\mathrm{RAR}t/24}) \qquad (3)$$

where n_0 is the initial amount of nutrient in the plant and RAR is the relative addition rate expressed on a per day basis.

The average initial plant N concentration after the pre-treatment phase (analysed from the destructive harvest of a subset of seedlings) was 6 mg g^{-1} fresh mass. The initial N contents in each biotronic unit were calculated as:

$$N_0 = 6 \times (W_0/W_1) \quad (4)$$

where W_0 was the initial fresh mass of all the seedlings in a unit and W_1 was the fresh mass of seedlings following either the FA or the 4% RAR treatment period. N_0 was recalculated after each harvest to account for the biomass removed.

Nitrogen productivity (NP) was calculated from relative growth rate and plant N concentration according to Ingestad (1981) as:

$$NP = (e^{RGR} - 1)/wpN, \quad (5)$$

where wpN is whole-plant nitrogen concentration in g N g^{-1} total plant dry mass.

Nutrient uptake rate (NUR) (the net uptake rate of nitrogen per unit root mass) (g wpN g^{-1} root dry mass day^{-1})) was calculated as:

$$NUR = ((N_2 - N_1)/ t_2 - t_1) \times ((\ln R_2 - \ln R_1)/R_2 - R_1), \quad (6)$$

Where N is whole plant nitrogen content (g), t is time (days) and N_2 and N_1 are plant nitrogen contents at time t_2 and t_1, respectively. R is root biomass (g) and R_2 and R_1 are root biomass at time t_2 and t_1, respectively.

Unit leaf rate (ULR), which is the rate of increase in total plant biomass per unit leaf area (g total plant dry mass cm^{-2} leaf area day^{-1}) was calculated as:

$$ULR = ((W_2 - W_1)/ t_2 - t_1) \times ((\ln LA_2 - \ln LA_1)/LA_2 - LA_1), \quad (7)$$

where W is plant biomass (g), t is time (days) and W_2 and W_1 are plant biomass at time t_2 and t_1, respectively. LA is leaf area (cm^2) and LA_2 and LA_1 are leaf area at time t_2 and t_1, respectively.

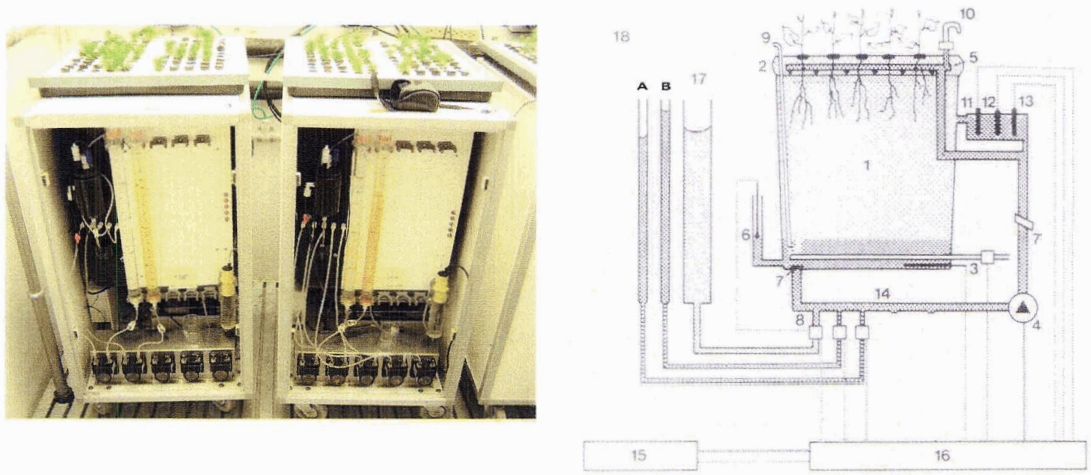


Figure 2.1. Biotronic units. **Left:** Two of the Biotronic units used in this study (contained inside a Conviron growth chamber at the Pacific Forestry Centre). **Right:** A schematic diagram of a Biotronic unit. 1) black plastic container to hold nutrient solution and contain roots, 2) cover with holes for seedlings, 3) cooling coil and heat source, 4) pump for circulation of nutrient solution, 5) tubes with nozzles to spray nutrient solution, 6) solution level sensor, 7) filters, 8) magnetic valve for addition of distilled water, 9) aeration nozzle, 10) sampling tube, 11) pH transmitter with measurement cell, 12) conductivity transmitter with measurement cell, 13) temperature sensor, 14) magnetic valves for nutrient addition, 15) PC computer with 16) interface, 17) burette for distilled water, 18)(A,B) burettes for nutrient stock solutions (adapted from Ingestad and Lund 1986).

Measurements

The Sitka spruce seed took longer to germinate than the lodgepole pine seed, thus spruce germinants were moved from the pre-treatment to the treatment units 7 days after the pine in both the free access and 4% RAR treatment. Seedlings were harvested three times during the growth period when they approximately doubled in size. The delay between lodgepole pine and Sitka spruce harvests was maintained throughout the free access

treatment. However, due to Christmas scheduling difficulties, the lodgepole pine seedlings were harvested at the same time as the Sitka spruce for the 4% RAR treatment. These seedlings had more than doubled in size between harvests. Specifics on the dates of each harvest are listed in Table 2.3.

Table 2.3. Harvest times for free access and 4% relative addition rate (RAR) nutrient treatment experiments. 12 seedlings from each of 4 Biotronic units (24 pine and 24 spruce) were measured at each harvest. Harvests occurred after the seedlings approximately doubled in size.

		Free Access	4% RAR
Species	Harvest number	Day from start of treatment	Day from start of treatment
Lodgepole pine	1	20	27
	2	32	62
	3	48	91
Sitka spruce	1	18	21
	2	33	56
	3	46	85

Twelve seedlings per Biotronic unit were weighed fresh every three days for the free access treatment, and weekly for the 4% RAR nutrient stress treatment. Each seedling was removed from the biotronic unit, gently blotted and weighed, then immediately replaced. At each harvest, photosynthesis, leaf dark-respiration and root respiration were measured for 12 trees per unit using a LCA4, closed system, dual gas exchange analyser (Analytical Development Company Ltd. Hoddesdon, Hertfordshire, UK). For all measurements, air flow through the chamber was 4 m s^{-1}; air inside the chamber was mixed by a small fan. Reference air was drawn from a large bag to maintain a stable CO_2 concentration. Each shoot was placed into the measurement chamber in such a way as to leave its roots within the Biotronic unit. CO_2 flux data were recorded after the difference in CO_2 concentration between the reference air and the chamber air had stabilized. Measurements of photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}), chamber temperature (^{o}C), and transpiration (measured as the difference in water vapour (millibars) entering and leaving the shoot chamber) were also taken when readings stabilized (5-10 min). The cuvette was then covered with an aluminium foil lid and dark-

respiration was measured after the difference in CO_2 concentration between the reference air and the chamber air had stabilized. Root respiration was measured for each of the 12 seedlings per unit by removing the roots from the unit, gently coiling them together, tying the root bundle with a twist tie, and inserting them into the LCA4 cuvette. Roots were kept in the dark during all measurements.

After photosynthesis and respiration measurements, the 12 seedlings from each unit were destructively harvested. Fresh mass of roots, stems and needles, and root length, root collar diameter, stem length and leaf area were measured. Roots, stems and needles of each seedling were individually packaged in coin envelopes and placed in a drying oven at 60 °C for 72 h. Dry mass of all samples was measured. Each dried root, stem and needle sample was ground to a fine dust using a Wig-L-Bug Amalgamator (model 3110-3A, Crescent Dental MFG Co., Illinois, USA). Ground samples were stored in 1 ml glass vials until they could be packaged for N content analysis. The glass vials were opened and samples were placed in a drying oven at 60 °C for 24 h prior to packaging. 8 mg of tissue was packaged into a 10 mm diameter tin capsule and analysed for N and C contents using a FlashEA 1112 Elemental Analyser (ThermoQuest Corp., Italy).

Statistics

To test the significance ($p<0.05$) of species differences within nutrient treatments standard one-way analyses of variance were conducted (SAS Institute Inc. 1988). The following linear model was used to represent individual tree values for each trait measured:

$$Yijlk = \mu + s_i + u_{l(i)} + h_j + sh_{ij} + shu_{jl(i)} + e_{ijlk} \qquad (7)$$

where:

$Yijlk$ is the value of the kth tree, of the ith species within the lth unit in the jth harvest

μ is the overall mean

s_i is the fixed effect of species i

$u_{l(i)}$ is the random effect of the ith species within the lth unit

h_j is the fixed effect of harvest j

sh_{ij} is the random interaction effect of the ith species and the jth harvest

$shu_{jl(i)}$ is the random interaction effect of the jth harvest and the ith species within the lth unit

e_{ijlk} is the random error of the kth tree of the ith species within the lth unit in the jth harvest

Due to the split plot experimental design, hypothesis tests for species differences were made with $u_{l(i)}$ as the error term. Hypothesis tests for harvest, and harvest × species interactions were made with $shu_{jl(i)}$ as the error term.

There were significant harvest × species interactions in the overall model (equation 7) for all growth parameters analysed (i.e. biomass allocation, specific leaf area, specific root length, N allocation, photosynthesis, respiration), thus harvests 2 and 3 were analysed separately with tests of hypothesis made using ANOVA mean squares for unit(spp) as the error term. There were no significant Biotronic unit × species interactions for any of the growth parameters allowing data from seedlings of the same species, grown in separate Biotronic units, to be combined for each nutrient treatment

For mass fraction comparisons (i.e. LMF, SMF, RMF), and any other tests with proportions as the response variable, data were arcsin transformed (arcsin × (√proportion)) to account for their non-normality prior to all statistical analyses.

T–tests were used to make comparisons between the free access and 4% RAR nutrient treatments. Bonferroni's adjustment was applied to account for multiple hypothesis tests.

Correlation analyses were carried out using the PROC CORR procedure in SAS (SAS Institute Inc. 1988). Correlations involving component nitrogen concentrations, net photosynthesis (A), root respiration (R), specific leaf area (SLA), and specific root length (SRL) were calculated using the combined data sets (including harvests 2 and 3) from both nutrient treatments (n=192). If the above variables were correlated within nutrient treatments, within species and within harvests, n=24. Because RGR, NP, NUR and ULR were calculated (rather than directly measured) parameters, all data used in correlations between these parameters were the mean values of 4 seedlings. Thus, n=48 for correlations across species and nutrient treatments. Similarly when RGR was correlated with A, R, R:A, SLA or SRL, data were the mean of 4 seedlings (n=48).

Allometric relationships were determined using the general equation:

$$\ln y = b_0 + b_1 \ln x, \quad (8)$$

where x and y are either total plant biomass, morphological characteristics such as SLA or SRL, or the biomass or N concentration of different plant parts. This transformation allows an assessment of treatment effects with linear regression techniques, by testing for significant differences between slopes (b_1). Heterogeneity of slopes was tested using a SAS general linear models (GLM) procedure (Little *et al.* 1991). Significant interactions between species or N treatment and ln x indicated differences between slopes. Both harvests 2 and 3 were included in analyses.

RESULTS

Stability of relative growth rates and N nutrition

There was a > 7 fold increase in seedling mass during the experimental period for both lodgepole pine and Sitka spruce in the two nutrient treatments (Figure 2.2). RGR was constant over time for both species in the free access (FA) treatment; however, the slight decrease in RGR from harvest 1 to harvest 2 for both lodgepole pine and Sitka spruce in the 4% relative addition rate (4% RAR) treatment (Figure 2.3 a) indicated seedlings had not yet equilibrated to the nutrient treatment and that steady state was not achieved until after the first harvest. Harvest 1 was not included in statistical analyses. The constancy of whole plant nitrogen concentration (wpN) over time for both species in the FA treatment, indicated that seedlings maintained steady state nitrogen nutrition across all three harvests. The increase in wpN from harvest 1 to harvest 2 for both species in the 4% RAR treatment again indicated that the seedlings had not yet achieved steady state. Consistent with RGR comparisons, harvest 1 was excluded from statistical analysis.

RGRs between FA and 4% RAR nutrient stress treatments were significantly different for both species (Figure 2.3 a). The RGR, averaged for harvests 2 and 3, of lodgepole pine at FA was (mean ± SE), 0.0797 ± 0.0009 g g^{-1} day^{-1}, while at 4% RAR it was 0.0432 ± 0.0018 g g^{-1} day^{-1} ($p = 0.0031$). The mean RGR for Sitka spruce at FA was

0.0794 ± 0.0017 g g^{-1} day^{-1}, while at 4% RAR it was 0.0475 ± 0.0009 g g^{-1} day^{-1} (p= 0.0037). Within each treatment the RGRs of lodgepole pine and Sitka spruce were not significantly different (FA p= 0.8738; 4% RAR p= 0.2084).

For both species, whole plant nitrogen concentrations (wpNs) were significantly different between the FA and 4% RAR nutrient treatments (Figure 2.3b). The mean wpN (harvests 2 and 3) for lodgepole pine at free access was (mean ± SE), 2.26 ± 0.032%, while at 4% RAR it was 1.14 ± 0.033% (p< 0.001). The mean wpN for Sitka spruce at free access was 2.31 ± 0.051%, while at 4% RAR it was 1.047 ± 0.026% (p< 0.001). Within each treatment the wpNs of lodgepole pine and Sitka spruce were not significantly different (free access, p= 0.6095; 4% RAR, p= 0.5451).

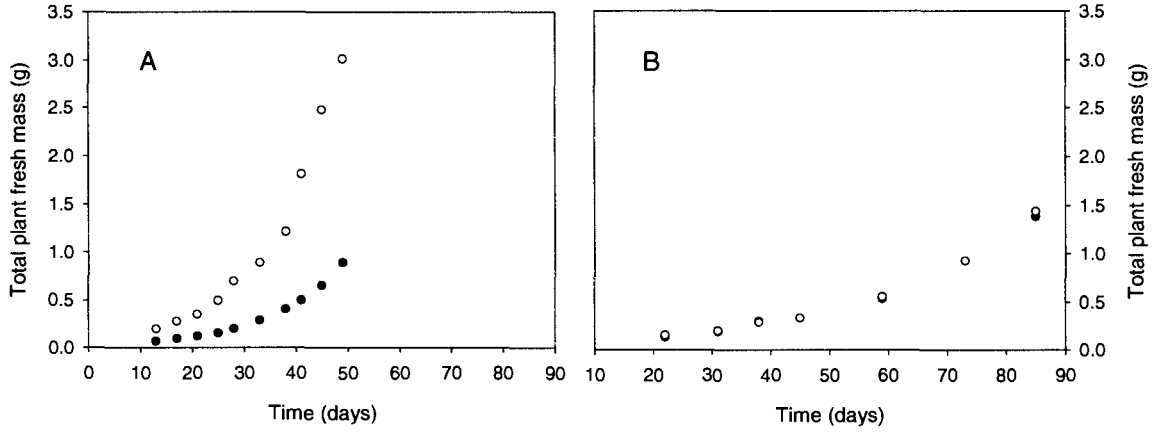


Figure 2.2. Growth curves for lodgepole pine (closed circles) and Sitka spruce (open circles) grown with **A.** free access to nutrients and **B.** 4% RAR nutrient stress. Each point represents the mean of 24 seedlings. Harvest and experimental end dates are indicated in Table 2.3.

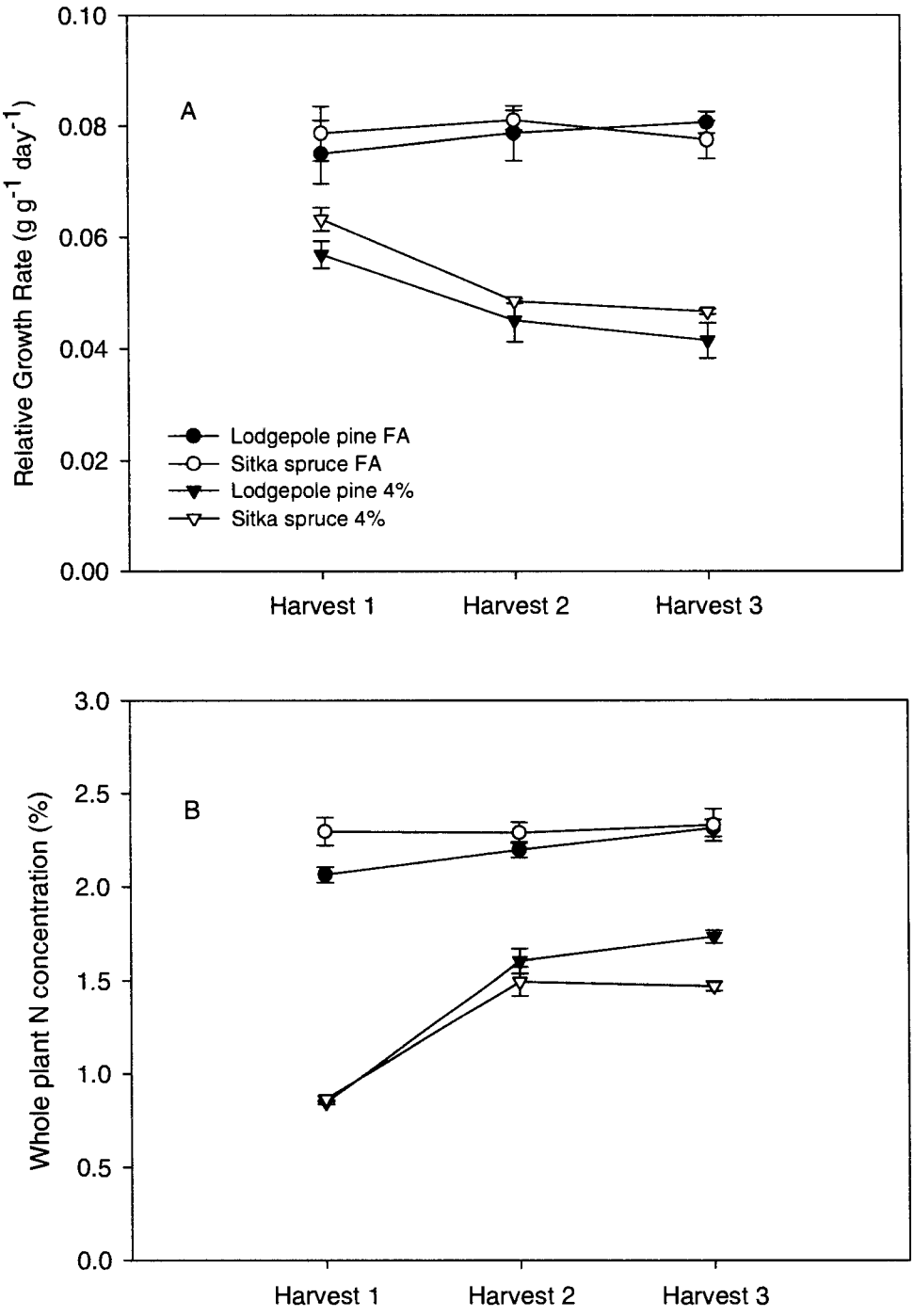


Figure 2.3. Changes in **A.** relative growth rate (RGR) and **B.** whole plant N concentrations (wpN) over time at harvests 1, 2 and 3 (harvest dates are given in Table 2.3), for lodgepole pine (closed symbols) and Sitka spruce (open symbols) seedlings grown with free access to nutrients (circles) or under 4% RAR nutrient stress (triangles). Points represent the mean of 24 seedlings. Vertical bars = standard error of the mean.

Biomass allocation

Biomass allocation was analysed for three components: roots, stems, and leaves (Figure 2.4). For harvest 2 (H2), in both the FA treatment and the 4% RAR treatment, root mass fraction (RMF), stem mass fraction (SMF) and leaf mass fraction (LMF) were all statistically different between species (Table 2.4, Figure 2.4). For harvest 3 (H3) component mass fractions were significantly different between species within nutrient treatments except for SMF in the 4% RAR treatment. Tests for statistical significance were carried out on arc sine transformed data. Values given in text, figures and tables are based on untransformed data.

In the FA treatment, both species allocated the greatest proportion of their total biomass to leaves. Sitka spruce, however, allocated a significantly larger proportion of biomass to leaves and stems than lodgepole pine, while lodgepole pine seedlings allocated a significantly greater proportion of biomass to roots (Table 2.4, Figure 2.4). In the 4% RAR nutrient stress treatment, both species allocated a greater proportion of biomass to roots than they did in the FA treatment. T–tests indicated that all component mass fractions were significantly different ($p<0.001$) between free access and 4% RAR nutrient stress treatments for both lodgepole pine and Sitka spruce. While Sitka spruce seedlings allocated significantly more biomass to roots under conditions of nutrient stress than they did in the free access nutrient treatment, seedlings still allocated the greatest proportion of their biomass to needles ((Mean ± SE) H2 LMF: 0.4812 ± 0.008; H3 LMF: 0.5172 ± 0.008). Lodgepole pine seedlings, however, allocated the greatest proportion of their total biomass to roots in the 4% RAR nutrient treatment (H2 RMF: 0.5414 ± 0.007; H3 RMF: 0.5054 ± 0.013) (Table 2.4, Figure 2.4).

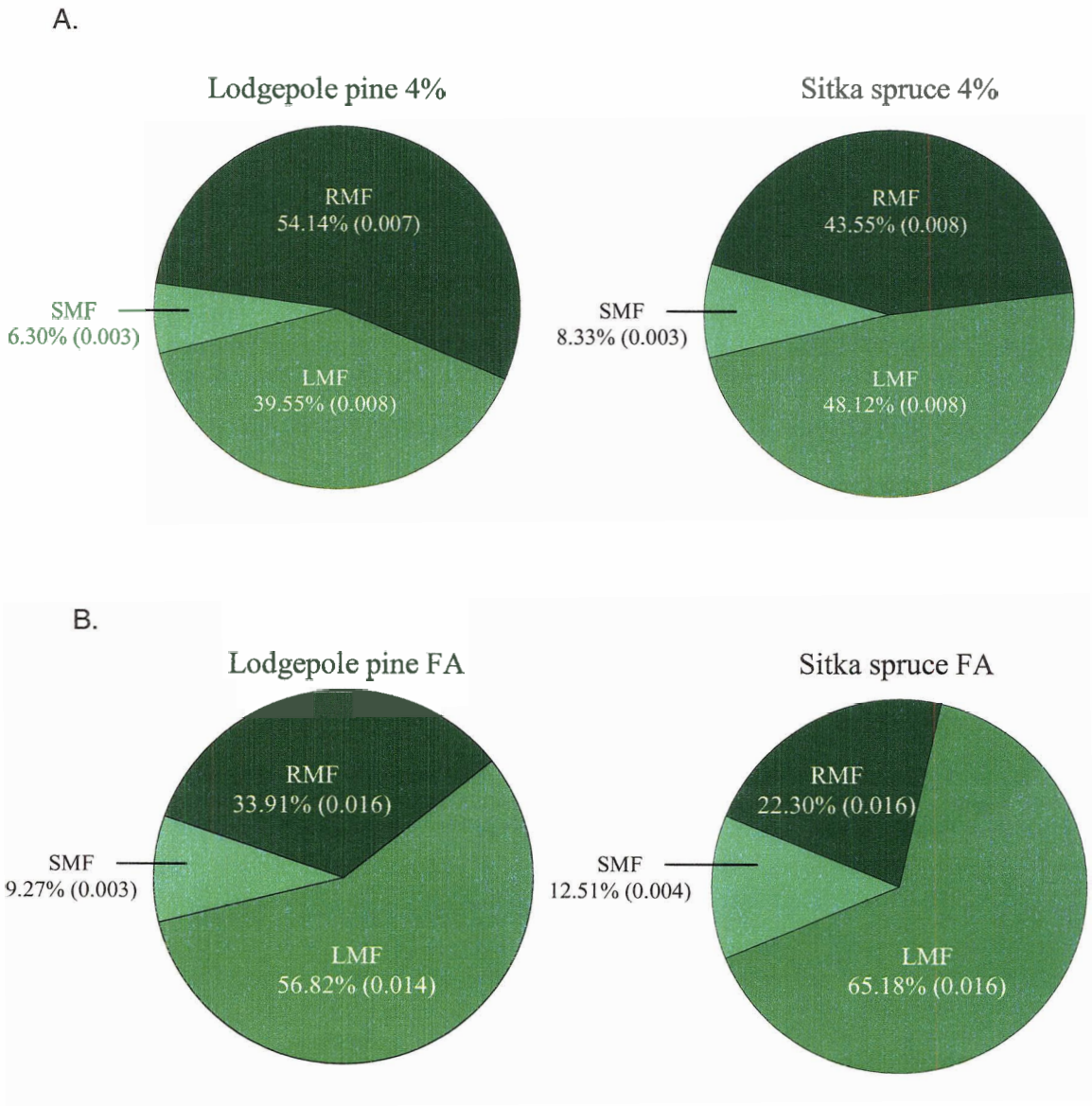


Figure 2.4. Mean biomass allocation data depicted as percent total seedling dry mass allocated to roots (RMF), stems (SMF) and leaves (LMF) of lodgepole pine and Sitka spruce seedlings grown under conditions of **A.** 4% RAR nutrient stress and **B.** free access to nutrients (values in brackets = standard error of the mean). Data is from harvest 2, n=24 for each species.

Table 2.4. Means and probabilities for ANOVA *F*-tests on lodgepole pine and Sitka spruce biomass allocation components (g g^{-1}) in relation to Biotronic unit and species. RMF = root mass fraction, SMF= stem mass fraction, LMF =leaf mass fraction. There were significant harvest x species interactions in the overall model thus harvests 2 and 3 were analysed separately with tests of hypothesis made using ANOVA ms for unit(spp) as the error term. Comparisons were made between species within the free access (FA) and 4% RAR nutrient stress (4% RAR) treatment. There were 4 units with 12 trees/unit /harvest. * = significant at the 0.05 level.

Harvest 2, FA	RMF	SMF	LMF
	(Mean biomass fraction (g g^{-1}) ± SE)		
lodgepole pine	0.34 ± 0.008	0.093 ± 0.003	0.57 ± 0.008
Sitka spruce	0.22 ± 0.006	0.13 ± 0.004	0.65 ± 0.007
	ANOVA P value		
	RMF	SMF	LMF
Species	0.0099*	0.0186*	0.0120*
Unit(species)	0.2986	0.4327	0.4563
Harvest 3, FA	RMF	SMF	LMF
	(Mean biomass fraction (g g^{-1}) ± SE)		
lodgepole pine	0.38 ± 0.007	0.079 ± 0.002	0.55 ± 0.007
Sitka spruce	0.22 ± 0.009	0.11 ± 0.004	0.67 ± 0.010
	ANOVA P value		
	RMF	SMF	LMF
Species	0.0173*	0.0089*	0.0174*
Unit(species)	0.0905	0.6008	0.2207
Harvest 2, 4% RAR	RMF	SMF	LMF
	(Mean biomass fraction (g g^{-1}) ± SE)		
lodgepole pine	0.54 ± 0.007	0.063 ± 0.003	0.40 ± 0.008
Sitka spruce	0.44 ± 0.008	0.083 ± 0.003	0.48 ± 0.008
	ANOVA P value		
	RMF	SMF	LMF
Species	0.0316*	0.0015*	0.0441*
Unit(species)	0.0608	0.9658	0.0726
Harvest 3, 4% RAR	RMF	SMF	LMF
	(Mean biomass fraction (g g^{-1}) ± SE)		
lodgepole pine	0.51 ± 0.012	0.085 ± 0.003	0.41 ± 0.012
Sitka spruce	0.39 ± 0.009	0.090 ± 0.003	0.52 ± 0.008
	ANOVA P value		
	RMF	SMF	LMF
Species	0.0345*	0.4385	0.0434*
Unit(species)	0.1150	0.3589	0.1166

Biomass allocation: Optimal partitioning or ontogenetic drift?

Root mass fractions were higher for both species when grown at 4% RAR, and leaf and shoot mass fractions were higher for both species grown at FA (Figure 2.4). Next, we asked if the observed differences in allocation patterns were independent of seedling size, or if they were brought about indirectly by nutrient treatment- and/or species-induced size differences.

Allometric analyses

Figure 2.5 depicts allometric analyses of leaf biomass, stem biomass and root biomass plotted against the biomass of the whole plant. For LMF in the FA treatment, the test for heterogeneity of slopes revealed that the slopes of the regression lines fit to the data for Sitka spruce and lodgepole pine were significantly different from one another ($p=0.0021$). For all other plots of component biomass against total plant biomass, in both the FA and 4% RAR treatments, slopes of the regression lines were not significantly different between species (i.e there were no significant spp × ln dry mass interaction terms). This indicates that for FA seedlings, differences in stem and root mass fractions were due to indirect effects of seedling size and not to species differences in allocation *per se*. The same is true for all mass fraction (stem, root, and leaf dry mass) differences between lodgepole pine and Sitka spruce in the 4% RAR nutrient treatment.

There were direct nutrient treatment effects on biomass allocation, independent of seedling size. When the biomass allocation of seedlings within species was compared between FA and 4% RAR treatments, the slopes of the regression lines for leaf biomass, stem biomass and root biomass plotted against the biomass of the whole plant, were all significantly different ($p<0.01$). Thus, while component dry masses of the two species did not differ significantly within a nutrient treatment when compared at a common size (total plant dry mass), differences in biomass allocation between nutrient treatments (within species) remained.

Allometric analyses of leaf dry mass plotted against root dry mass, and shoot dry mass plotted against root dry mass revealed that there were no species differences in relative allocation to above vs. below ground biomass, within either the FA or the 4%

RAR treatments (i.e. there were no significant spp × ln root dry mass interactions, or within a nutrient treatment, the slope of the lines fit to the data for lodgepole pine and Sitka spruce were not significantly different from one another) (Figure 2.6). Within species, however, both lodgepole pine and Sitka spruce differed significantly ($p<0.01$) in above and below ground biomass allocation between nutrient treatments. Although absolute leaf to root ratios were higher for free access seedlings, due primarily to lower root biomass, ((mean ratio ± SE) lodgepole pine, FA = 1.598 ± 0.05, lodgepole pine 4% RAR = 0.788 ± 0.03; Sitka spruce, FA = 3.176 ± 0.19; Sitka spruce 4% RAR = 1.231 ± 0.03) the steeper slopes of the lines fit to the data for 4% RAR seedlings indicates that the relative increase in leaf growth relative to root growth was actually higher in low-N grown plants (Figure 2.6 a).

The same relationships seen for leaf:root ratios are seen for stem:root ratios (Figure 2.6 b). FA seedlings had less root dry mass than 4% RAR seedlings and the absolute stem:root ratios were higher for FA seedlings ((mean ratio ± SE) lodgepole pine, FA = 0.2463 ± 0.01, lodgepole pine 4% RAR = 0.1445 ± 0.006; Sitka spruce, FA = 0.568 ± 0.03; Sitka spruce 4% RAR = 0.213 ± 0.007). The steeper slopes of the lines fit to the data for 4% RAR seedlings, however, indicate that the relative increase in stem growth compared to root growth was higher in low-N grown plants (Figure 2.6 b).

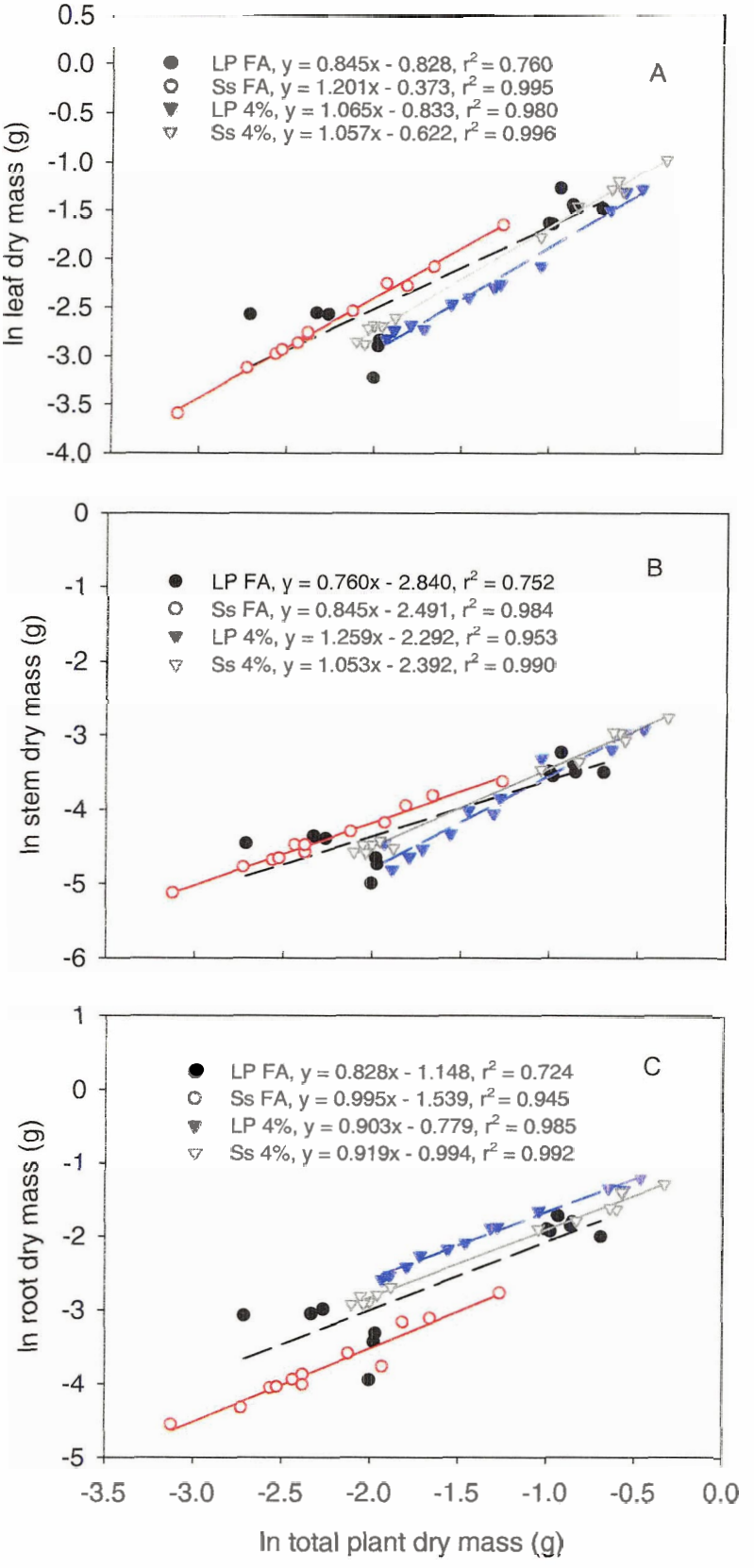


Figure 2.5. Allometric analyses of **A.** leaf, **B.** stem and, **C.** root dry mass against total plant biomass for lodgepole pine (LP, black and blue closed symbols) and Sitka spruce (Ss, red and grey open symbols) seedlings grown with free access to nutrients (FA, circles) and under conditions of 4% RAR nutrient stress (4%, triangles). Each point represents the mean of 4 seedlings.

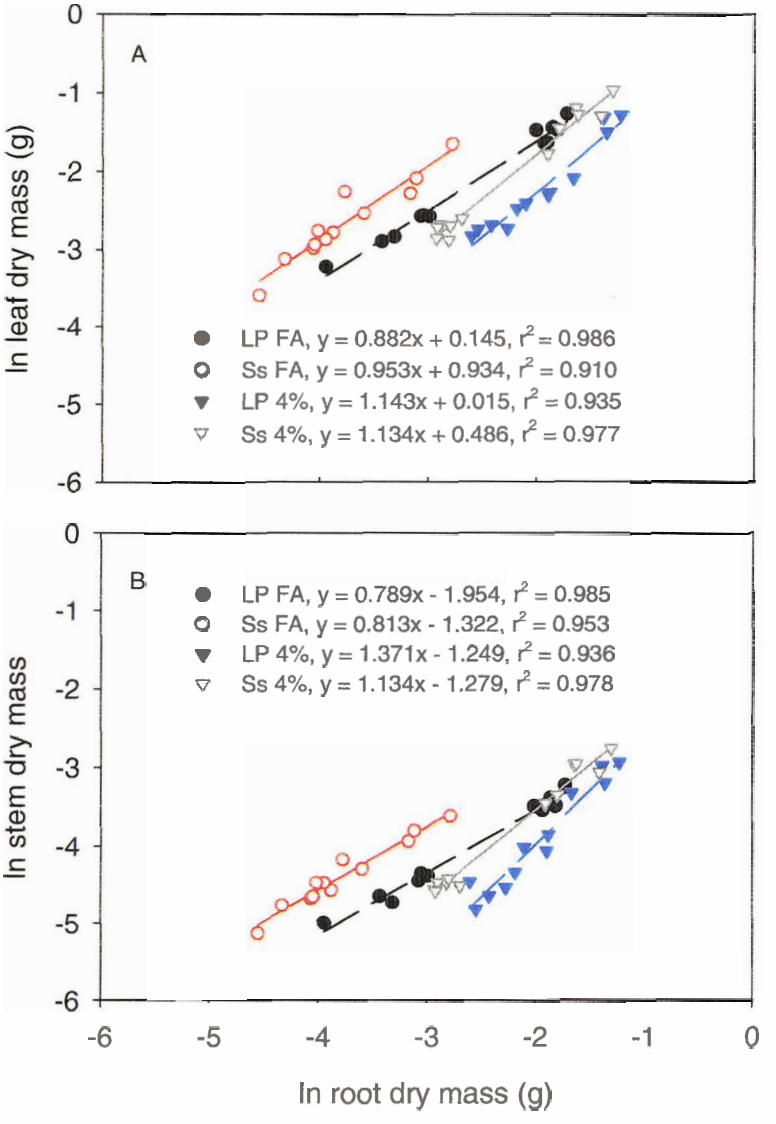


Figure 2.6. Allometric plots of **A.** leaf vs. root mass and, **B.** stem vs. root mass for lodgepole pine (LP: black and blue closed symbols) and Sitka spruce (Ss: red and grey open symbols) seedlings grown with either free access to nutrients (FA: circles) or under conditions of 4% relative addition rate nutrient stress (4%: triangles). Each point represents the mean of 4 seedlings.

Morphological comparisons: Specific leaf area and specific root length

To compare species and nutrient treatment differences in an above-ground and a below-ground morphological parameter, specific leaf area (SLA, leaf area per unit leaf mass) and specific root length (SRL, root length per unit root mass) were analysed.

Specific Leaf Area

Analysis of SLA (Figure 2.7) revealed that leaf area per unit leaf mass remained relatively constant both between species within a nutrient treatment and between nutrient treatments. For Sitka spruce, there were no significant differences in SLA from H2 to H3 within either nutrient treatment. SLA was slightly lower for seedlings in the FA nutrient treatment than seedlings in the 4% RAR nutrient treatment (mean ± SE; FA: H2, 21.78 ± 0.60, 4% RAR H2, 24.31 ± 0.52, ($p= 0.003$); FA H3 21.58 ± 0.26, 4% RAR H3 23.79 ± 0.64, ($p=0.002$)). Lodgepole pine seedlings, however, showed a significant decrease in SLA from H2 to harvest H3 in both treatments ((Mean ± SE) FA: H2, 23.34 ± 0.47; H3, 19.95 ± 0.31 ($p<0.001$); 4% RAR: H2, 24.21 ± 0.54; H3, 21.83 ± 0.62 ($p=0.006$)). This is likely due to the production of secondary needles (needles in pairs of two, borne in fascicles) between H2 and H3. As seen for Sitka spruce, SLA was slightly lower for lodgepole pine FA seedlings than for seedlings subject to 4% RAR nutrient stress, though these differences were only statistically significant for H3 (H2 $p= 0.230$; H3 $p=0.009$).

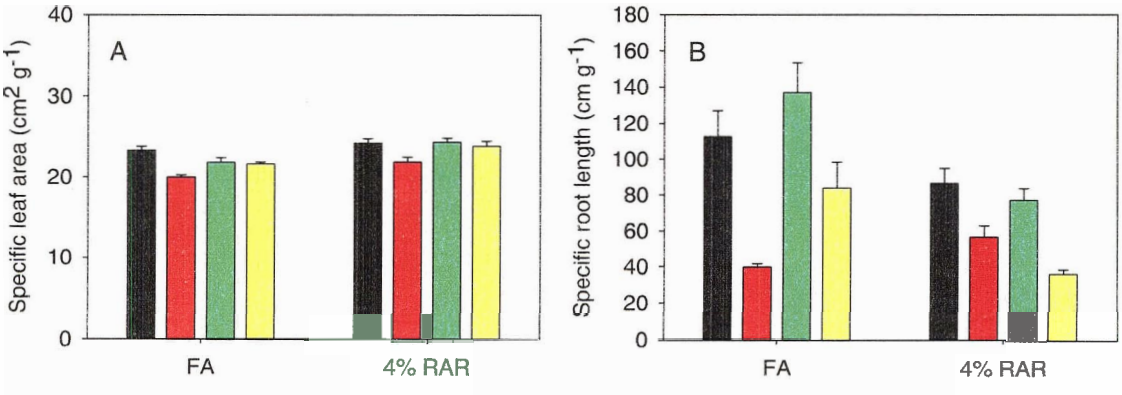


Figure 2.7. Morphological parameters **A.** specific leaf area (leaf area per unit leaf mass) and, **B.** specific root length (root length per unit root mass) for lodgepole pine and Sitka spruce grown with free access to nutrients (FA) or under conditions of 4% relative addition rate nutrient stress (4% RAR). n=24. Black bars = lodgepole pine H2; Red bars = lodgepole pine H3; Green bars = Sitka spruce H2; yellow bars = Sitka spruce H3.

Specific Root Length

Species and nutrient treatment comparisons

There were significant species and treatment differences in the below-ground morphological parameter, specific root length. A high SRL value indicates that roots are long and thin. In the FA treatment, Sitka spruce seedlings had higher SRL than lodgepole pine, although this difference only became statistically significant at H3 ($p=0.0036$). The reverse was true for the 4% RAR treatment. Lodgepole pine seedlings had higher SRL than Sitka spruce seedlings, but again, the difference only became statistically significant at H3 ($p=0.0017$).

Comparisons of SRL between FA and 4% RAR nutrient treatments indicated that both lodgepole pine and Sitka spruce seedlings had significantly greater SRL at FA than at 4% RAR ($p<0.01$), with one exception. SRL of lodgepole pine seedlings at H3 was significantly greater at 4% RAR than it was at FA ($p=0.015$).

Harvest comparisons

Both lodgepole pine and Sitka spruce seedlings seem to be subject to ontogenetic drift in SRL. SRL decreases as plants increase in size from H2 to H3 in both the FA and 4% RAR nutrient treatments ((Mean ± SE) lodgepole pine, FA, H2: 112.45 ± 14.58, H3: 39.66 ± 1.82 ($p<0.001$); Sitka spruce, FA, H2: 136.94 ± 16.59, H3: 83.83 ± 14.55 ($p<0.001$); lodgepole pine, 4% RAR, H2: 86.46 ± 8.46, H3: 56.55 ± 6.43 ($p<0.001$); Sitka spruce, 4% RAR, H2: 76.84 ± 6.62, H3: 35.81 ± 2.31 ($p<0.001$)). In the FA treatment the decrease in SRL between harvests was larger for lodgepole pine than Sitka spruce (Δ SRL= 72.79 cm g^{-1} for lodgepole pine vs. 53.51 cm g^{-1} for Sitka spruce), while in the 4% RAR treatment the decrease in SRL was greater for Sitka spruce (Δ SRL= 29.91 cm g^{-1} for lodgepole pine vs. 41.03 cm g^{-1} for Sitka spruce). Thus, lodgepole pine may be better able to alter SRL in response to the nutrient environment (decreasing SRL at high N, and maintaining relatively higher SRL at low N).

Allometric analyses

Specific Leaf Area

Allometric analyses of SLA vs total plant dry mass (Figure 2.8a), compliment the results obtained from the analysis of the untransformed data (Figure 2.7a). For both lodgepole pine and Sitka spruce seedlings there were no significant differences in the slopes of the lines fit to the FA and 4% RAR nutrient treatment data (i.e. there were no significant treatment × ln total dry mass interactions indicating that, within a species, SLA was not significantly different between treatments). As seen in Figure 2.7a, SLA remained relatively constant between nutrient treatments and the within-species treatment differences were slight. Within-species differences in SLA between FA and 4% RAR seedlings are more likely to be explained by differences in seedling size, rather than as species-specific morphological responses to the nutrient environment.

Within-nutrient-treatment comparisons of SLA vs. total plant dry mass allometric plots, revealed significant differences between species. The test for heterogeneity of

slopes indicated that the lines fit to the data for lodgepole pine and Sitka spruce seedlings grown at both FA and 4% RAR were significantly different from one another (FA: $p=0.033$, 4% RAR $p=0.0086$). While, when both H2 and H3 were combined, mean SLA's were not significantly different between species within both FA and 4% RAR treatments (FA: lodgepole pine, 21.64 ± 0.37, Sitka spruce, 21.68 ± 0.32 ($p=0.9433$); 4% RAR: lodgepole pine, 23.02 ± 0.44, Sitka spruce 24.05 ± 0.41 ($p=0.4292$)), it can be seen in both Figures 2.7a and 2.8a that the SLA of lodgepole pine decreased with increasing total plant dry mass (from H2 to H3) while the SLA of Sitka spruce remained constant (slopes of the lines are not significantly different from 0, $p<0.01$).

The production of secondary needles by lodgepole pine seedlings between H2 and H3 may be related to the ontogenetic drift in SLA (Figure 2.8a). The production of these needles is a developmental constraint that may override morphological changes that may be occurring due to nutrient treatment. Sitka spruce seedlings do not produce secondary needles, and are not influenced by ontogenetic drift in SLA, but these seedlings' SLAs were also not greatly affected by nutrient treatment. Thus, leaf morphology, as a general growth characteristic of these two species, may not be responsive to changes in the nutrient environment.

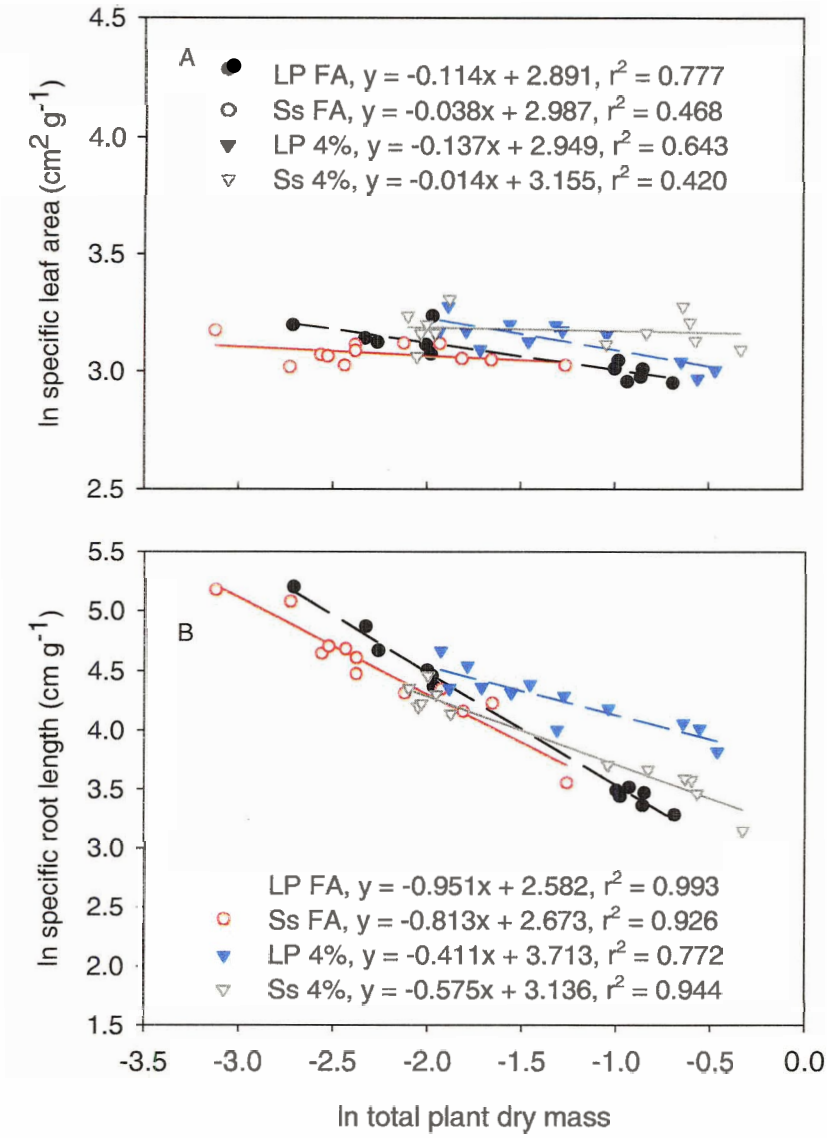


Figure 2.8. Allometric plots of **A.** specific leaf area vs. total plant dry mass and, **B.** specific root length vs. total plant dry mass for lodgepole pine (closed symbols) and Sitka spruce (open symbols) seedlings grown with either free access to nutrients (circles) or under conditions of 4% relative addition rate nutrient stress (triangles). Each point represents the mean of 4 seedlings.

Specific Root Length

In both FA and 4% RAR nutrient treatments, lodgepole pine and Sitka spruce seedlings differed significantly in their SRL when compared at a common plant size. In both FA and 4% RAR nutrient treatments there were significant differences in the slopes of the lines fit to the data for the two species (heterogeneity of slopes test, FA: $p=0.0147$, 4% RAR: $p=0.0046$) (Figure 2.8b)

It appears as though lodgepole pine seedlings' SRL response to the nutrient environment is more plastic than that of Sitka spruce. Complementary to the within-nutrient-treatment changes in SRL calculated as the difference between H2 and H3 (see above), the slope of the regression line fit to the data for lodgepole pine in the FA treatment was steeper than that for Sitka spruce (-0.951 vs. -0.813, see Figure 2.8a), while the slope of the regression line fit to the lodgepole pine data was shallower than that for Sitka spruce at 4% RAR (-0.411 vs. -0.575). The relative decrease in SRL from H2 to H3 was not only lower for lodgepole pine seedlings in the 4% RAR treatment, but the mean SRL of lodgepole pine seedlings at H3 was actually higher in the 4% RAR treatment than the FA treatment (Figure 2.7b).

Within-species comparisons of allometric plots of SRL vs. total plant biomass revealed that for both lodgepole pine and Sitka spruce there were significant differences in slopes of the lines fit to the FA data and the lines fit to the 4% RAR data ($p<0.001$). Thus, the SRL differences seen in Figure 2.7b were in response to the N environment, over and above any treatment induced differences in seedling size. The decrease in SRL from H2 to H3 for both species in both treatments is indicative of ontogenetic drift in this character. However, the fact that SRL of both species grown at 4% RAR nutrient stress decreased to a lesser degree with increasing plant size than the SRL of seedlings grown at FA, indicates that both species responded to nutrient stress by maintaining relatively higher SRL. Given that greater root length per unit root mass is generally assumed to increase root extension into the soil and benefit the plant in terms of nutrient acquisition, maintaining a greater SRL would be an advantage for nutrient stressed seedlings.

Tissue nitrogen concentrations

In the following section the N concentrations of roots, stems, and leaves, of lodgepole pine and Sitka spruce seedlings, subject to high and low N environments are presented (Figure 2.9). Tests for statistical significance were carried out on arc sine transformed data. Values given in text, figures and tables are based on untransformed data.

N comparisons between nutrient treatments

The most unequivocal, and least surprising, result of N analyses was that free access and 4% RAR nutrient stress treatments induced statistically significant different whole plant nitrogen concentrations (wpNs) for both species at both harvests ($p<0.01$) (see means Appendix III, and Figure 2.9). Seedlings grown with free access to nutrients had higher wpN's than seedlings grown under conditions of 4% RAR nutrient stress. As well, with the exception of leaf N concentration at H2 for Sitka spruce, which was not significantly different between FA and 4% RAR nutrient treatments ($p=0.283$), all component nitrogen concentrations were also significantly higher for seedlings from the FA nutrient treatment than seedlings from the 4% RAR nutrient treatment ($p<0.01$).

N comparisons between species

The only consistent species difference was that lodgepole pine had lower root N concentration than Sitka spruce in both harvests of the FA nutrient treatment, and at H2 in the 4% RAR nutrient treatment (Figure 2.9). At H2 in the 4% RAR treatment, Sitka spruce seedlings had higher leaf N concentration than lodgepole pine, while at H3, lodgepole pine seedlings had both higher leaf and higher stem N concentration than Sitka spruce. These changes in N concentration-relationships between species, highlight the importance of analysing N concentration in relation to plant growth over time.

N comparisons between harvests, within species

As required to meet the assumptions of steady state (mentioned previously in regard to Figure 2.3b), the wpNs of both lodgepole pine and Sitka spruce were not significantly different from H2 to H3 within each treatment (see means Appendix III). There were also no significant species differences in wpN, except for H3 in the 4% RAR treatment where lodgepole pine had significantly higher wpN than Sitka spruce ($p=0.003$) (Figure 2.9)

While wpN's were constant across harvests, component nitrogen concentrations showed more variable patterns over time from H2 to H3 (Figure 2.9, see means Appendix III). The most consistent of these changes was that root N concentration decreased significantly for both species from H2 to H3, in both the FA and 4% RAR nutrient treatments ($p<0.001$) (Figure 2.9). In the FA treatment, leaf N concentration was significantly higher at H3 than H2 for lodgepole pine ($p<0.001$), while stem N concentration was significantly lower at H3 than H2. There was no change in leaf or stem N concentration between harvests for Sitka spruce in the FA treatment ($p=0.275$, $p=0.065$, respectively) (Figure 2.9 a, b). In the 4% RAR nutrient treatment leaf N concentration was significantly higher at H3 than H2 for lodgepole pine ($p<0.001$), while the reverse was true for Sitka spruce with lower leaf N concentration at H3 than H2 ($p<0.001$) (Figure 2.9 c, d). Stem N concentration did not change significantly from H2 to H3 for lodgepole pine seedlings ($p=0.284$), but it decreased significantly over time for Sitka spruce ($p<0.001$).

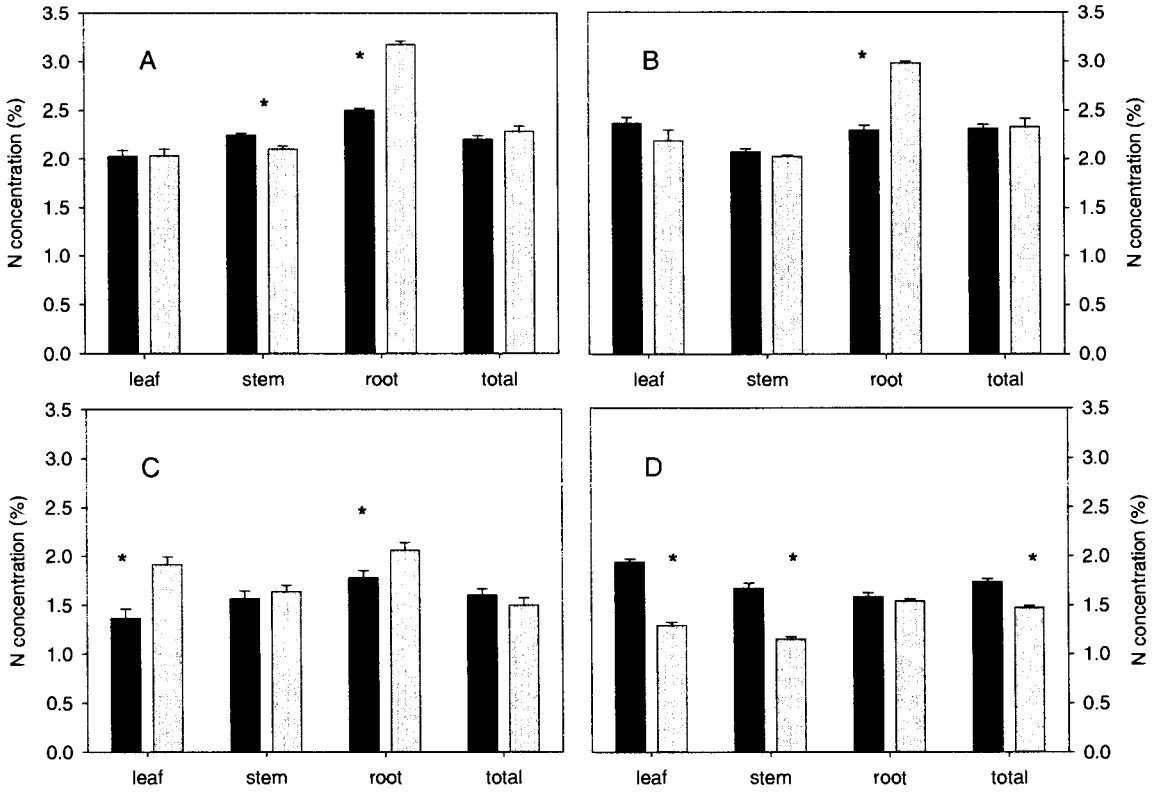


Figure 2.9. Nitrogen allocation of lodgepole pine (black bars) and Sitka spruce (grey bars) seedlings grown with free access to nutrients (A,B) or under conditions of 4% RAR nutrient stress (C,D). Graphs A & C depict percent nitrogen concentration of leaves, stems, roots for harvest 2. Graphs B & D depict percent nitrogen concentration of leaves, stems, roots for harvest 3. n=24, lines represent standard error of the mean. * indicate significant species differences ($p<0.05$).

N comparisons between plant compartments, within species

Within-species N concentrations of leaf, stem and root plant compartments were variable between harvests and nutrient treatments (Figure 2.9). When only statistically significant component N concentrations were considered (Table 2.5), it was evident that Sitka spruce seedlings demonstrated relatively consistent N concentration patterns across both harvests and both nutrient treatments. Roots were always the most N-rich component, and when differences between stem and leaf N concentrations were

significant, leaves were the second most N-rich component. Lodgepole pine seedlings on the other hand, showed distinct differences in component N concentration from H2 to H3, but, as with spruce, these changes were relatively consistent between nutrient treatments. At H2 in both FA and 4% RAR treatments, roots were the most N-rich plant component and leaves were the most N-poor. At H3 in the 4% RAR treatment the situation was reversed and leaves were the most N rich, while roots were the most N-poor (at H3 in the FA treatment, the N concentration of leaves was not statistically different than the N concentration of roots).

Table 2.5. Significance tests for differential allocation of N to leaf, stem and root plant compartments by lodgepole pine and Sitka spruce seedlings grown with either free access to nutrients or under conditions of 4% relative addition rate nutrient stress. *P*-values <0.05 (t-tests) indicate significant differences in N allocation between listed compartments within each species. Means are listed in Appendix III.

	FA, Harvest 2		FA, Harvest 3	
	P- value, N allocation comparisons		P- value, N allocation comparisons	
Compartment	Lodgepole pine	Sitka spruce	Lodgepole pine	Stika spruce
Leaf N vs. Stem N	0.110	0.196	<0.001	0.001
Root N vs. Stem N	0.055	0.011	<0.001	<0.001
Leaf N vs. Root N	0.001	<0.001	0.099	<0.001
	4% RAR, Harvest 2		4% RAR, Harvest 3	
	P- value, N allocation comparisons		P- value, N allocation comparisons	
Compartment	Lodgepole pine	Sitka spruce	Lodgepole pine	Stika spruce
Leaf N vs. Stem N	0.001	<0.001	<0.001	0.196
Root N vs. Stem N	<0.001	<0.001	0.358	<0.001
Leaf N vs. Root N	<0.001	0.372	<0.001	<0.001

Photosynthesis and respiration

Photosynthesis relationships did not change significantly when values were calculated on a leaf dry mass or a leaf area basis. In the following analyses, photosynthesis is reported on a leaf mass basis (A) when compared to other parameters involving plant biomass (i.e root respiration), and it is reported on a leaf area basis (A_{la}) when compared to parameters involving leaf area (i.e. unit leaf rate, ULR).

At H2 in the FA treatment lodgepole pine had significantly higher rates of both photosynthesis and root respiration than Sitka spruce (t-tests, $p<0.001$). At H3, however, there was no significant species difference in either of these parameters (Figure 2.10, Table 2.6). While, Sitka spruce seedlings showed no significant change in photosynthetic rates from H2 to H3, there was a significant decrease in net photosynthetic rate from H2 to H3 for lodgepole pine seedlings ($p<0.001$). This reduction in net photosynthetic rate did not translate into decreased nitrogen productivity or relative growth rate. As discussed below, the rate of increase in plant biomass per unit leaf area (ULR) of the lodgepole pine seedlings actually increased at H3.

Table 2.6. Photosynthetic rates, leaf dark respiration rates, root respiration rates, and the ratio of root respiration to net photosynthesis (R:A) of lodgepole pine and Sitka spruce seedlings grown with free access to nutrients (FA) or under conditions of 4% relative addition rate (4% RAR) nutrient stress. Values are means ± SE for each harvest, n=24.

Nutrient treatment	Species	Harvest	Photosynthetic rate (μmol CO_2 g^{-1} leaf dry mass s^{-1})	Shoot respiration rate (μmol CO_2 g^{-1} leaf dry mass s^{-1})	Root respiration rate (μmol CO_2 g^{-1} root dry mass s^{-1})	R:A ratio
FA	lodgepole pine	H2	0.099 ± 0.004	0.030 ± 0.001	0.062 ± 0.003	0.65 ± 0.03
		H3	0.070 ± 0.004	0.027 ± 0.001	0.056 ± 0.013	0.80 ± 0.12
	Sitka spruce	H2	0.078 ± 0.002	0.026 ± 0.002	0.043 ± 0.003	0.56 ± 0.06
		H3	0.072 ± 0.007	0.022 ± 0.002	0.058 ± 0.002	0.89 ± 0.10
4% RAR	lodgepole pine	H2	0.062 ± 0.003	0.016 ± 0.001	0.034 ± 0.001	0.56 ± 0.02
		H3	0.061 ± 0.005	0.019 ± 0.001	0.027 ± 0.001	0.55 ± 0.10
	Sitka spruce	H2	0.048 ± 0.002	0.016 ± 0.001	0.030 ± 0.001	0.67 ± 0.04
		H3	0.045 ± 0.002	0.011 ± 0.001	0.027 ± 0.001	0.65 ± 0.05

In contrast to the FA nutrient treatment, in the 4% RAR treatment lodgepole pine seedlings had significantly higher rates of photosynthesis than Sitka spruce seedlings at both harvests and there were no significant differences in photosynthetic rates from H2 to H3 for either species. At H2, root respiration was higher for lodgepole pine seedlings than Sitka spruce seedlings ($p=0.023$), however there was no significant difference between species at H3 ($p=0.535$). Root respiration decreased slightly for both species from H2 to H3 (see means Table 2.6, lodgepole pine $p<0.001$; Sitka spruce $p=0.026$).

The ratios between root respiration and net photosynthesis increased from H2 to H3 for both species in the FA treatment, but they remained relatively constant across harvests for seedlings in the 4% RAR treatment. These differences in carbon balance over time contribute to the lack of a significant relationship between RGR and R:A ratio when compared across species and nutrient treatments (see below).

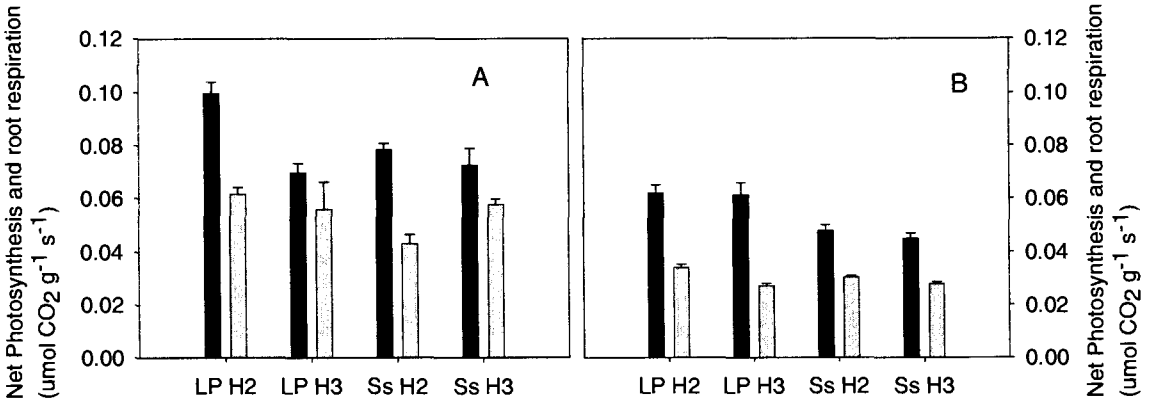


Figure 2.10. Rates of net photosynthesis (black bars) and root respiration (grey bars) for lodgepole pine (LP) and Sitka spruce (Ss) seedlings grown with **A.** free access to nutrients, and **B.** under conditions of 4% relative addition rate nutrient stress. H2 = harvest 2, H3 = harvest 3. n = 24, lines represent standard error of the mean.

Across both harvests, there were no species-specific differences in gas exchange within the FA nutrient treatment. Analysis of photosynthetic rates vs. root respiration rates (Figure 2.11) revealed that there was no significant difference between the slope of the line fit to the data for lodgepole pine and the line fit to the data for Sitka spruce within the FA nutrient treatment ($p=0.8343$).

In the 4% RAR treatment, however, the slopes of the lines fitted to the data for lodgepole pine vs. Sitka spruce were significantly different ($p=0.0452$). Thus, in this treatment, the two species differed in their gas exchange response to low N across both harvests. Lodgepole pine seedlings had a slightly greater relative increase in photosynthesis relative to root respiration than Sitka spruce seedlings.

When the slopes of the plots were compared between treatments, the relationship between photosynthesis and respiration was significantly different for both species (lodgepole pine $p<0.001$, Sitka spruce $p=0.024$). The steeper slopes of the lines fit to the data for 4% RAR seedlings indicate that the relative increase in photosynthesis verses root respiration was higher in low-N grown plants.

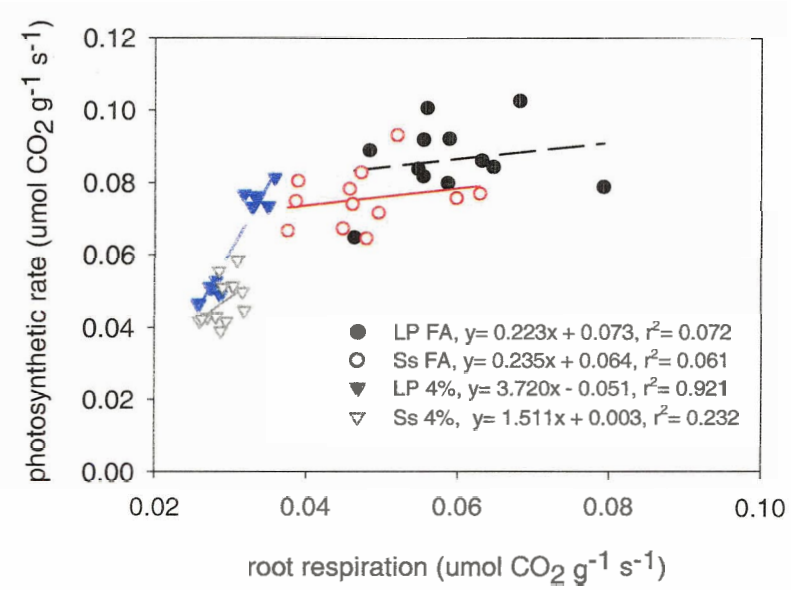


Figure 2.11. Net photosynthesis vs. root respiration for lodgepole pine (closed black and blue symbols) and Sitka spruce (open red and grey symbols) grown with free access to nutrients (circles) or under conditions of 4% relative addition rate nutrient stress (triangles). Each point represents the mean of 4 seedlings.

Gas exchange and seedling growth

The first of the following two sections presents relationships between photosynthesis (A), plant N concentrations, SLA, and ULR, while the second section presents relationships between root respiration, rN and SRL.

Photosynthesis, N, SLA and ULR

There were significant positive correlations between photosynthesis and the nitrogen concentrations of leaves, stems, and roots when compared across both species and both nutrient treatments ((correlation coefficient, p-value) wpN: 0.4929, $p<0.001$, lN: 0.3941, $p=0.008$, stmN: 0.6167, $p<0.001$, rN: 0.4906, $p<0.001$). When the A was plotted against leaf N concentration differentiating between species, nutrient treatments, and harvests within nutrient treatments (Figure 2.12), some complex patterns emerged. At H2 in the FA treatment, the leaf nitrogen concentrations of both lodgepole pine and Sitka spruce were significantly correlated with A (lodgepole pine: 0.977, $p=0.0008$; Sitka spruce: 0.866, $p=0.0259$). At H3 in the FA treatment, the rate of photosynthesis dropped for both species, likely due to self-shading, as well as leaf morphology changes in pine. The correlation between A and leaf N concentration remained positive for both species but was no longer statistically significant.

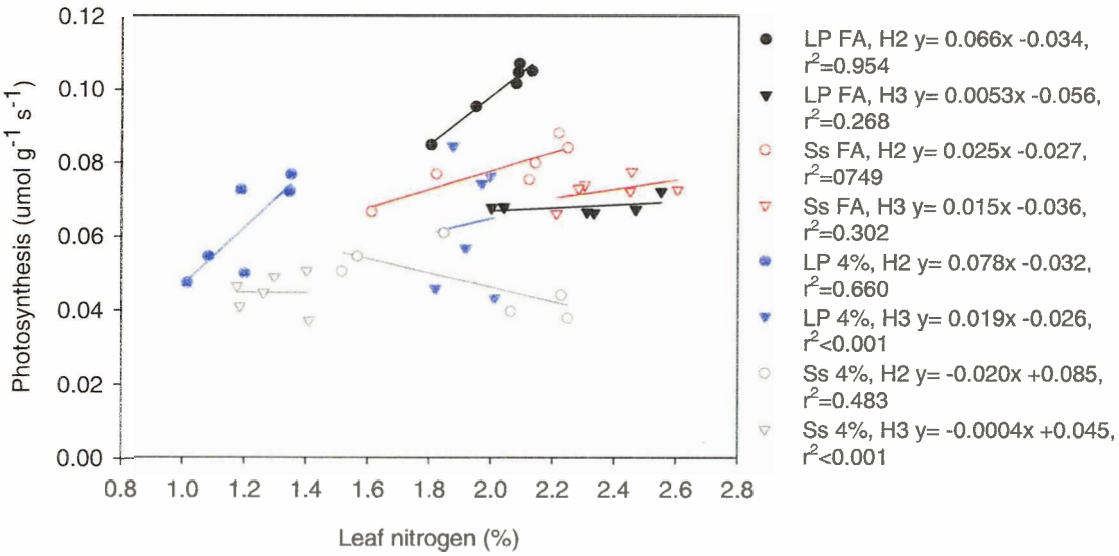


Figure 2.12. Relationships between photosynthesis and leaf nitrogen concentration of lodgepole pine (black and blue symbols) and Sitka spruce (red and grey symbols) seedlings differentiated by harvest (H2=circles, H3=triangles) and nutrient treatment (FA= black and red symbols, 4% RAR= blue and grey symbols). Each point represents the mean of 4 seedlings.

In the 4% RAR nutrient treatment, the only statistically significant correlation between A and lN was for lodgepole pine seedlings at H2 (0.8143, p=0.0493). Contrary to the general trend, lN was lower at H3 than H2 for Sitka spruce seedlings in the 4% RAR treatment, and lN was negatively (thought not significantly) correlated with A.

SLA was not significantly correlated with net photosynthesis (on a leaf dry mass or a leaf area basis) when compared across both species and treatments, nor when compared within treatments on a species- and harvest- specific basis. ULR, however, was strongly correlated with photosynthetic rates for both species ($p<0.001$) (Figure 2.13).

The relationships between mean photosynthesis on a leaf area basis (A_{la}) and ULR did not differ significantly between H2 and H3 for either species grown at 4% RAR, nor for Sitka spruce at FA. For lodgepole pine seedlings grown at FA, however, the relationship between mean ULR and mean A_{la} was significantly different between harvests ($p<0.001$, Figures 2.13a). ULR values were higher for a given rate of photosynthesis at H3 than at H2. This indicates that less photosynthesis was required for a given rate of increase in plant biomass (recall that unit leaf rate is defined as the increase in total plant biomass per unit leaf area per unit time) at H3 than at H2. Interestingly, during the period of growth from H2 to H3 the lodgepole pine seedlings began to develop secondary needles. Increased photosynthetic efficiency with the production of secondary needles may explain the high ULR maintained at lower mean photosynthesis in H3. This trend is likely not seen in the 4% RAR seedlings as they had not developed as many secondary needles by H3 (mean number of fully extended fascicle needles ± SE: H3, 4% RAR = 2.56 ± 0.30; H3 FA = 6.42 ± 0.43, t-test $p<0.001$).

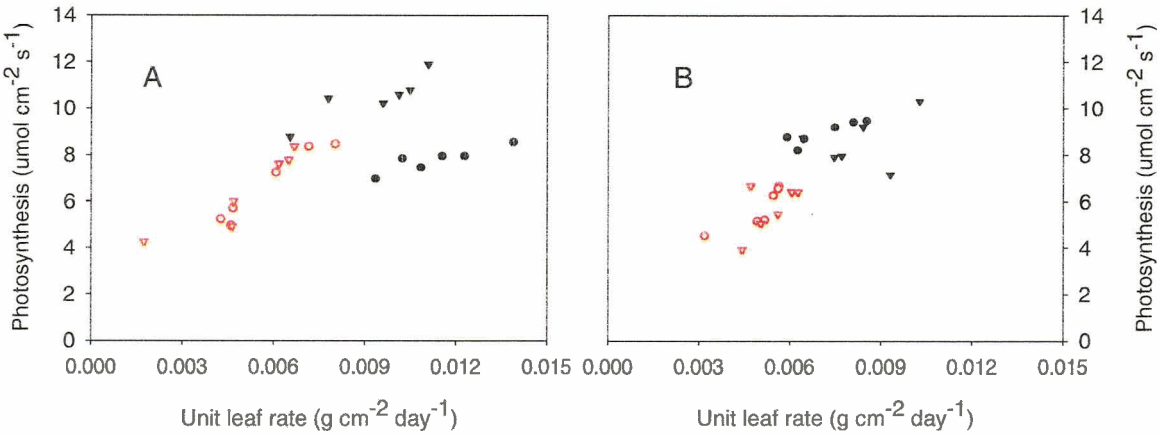


Figure 2.13. The relationship between net photosynthesis on a leaf area basis (A_{la}) and unit leaf rate (ULR) for **A.** lodgepole pine and **B.** Sitka spruce seedlings grown with either free access to nutrients (closed black symbols) or under conditions of 4% RAR nutrient stress (open red symbols). Harvest 2 data points are indicated by triangles, harvest 3 by circles. Each data point is the mean of 4 seedlings.

Root respiration, tissue N concentrations, and SRL

There were significant positive correlations between root respiration and the nitrogen concentrations of leaves, stems, and roots when data from both species and both nutrient treatments were included ($p<0.001$). However, when root respiration was plotted against root N concentration (rN) differentiating between species, nutrient treatments, and harvests within nutrient treatments (Figure 2.14), there were no significant relationships between rN and root respiration.

Both lodgepole pine and Sitka spruce had similar rN and similar root respiration rates at both harvests in the 4% RAR nutrient treatment. In the FA treatment, on the other hand, lodgepole pine had lower rN but higher root respiration than Sitka spruce at H2, while at H3 lodgepole pine had both lower rN and lower root respiration than spruce. The species differences in rN are statistically significant for both harvests (see Figure 2.9) while the root respiration differences are only significant at H2 (Figure 2.10).

When root respiration and SRL were compared across both species and both nutrient treatments, they were not significantly correlated ((correlation coefficient, p-value) 0.2196, p=0.1521). When root respiration was plotted against SRL differentiating between nutrient treatments and species, the two parameters were only significantly correlated in the 4% RAR treatment (lodgepole pine, FA: 0.084, p=0.8169; 4% RAR: 0.7935, p=0.0036; Sitka spruce, FA: -0.2638, p=0.4613; 4% RAR: 0.5961, p=0.0408).

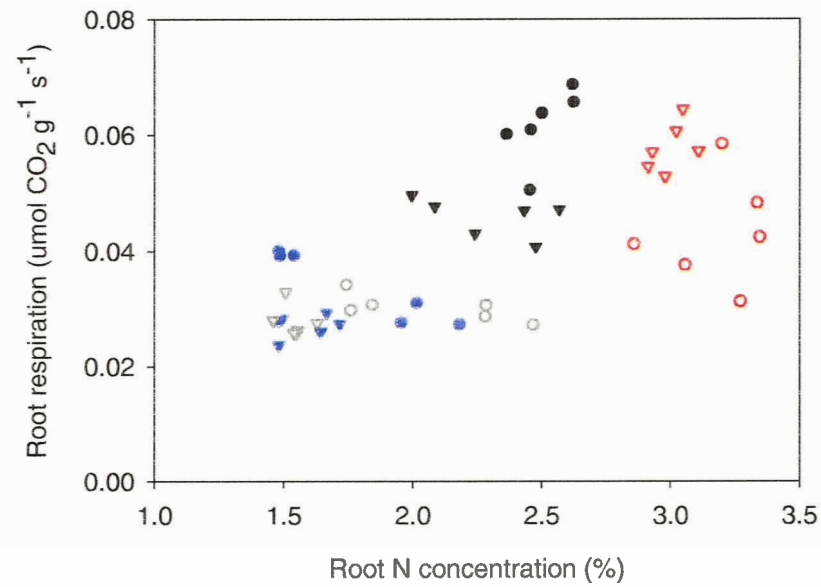


Figure 2.14. Relationships between root respiration and root nitrogen concentration of lodgepole pine (closed black and blue symbols) and Sitka spruce (open red and grey symbols) seedlings differentiated by harvest (H2=circles, H3=triangles) and nutrient treatment (FA= black and red symbols, 4% RAR= blue and grey symbols).

Putting the pieces together: overall measures of plant productivity

The above sections have provided insight into lodgepole pine and Sitka spruce seedling responses to high and low levels of N supply in terms of biomass allocation, morphology, tissue N concentrations, and gas exchange. The question remains as to how these varied responses translate into overall differences in seedling growth. The following sections

present the relationships among the above growth parameters and relative growth rate (RGR), nitrogen productivity (NP, plant biomass produced per unit wpN per day), nitrogen uptake rate (NUR, rate of increase in wpN per unit root mass), and unit leaf rate (ULR, the rate of increase in total plant biomass per unit leaf area). These variables were chosen because they integrate aspects of both above and below-ground tissues, and give an indication of overall seedling N uptake and use efficiency.

RGR relationships with N, NP, NUR and gas exchange

Correlations between RGR and all component N concentrations were statistically significant ((correlation coefficient, p-value) leaf N concentration: 0.716, p=0.046; stem N concentration: 0.847, p=0.008; root N concentration: 0.854, p=0.007), but the strongest correlation was between RGR and whole plant N concentration (wpN: 0.918, p=0.0013). RGR was also strongly correlated with NP (0.965, p<0.001), NUR (0.917, p=0.0013) and ULR (0.856, p=0.0081) (means Table 2.7).

RGR was significantly correlated with photosynthesis, expressed both on a leaf area (0.743, p=0.0273) and a leaf dry mass basis (0.796, p=0.0335) but this correlation was weaker than the correlation between either NP, NUR or wpN. When overall seedling carbon balance was represented as the balance between net photosynthesis (A) and root respiration (R), expressed as a R:A ratio, RGR and R:A were not significantly correlated. RGR was also not significantly correlated with SLA nor SRL.

Nitrogen productivity, nitrogen uptake rate, and unit leaf rate

There were no significant nitrogen productivity differences (NP) between harvests or species, within either the FA or the 4% RAR nutrient treatments. All seedlings in the FA treatment had significantly higher nitrogen productivity (i.e. produced significantly more dry mass per unit N per day) than seedlings in the 4% RAR nutrient treatments (t-test, p<0.001).

There were no significant nitrogen uptake rate differences (NUR) between H2 and H3 for either species. However, lodgepole pine had significantly lower NUR than Sitka spruce in the FA nutrient treatment (p=0.021). This is likely due to lower SRL and rN (N being an important component of proteins involved in nutrient uptake) of lodgepole pine

seedlings in this treatment. There were no differences in NUR between species in the 4% RAR treatment. All seedlings grown at 4% RAR nutrient stress had significantly lower net uptake rates of nitrogen per unit root mass (NUR) than FA seedlings ($p<0.001$).

Table 2.7. Mean nitrogen productivity (NP), nitrogen uptake rate (NUR), unit leaf rate (ULR), and whole-plant nitrogen concentration (wpN) for each relative growth rate at harvests 2 and 3, for lodgepole pine and Sitka spruce seedlings grown with free access to nutrients (FA) or under conditions of 4% relative addition rate nutrient stress (4% RAR). n=24.

Nutrient treatment	Species	Harvest	RGR ($g\ g^{-1}\ day^{-1}$)	NP (g total dry mass g^{-1} wpN day^{-1})	NUR (g wpN g^{-1} root mass day^{-1})	ULR (g total dry mass cm^{-2} leaf day^{-1})	wpN ($gN\ g^{-1}$ total dry mass)
FA	lodgepole pine	H2	0.0788	0.0377	0.003305	0.00877	0.0212
		H3	0.0806	0.0366	0.003326	0.0114	0.0232
	Sitka spruce	H2	0.0811	0.0374	0.005080	0.00827	0.0229
		H3	0.0776	0.0365	0.004887	0.00712	0.0233
4% RAR	lodgepole pine	H2	0.0450	0.0298	0.001010	0.00508	0.0160
		H3	0.0414	0.0246	0.000993	0.00581	0.0173
	Sitka spruce	H2	0.0485	0.0264	0.000808	0.00537	0.0149
		H3	0.0486	0.0266	0.000679	0.00501	0.0137

Similar to NP and NUR, all seedlings grown at 4% RAR nutrient stress had significantly lower rates of increase in total plant biomass per unit leaf area (ULR) than FA seedlings ($p<0.001$). There were no differences in ULR between species, nor between harvests within-species in the 4% RAR treatment. ULR showed unique species differences within the FA treatment (Table 2.7). The ULR of lodgepole pine seedlings increased significantly from H2 to H3 in the FA treatment ($p<0.001$), while the ULR of Sitka spruce seedlings decreased slightly ($p=0.031$). The two species only had significantly different ULR's at H3 ($p<0.001$).

DISCUSSION

Experimental approach

Biotronic units

To allow for comparisons between species, as well as nutrient treatments, seedlings were grown in Biotronic units using relative-nutrient-addition-rate growth techniques. Nutrient solutions were continuously sprayed on roots and circulated through automatic monitoring and replenishment devices that maintained conductivity or relative addition rate while calculating nutrient uptake. This type of spray aeroponic system creates thin boundary layers, allowing for good physical contact between solution and the roots (Ingestad and Lund 1986). Close control of nutrient uptake is obtained because uptake occurs rapidly following hourly nutrient titrations, with little or no storage of nutrients in the solution for subsequent uptake. In their studies using Biotronic units and steady-state nutrition techniques, Ericsson (1981) and Ingestad (1981) found that, even at high growth rates, uptake of added nutrients by willow, birch and alder seedlings was complete within one hour, leaving a solution low in mineral ions (conductivity of $< 20\ \mu S\ cm^{-1}$) before the next addition.

In addition to controlling nutrient addition rate, and thus relative growth rate and internal nutrient concentrations, Biotronic units were contained within a Conviron growth chamber where humidity, day-length, temperature and light levels were kept constant. Although this type of experimental set-up allows researchers to create highly controlled nutrient environments, such systems are not easily assembled without specialized knowledge. The expense and technical complexity of computer controlled aeroponic systems is prohibitive to their widespread used, and they do not readily accommodate large numbers of replicate plants. Relative addition rate techniques have not been widely adopted because of these requirements (Coleman *et al.* 1998). Furthermore, it can be argued that root systems grown in aeroponics are not representative of soil grown roots because of differences in soil resistance encountered and the availability of nutrients at the root surface (see Taylor 1974; Nye and Tinker 1977). In soil-grown plants, factors such as root penetration resistance and soil oxygen supply have large formative effects on

root growth, which may be more important for root morphology than local nutrient supply (Caldwell 1994). That said, the differences in supply of nutrients for uptake between soil and solution culture make it much easier to control the actual nutrient levels encountered by the roots and thus to maintain steady state nutrition (though see Coleman *et al.* 1998 for the application of steady state techniques to soil-grown trees). In soil, nutrients come in contact with roots via root growth, diffusion and mass flow. Root growth and extension into unexploited soil are most important for the supply of highly immobile nutrients such as phosphorous, while diffusion and mass flow are more important for relatively mobile nutrients such as nitrate and ammonium (Clarkson 1985). For more mobile N compounds, depletion zones can develop for quite a distance from roots because N uptake is greater than N supply at the root surface. Overlap of depletion zones and root competition can occur at high rooting density (Nye and Tinker 1977) and exponential growth into unexploited soil volumes may not meet exponential nutrient uptake required to achieve steady-state nutrition. The Biotronic units used in this study eliminated these complications.

Ectomycorrhizal associations are ubiquitously found in the roots of field-grown coniferous trees (Wilcox 1991; Marschner 1995). For this reason, the validity of tree growth studies where these associations are lacking has frequently been questioned. In the context of the controlled nutrient environments provided by the Biotronic units, mycorrhizal fungi would make the assumption that added nutrients were taken up solely by the experimental seedlings untenable. Further, the spray aeroponic system essentially assumes the role that mycorrhizal associations have in the field (i.e. increasing the effective root contact with nutrients in the soil), without taking photosynthates from the plant and complicating experimental calculations of carbon balance.

To analyse the effects of N availability on plant growth, comparisons have generally been made between two levels of N solution concentrations representing high and low N environments. However, as noted by Gebauer *et al.* (1996), it is likely that growth and allocation patterns change in a non-linear manner with resource availability. It would be better to measure plant responses along a continuum of N solution concentrations and to establish nutrient response curves. While the nutrient solution N concentrations listed in Table 2.2 are 712.37 μM N for the FA treatment, and 71.24 μM

N for the 4% RAR treatment (representing high N and low N, respectively), it is important to remember that plants were exposed to exponentially increasing amounts of nutrient solution, concurrent with growth; thus nutrient uptake rate, rather than solution concentration, controlled plant growth. There is no doubt that future studies would benefit from comparing plant growth response to nutrient stress over a wider range of relative nutrient addition rates.

Steady-state nutrition

To obtain reproducible experimental results, laboratory or greenhouse experiments generally control external environmental conditions such as light, humidity, temperature and the chemical composition of nutrient media. However, these controls are of little value if they are changed by plant activity (Ingestad and Ågren 1995). Factors such as variable plant growth rates and fluctuating internal nutrient concentrations may confound plant responses to environmental stress despite well-controlled external environments. For instance, many studies which have assessed plant responses to elevated atmospheric CO_2 and ozone have shown that plant nutritional status is an important interacting factor; yet, these experiments have used traditional nutrient application regimes where plant growth rate and/or internal nutrient concentration are not constant over the experimental period (Brown 1991; Tjoelker and Luxmoore 1991; Sinclair 1992; Bazzaz and Miao 1993; Coleman *et al.* 1993).

If larger, faster-growing plants are grown in a set volume of soil or nutrient solution, they will deplete the nutrients to a greater extent than smaller, slower growing individuals grown in the same volume. In other words, when nutrients are added at fixed rates, fast-growing species or individuals may experience more nutrient limitation than slow growing ones (Boot 1990) confounding comparisons of biomass allocation. If no allowance is made for the size of the plant, a size-by-nutrient interaction may occur which will interfere with the outcome of subsequent growth, biomass allocation, plasticity analysis etc. (Boot 1990; Boot and Mensink 1990; Van de Vijver *et al.* 1993).

Two studies involving allometric analyses of biomass allocation in *Abutilon theophrasti* provide an excellent example of this phenomenon. Gedroc *et al.* (1996) and McConnaughay and Coleman (1999) report conflicting results regarding the plasticity of

root vs. shoot allocation in response to nutrient availability. Gedroc *et al.* (1996) found that R:S ratios were higher in low-nutrient-grown plants irrespective of whether comparisons were made at a common time or at a common plant size. McConnaughay and Coleman (1999), on the other hand, found that R:S allocation in *A. theophrasti* did not respond to nutrient availability. Allocational shifts were solely due to increasing plant size. Both studies used similar nutrient levels and applied weekly additions of liquid soluble fertilizer. McConnaughay and Coleman (1999), however, applied nutrients in gradually increasing quantities commensurate with plant growth, while Gedroc *et al.* (1996) provided nutrients in equal doses. The biomass allocation response seen in the latter study, where nutrients were added in fixed doses, was likely due to the imposition of a greater degree of nutrient stress.

The relative nutrient addition rate techniques used in this study allowed steady-state plant growth and nutrition to be maintained. According to Ingestad (1982) and Ingestad and Lund (1986), plant mass must increase by at least 7 times during the experimental period to fulfil the theoretical requirement for assessment of steady-state, and experimental accuracy is acceptable if there is no systematic change in RGR or internal N concentration over time. Relative growth rates and whole plant N concentrations were constant over the period from harvest two to harvest three (Figure 2.3), and plant mass increased by >7 fold throughout the course of both the FA and 4% RAR nutrient treatments (Figure 2.2).

While other experiments have shown that plants growing at steady-state nutrition in solution culture have constant allocation patterns over time (Ingestad and Ågren, 1995), in this study, changes in biomass allocation to leaves stems and roots were observed for both species, over the course of both FA and 4% RAR experiments. These allocation changes occurred even though plants were growing at constant relative growth rates. Even though whole-plant nitrogen concentrations remained constant for both species over the course of both nutrient treatments, N concentrations of leaf, stem and root plant compartments were also not constant over time (Figure 2.9). For instance, root N concentration of both species decreased from H2 to H3 in both the FA and 4% RAR nutrient treatments.

In accordance with steady-state nutritional theory, all researchers using relative addition rate techniques comment that internal N concentrations should remain constant, but, to my knowledge, these have only been measured as wpN. The constancy of wpN in this study is indicative of steady-state, but the observed temporal shifts in N and biomass allocation may also be associated with drift of internal nutrient concentration resulting from imperfect steady-state conditions. Changing allocation in response to N and other environmental variables has been attributed to differences in plant size among treatments (Coleman and McConnaughay 1995; Gebauer *et al.* 1996; Gedroc *et al.* 1996; King *et al.* 1999). However, allometric analyses do not support such a conclusion. Differences in biomass and N allocation between the FA and 4% RAR treatments (i.e. between high and low relative growth rates) were independent of seedling size. Although allocation changes may be associated with imperfect steady-state conditions, changing allometric relationships have been reported in other relative addition rate experiments, conducted with solution culture, where whole-plant relative growth rate and internal nitrogen concentrations were constant (Freijsen and Otten 1984; MacDuff *et al.* 1993). Clearly, a closer look at both component biomass and N allocation changes in relative growth rate experiments is called for.

Harvest schedule

In an attempt to address the issue of ontogenic drift, plants were harvested when they doubled in size, making the average time between FA harvests 14 days, and the average time between 4% RAR harvests 32 days (Table 2.3). By taking weekly fresh mass measurements of a separate group of 12 seedlings within each Biotronic unit (to estimate when the seedlings had doubled in size), we eliminated the confounding effects that repeated handling could have on the growth of the experimental seedlings. This approach was not perfect, however, because the germinants of each species varied in size when they were put into the Biotronic treatment units. Small seedlings that double in size are not necessarily at the same developmental stage as large seedlings that double in size, thus, interpretation difficulties regarding species and treatment effects on biomass allocation still exist. To address the potential effects of seedling size on the allocational,

morphological and physiological variables, allometric regression analyses were conducted.

Species-specific or size dependent differences in biomass allocation?

When compared at H2 or H3, within either the FA or the 4% RAR nutrient treatments, lodgepole pine and Sitka spruce seedlings differed significantly in their biomass allocation to leaves, stems and roots. Lodgepole pine consistently allocated a greater proportion of biomass to roots than Sitka spruce, while Sitka spruce allocated more biomass to stems and leaves. However, allometric analyses of leaf biomass, stem biomass and root biomass plotted against the biomass of the whole plant, indicated that the only significant species difference was that Sitka spruce allocated more biomass to leaves than lodgepole pine in the FA treatment.

Lodgepole pine has a low potential for regeneration under conditions of low light (Klinka *et al.* 1998) while Sitka spruce seedlings commonly grow under canopies of deciduous species such as alder (Harris 1990). It may be that the FA treatment is more reflective of the native nutrient environment of Sitka spruce than lodgepole pine. Sitka spruce seedlings may be adapted to allocate biomass to plant tissues associated with light interception if light is more limiting than soil nutrients in most natural regeneration settings. Lodgepole pine seedlings successfully establish in the open, after fire. This species may have been subject to weaker selection pressure for light interception than Sitka spruce.

To date, only a handful of studies have explicitly distinguished between allocational changes that result as a normal consequence of plant growth and development (i.e. ontogenetic drift) and those that occur in response to environmental conditions. While biomass allocation of tree species such as loblolly pine (*Pinus taeda* L.) and trembling aspen (*Populus tremuloides* Michx.) in response to CO_2, N, and irrigation and has been studied using allometric analyses (Ledig *et al.* 1970; Li *et al.* 1991; Gebauer *et al.* 1996; Coleman *et al.* 1998; King *et al.* 1999), to my knowledge, there have been no between-species comparisons of biomass allocation, morphology or physiology of conifers using allometric techniques. A study involving plant species found in fertile and infertile habitats, respectively, has been conducted with the grasses

Holcus lanatus and *Deschampsia flexuosa* (Van de Vijver *et al.* 1993). Van de Vijver *et al.* (1993) found that, averaging across all nitrate concentrations, RMF was lower for *D. lanatus*, a species associated with infertile sites, than *H. lanatus,* a species associated with fertile sites. These findings are contrary to prediction of optimal partitioning theory that plants from infertile habitats would allocate more biomass to roots than those from fertile habitats. However, while the authors only comment on the inherent species differences in biomass allocation at high N availability, one can see from their plots of LMF and RMF against nitrate concentration (Van de Vijver *et al.* 1993, Figure 1), that at low N there were no significant species differences in either of these components. This is similar to the situation I saw here. In the 4% RAR nutrient treatment there was no difference in LMF, SMF, or RMF, between lodgepole pine and Sitka spruce seedlings when compared at a common plant size. It may be the case that species differences in plant biomass allocation patterns converge in low N environments because morphological and allocation plasticity are limited by nutrient supply (i.e. low N levels give plants fewer options for optimizing growth). It has been argued that in infertile habitats the costs of shifting biomass allocation exceed benefits gained in nutrient acquisition, particularly for species with large amounts of expensive structural tissue (Bloom *et al.* 1985, Crick and Grime 1987).

Specific root length more revealing than biomass alone

Considering the contrasting nutrient environments in which lodgepole pine and Sitka spruce are found, it is surprising that species differences in biomass allocation to roots were not evident in seedlings of common size. If, however, RMF is not indicative of absorptive area, a lack RMF plasticity differences between species may be understandable. A change in this parameter may not strongly reflect resource acquisition or important species responses to the N environment (Reynolds and D'Antonio 1996). This argument may appear contradictory to the finding that both species had increased root mass fractions in the 4% RAR treatment, whether compared at a common harvest or at a common size. However, the relative allocation to shoots vs. roots was actually greater in low-nutrient grown plants (Figure 2.6). It has been suggested that in woody

species, biomass fractions are not necessarily good indicators for resource acquisition because below- and above-ground parts also contain a large proportion of tissues with storage, support and transport function (Cromer and Jarvis 1989; Körner 1994). The seedlings in this study did not have lignified support roots, but SRL may still be a more meaningful indicator of allocation to enhanced nutrient uptake than RMF. Root structure as described by SRL is reported to be an important determinant for the capacity of resource acquisition, which can vary independently of biomass allocation (Körner and Renhardt 1987; Berntson *et al.* 1995)

In both the FA and the 4% RAR nutrient treatments, lodgepole pine and Sitka spruce seedlings had significantly different patterns of change in SRL over time. SRL was subject to ontogenetic drift in that both species had declining SRL as seedlings grew, regardless of nutrient treatment. However, both species also appeared to adjust SRL in response to the nutrient treatments. In the 4% RAR treatment, both lodgepole pine and Sitka spruce seedlings had smaller reductions in SRL from H2 to H3 than they did in the FA treatment, indicating that they responded to the low level of N supply by maintaining relatively greater root extension (within the bounds of the developmentally constrained decrease in SRL). Lodgepole pine seedlings, however, showed a smaller decrease in SRL between harvests than Sitka spruce in the 4% RAR treatment, as indicated by the smaller absolute change in SRL (Figure 2.7) and by the shallower slope of the regression line fit to the allometric data (Figure 2.8). In the FA treatment, lodgepole pine seedlings showed a larger reduction in SRL from H2 to H3 than Sitka spruce as indicated both by the greater absolute change in SRL (Figure 2.7) and by the steeper slope of the regression line fit to the allometric data (Figure 2.8). It can thus be concluded that lodgepole pine seedlings were more plastic in SRL than Sitka spruce seedlings.

Although most studies have compared species plasticity in terms of RMFs, those that have examined such features as SRL, and the length and density of root hairs (generally measured as 'effective root radius') suggest a pattern of slow-growing species from low fertility habitats having greater plasticity than faster-growing species of higher fertility habitats. Robinson and Rorison (1988) found that *Deschampsia flexuosa*, the slowest growing species examined in their study, was one of the most plastic in effective root radius while it was the least plastic in RMF. Boot and Mensink (1990) also found

that the slowest growing species examined in their study, *D. flexuosa* and *Festuca ovina,* were plastic with respect to root hair length and also root hair density. Both *D. flexuosa* and *F. ovina* are typically found on nutrient-poor, free draining soils of lowland acidic grasslands in Britain (Aerts *et al.* 1990). These patterns are similar to those seen here for lodgpole pine and Sitka spruce, which typically inhabit infertile and fertile sites respectively, though they are not as distinctive as slow or fast growing species.

Support for the importance of adjustments SRL in response to N supply in conifers comes from Gebauer *et al.*'s (1996) study on the growth and allometry of loblolly pine in response to changing CO_2 and N availability. These authors found that the most important direct compensatory adjustment to low N was a significant increase in total lateral root length per plant. This increase involved both greater biomass allocation to lateral roots, as well as an increase in SRL. With the exception of adjustments in allocation and root morphology at the lowest N concentration (0.5 mM NH_4NO_3), these authors found that loblolly pine exhibited relatively little phenotypic plasticity in biomass allocation.

Increased SRL in nutrient poor environments is likely to improve the acquisition of nutrients through more extensive exploitation of the soil (Chapin 1980; Lambers and Poorter 1992). Whether plants respond to N supply by altering biomass allocation patterns, by adjusting root morphology, or some combination of the two is likely to be a characteristic of individual species (Berntson *et al.* 1995). This study indicates that lodgepole pine, a species tolerant of infertile soils, is more likely to alter SRL in response to N supply than Sitka spruce.

Biomass allocation responses to the N environment: Optimal partitioning or ontogenetic drift?

To maintain an internal carbon-to-nutrient balance, plants are predicted to allocate biomass such that the acquisition of the resource that most strongly limits plant growth is maximized (Bloom *et al.* 1985). Do the results of this experiment support optimal partitioning models of plant growth? Supportive evidence includes the fact that both lodgepole pine and Sitka spruce seedlings grown with free access to nutrients allocated a relatively greater proportion of biomass to leaves and shoots, while seedlings grown

under conditions of 4% RAR nutrient stress allocated a relatively greater proportion of biomass to roots, regardless of whether comparisons were made at a common harvest, or at a common plant size (Figure 2.4, Figure 2.5). Thus, seedlings of both species clearly adjusted their patterns of biomass partitioning in response to nutrient availability. Optimal partitioning models predict that these adjustments in partitioning might lead to optimization of growth rate by making all resources equally limiting (Bloom *et al.* 1985). However, in order to test optimal partitioning, we would have to show that plants which allocated more biomass to roots at low N, for instance, would grow faster and larger than plants that did not. To do this, one would need to compare a range of genotypes that only differed in the extent to which they changed their allocation pattern under sub-optimal conditions. However, there are no such mutants or varieties whose biomass partitioning is constant throughout ontogeny. While we can say that the observed allocation responses are consistent with optimal partitioning theory, we cannot say whether the adjustments were actually optimal in terms of maximizing plant growth.

Evidence inconsistent with optimal partitioning theory is that the increases in both the rate of leaf growth relative to root growth, and the rate of stem growth relative to root growth were higher in low-N grown plants (Figure 2.6). King *et al.* (1999) found similar results in their study of irrigation and fertilization affects on stand-level allometry in loblolly pine. The lack of fit of their linear models to their fine root (ephemeral) data prevented them from interpreting the shifts in biomass between fine roots and foliage. However, contrary to King *et al.*'s (1999) hypothesis that increased nutrients would decrease biomass partitioning to roots relative to shoots for perennial (tap and coarse roots vs. stem and branches) they found an increase in perennial root biomass relative to perennial shoot biomass in response to fertilization. Similarly, allometric analyses conducted by Gedroc *et al.* (1996), indicated that after approximately 30 days, the relative growth of shoots exceeded that of roots under low nutrient conditions, whereas the relative allocation to roots vs. shoots was actually greater in high-nutrient grown plants. In addition, McConnaughay and Coleman (1999) found that when R:S ratios were compared as a function of plant size, the increased allocation to roots under nutrient stress observed for all of their study species was limited to the initial stage of plant

growth. As growth proceeded they found that nutrient stressed *Abutilion* and *Polygonium* plants allocated less biomass to roots than those grown at higher nutrient levels.

The findings of these studies suggest that (as observed by Bazzaz *et al.* (1989)) the ratio of root to shoot biomass may initially be very high because seedlings are adapted to establish a strong root base in the soil. After they have done so, root growth relative to shoot growth may decrease. More study is required to understand why plants grown at higher nutrient levels allocate more biomass to roots relative to shoots. The FA and 4% RAR seedlings had distinctly different growth rates of 0.0793 vs. 0.0454 g g^{-1} day^{-1}, respectively (grand means for both species over both H2 and H3). Therefore, even though plants are being compared at a common size, they may be at different developmental stages owing to different levels of nutrients controlling their relative growth rates.

Species differences in N concentration

With the exception of wpN, there was a high degree of variation in N concentration between species, between harvests and among plant compartments. However, the absolute differences in N concentrations between species and plant compartments were fairly small (< 2%). It is possible that N concentrations fluctuate within this percent range over time. The short duration of this experiment makes it difficult to assign functional significance to these changes.

Nonetheless, there were statistically significant species differences in N concentration to roots stems and leaves when compared both at a common plant size and common harvests. The most striking of these trends was that Sitka spruce seedlings had higher root N concentrations in the FA nutrient treatment than lodgepole pine. This difference was consistent over time (Figure 2.9). Presumably, this difference in rN (N-containing proteins play a major biochemical role in nutrient uptake and root respiration (Reich *et al.* 1998b)) in combination with the lower SRL of lodgepole pine seedlings in the FA treatment (Figure 2.7) contributed to the significantly lower nutrient uptake rates of lodgepole pine seedlings compared to those of Sitka spruce (Table 2.7). The lower rate of nutrient uptake did not translate into lower nitrogen productivity nor lower RGR

for lodgepole pine seedlings, suggesting that they are more efficient at converting N into biomass.

In the 4% RAR treatment, Sitka spruce had higher rN than lodgepole pine at H2 but there was no significant difference in rN between the two species at H3. Unlike the FA treatment, at 4% RAR lodgepole pine seedlings had greater NUR than Sitka spruce seedlings despite lower or equal rN (Table 2.8). Lodgpole pine seedlings did, however, have significantly greater SRL than Sitka spruce seedlings in the 4% RAR treatment. It may be that SRL is a more important determinant of NUR than rN. Despite species differences in rN, SRL and NUR there were no significant differences in NP or RGR between lodgepole pine and Sitka spruce seedlings within nutrient treatments. It is unclear if the observed species-specific variation in component N concentrations within nutrient treatments has any functional significance. Given that wpNs were stable within each nutrient treatment corresponding to stable RGRs and NPs, whole plant N concentration can be considered a better determinant of overall plant growth. Natural fluctuations in component N concentrations may be part of the reason why steady-state nutrition techniques have focused on wpN.

Tissue N concentrations and overall plant growth in response to N treatment

Data from both common-harvest comparisons and allometric analyses indicate that there were significant nutrient treatment-induced changes in N concentration for both lodgepole pine and Sitka spruce seedlings in this study. RGR was strongly positively correlated with wpN, and the N concentrations of all plant components decreased proportionally from the FA to the 4% RAR nutrient treatment. This is in agreement with data reported for other coniferous tree species. In their study of various *Pinus sylvestris, Picea abies* and *Pinus contorta* provenances Ingested and Kähr (1985) found that there were close linear relationships between wpN and RGR. Brown *et al.* (1996a) also reported that seedlings of *Picea sitchensis, Thuja plicata* and *Tsuga heterophyla* exhibited relative growth rates that increased linearly with wpN.

Although optimal partitioning theory suggests that plants may allocate more N to roots (to support nutrient uptake) under conditions of nutrient stress, it seems logical for

plants to have similar above ground and below ground N allocation in response to the nutrient environment. Plants in nutrient rich environments with high above ground biomass, photosynthesis and N concentrations, may require root systems to have high levels of N-containing proteins that potentially enhance nutrient acquisition required to support a high RGR. Nutrient stressed plants may be allocating N in such a way as to maximize growth rates, but in the absence of genotypes that only differ in their N allocation patterns in response to the nutrient environment we do not know if increased N allocation to roots under nutrient stress would indeed be optimal.

Growth rates in this study increased with increasing wpNs and wpN was positively correlated with NP, NUR, and ULR. Brown *et al.* (1996a) found that at higher wpNs (>0.02 g g^{-1}), the NP of *Picea sitchensis, Thuja plicata* and *Tsuga heterophyla* seedlings tapered off due to a lack of subsequent increase in leaf N concentration. In this study, leaf N and all other component N concentrations increased fairly proportionally with increasing wpN, and NP showed no decline with increasing wpN. However, this study covered a smaller range of seedling wpNs and the low N treatment was 4% RAR while Brown *et al.*'s (1996a) lowest RAR was 2.5%. In their study, the increase in NP with increasing wpN was primarily observed at wpNs resulting from the 2.5% RAR. The wpNs induced by the FA treatment may be in the range above which NP does not increase significantly. An appropriate wpN target for seedling production (where NP is maximized) of lodgepole pine and Sitka spruce may thus be wpN's of ~ 0.02 g g^{-1} as seen in the FA treatment of this study.

While experimental evidence suggests that increased plant nitrogen concentrations will lead to improved growth rate, it is important to consider that seedling growth and survival in the field is not necessarily improved by increasing wpN. An increase in wpN may decrease foliar concentrations of carbon-based secondary metabolites, e.g. terpenes (Muzika *et al.* 1989), and increase plant susceptibility to moisture stress (Etter 1969), frost damage (L'Hirondelle *et al.* 1992), and herbivory (Larsson *et al.* 1986), as well as decreasing root:shoot ratios (Ingestad and Ågren 1991). Lower root:shoot ratios with high wpN may provide inadequate root structure for resistance to high winds and moisture stress. Appropriate fertilization regimes need to

take into account both the biotic and abiotic stresses to which seedlings will be exposed upon outplanting.

Photosynthesis, N and SLA

Many studies have reported positive correlations between leaf nitrogen concentrations and net photosynthetic rates (e.g. Gulmon and Chu 1981; DeJong 1983; Field and Mooney 1986; Hirose and Kitajima 1986; Hirose and Werger 1987; Evans 1982; Reich *et al.* 1998b; Rosati *et al.* 1999). The relationship between photosynthetic capacity and foliar N concentration is less well documented for evergreen conifers than other higher plants. Strand (1997) found that a major part of the variation in the gross photosynthesis of current-year and one-year-old needles of *Picea abies* across control, irrigated, and irrigated and fertilized treatments was associated with differences in needle N concentration. Vapaavuori *et al.* (1995) found a significant correlation between the light saturated rate of CO_2 assimilation and foliar N-concentration in one-year-old shoots of *Pinus sylvestris* collected from four sites with different fertility. Similarly, Smolander and Oker-Blom (1989) found that foliar N-concentration and photosynthesis were correlated in one-year-old needles of *Pinus sylvestris.* Linder and Troeng (1980) found that one-year-old shoots of irrigated and fertilized trees of *Pinus sylvestris* had an *in situ* rate of CO_2 assimilation approximately 20 % greater than corresponding shoots of irrigated trees over a wide range of temperatures and incident irradiances. In agreement with these studies, there were significant positive correlations between leaf N concentration and photosynthesis for both lodgepole pine and Sitka spruce seedlings across nutrient treatments. Although the correlation between photosynthesis and leaf N concentration does not prove causation, there is evidence that leaf N concentration usually reflects the level of nitrogenous compounds (e.g. RubisCO) in leaves responsible for maximum assimilation rates. Fertilization treatments that increase leaf N concentration are likely to have positive effects on rates of photosynthesis. However, research has shown that fertilization of conifers in the field can have little or no effect on the rate of net photosynthesis (e.g. Sheriff *et al.* 1986; Teskey *et al.* 1994a) possibly due to imbalances between nutrient elements within the foliage that are induced by the fertilization (Teskey

et al. 1994b). Ideal fertilization regimes involve applications of complete nutrient solutions.

Researchers have found that variation in the photosynthesis-leaf N relationship among different species is often correlated with differences in SLA (Reich and Walters 1994, Reich *et al.* 1997, Poorter and Evans 1998). While there were no significant nutrient treatment differences in SLA for either lodgepole pine or Sitka spruce (see the last paragraph of the 'specific leaf area' results section), allometric analysis revealed that there were significant species differences in SLA. The SLA of lodgepole pine decreased with increasing total plant dry mass (from H2 to H3), while the SLA of Sitka spruce remained constant in both 4% RAR and FA nutrient treatments. The decrease in SLA of the lodgepole pine seedlings corresponds to the production of secondary needles that occurred in the time period from H2 to H3. The decrease in photosynthesis from H2 to H3 for lodgepole pine seedlings in the FA treatment (Figure 2.10) may have been due to secondary needle production and decreased SLA. Nonetheless, there were no significant correlations between A and SLA and differences in SLA alone do not explain the observed variation in photosynthesis-leaf N concentration relationships. While lodgepole pine and Sitka spruce seedlings showed variable patterns of A, lN and SLA these did not translate into distinct species differences in RGR (Table 2.8).

Reviewing 25 years of research, Nelson (1988) concluded that "progress in improving crop yield by improving photosynthesis has not been rapid or encouraging." Garnier (1991) comments that "[t]he path between leaf photosynthesis and yield is long but, even the first step - the link between the rate of leaf photosynthesis and plant growth rate - is confusing." I concur. In this study, both species had higher wpNs, photosynthetic rates, and growth rates in the FA as opposed to the 4% RAR nutrient treatments, but I was unable to tease apart the complex relationships between A, lN and SLA in such a way as to identify how species-specific physiological and morphological differences led to similar overall seedling growth.

Photosynthesis and ULR

In this study, ULR was a variable with a clearer species-specific relationship to photosynthesis than either leaf N concentration or SLA. ULR is the rate of total increase

in plant biomass per unit leaf area. This parameter has been used to represent the difference between whole-plant photosynthesis and respiration expressed per unit leaf area, integrated over time (Hunt 1990; Brown *et al.* 1996a. Note: these authors refer to NAR but it is equivalent to ULR as calculated in this study). Relationships between ULR and photosynthesis did not differ significantly with increasing plant size for Sitka spruce seedlings in either the FA or the 4% RAR treatment (Figure 2.13). Lodgepole pine seedlings however, showed distinct differences in ULR from H2 to H3, which were related to treatment-induced differences in seedling development. In the FA treatment lodgepole pine seedlings had a considerable number of fully extended secondary needles at H3 (mean of ~6). Mean ULR was significantly greater at H3 than H2 in this treatment, and ULR values for a given rate of photosynthesis were higher at H3. It appears as though secondary needles are more photosynthetically efficient, allowing higher ULR to be maintained at lower rate of photosynthesis. Lodgepole pine seedlings in the 4% RAR treatment did not have as many fully extended secondary needles at H3 (mean of ~3). Thus, while secondary needles may indeed be more photosynthetically efficient and their production advantageous in terms of enhanced seedling growth, the metabolic costs associated with their production may have meant that seedlings in the 4% RAR treatment did not have the requisite N for their growth.

Whether or not pine seedlings with primary as opposed to secondary needles differ in growth rates and field-performance is a question of interest to nurseries producing seedlings for reforestation purposes and to the individuals purchasing the seedlings. To produce secondary-needle pine, nurseries generally need to install more lighting and as a consequence, these seedlings can cost 5-10 % more than primary-needle pine (Steven Kiiskila, New Forest Tree works, personal communication, May 26, 2004). However, if secondary needles enhance field performance these costs may be outweighed by better growth and survival. The limited literature published on this topic provides no conclusive support for the enhanced performance of one needle type over the other (see Thompson 1976, 1981; McGilvray and Barnett 1982; Omi *et al.* 1992; Mustard *et al.* 1998). In this study, although lodgepole pine seedlings produced more biomass per unit leaf area per unit time at H3 in the FA treatment, this increased efficiency associated with the production of secondary needles did not translate into greater mean NP's nor greater

RGR's than seen for seedlings at H2. This may be due to the fact that the secondary needles were newly formed. Had the study continued for a longer period of time, allowing seedlings to develop a full set of photosynthetically active secondary needles, differences in NP and RGR may have become evident.

Root respiration and root N concentration

In agreement with previous studies which have shown that a linear relationship often exists between N concentration and respiration for plant tissues (Ryan 1991, 1995; Reich *et al.* 1996, 1998a; Maier *et al.* 1998), including the roots of trees (Burton *et al.* 1996, 2002; Zogg *et al.* 1996; Pregitzer *et al.* 1998; Reich *et al.* 1998b), there were significant positive correlations between root respiration and the nitrogen concentrations of seedling roots in this study when compared across both species and both nutrient treatments. In a 2002 study on the effects of nitrogen concentration and temperature on the root respiration of forest tree species from 10 forested study sites across North America, Burton *et al.* (2002) found that a basic relationship between root respiration, temperature and nitrogen was apparent across species and biomes. A great deal of the variation among the forest sites was explained by site-to-site variation in fine root N concentration; root respiration rates were highly correlated with fine root N concentrations at all measurement temperatures. As well, Burton *et al.*'s (2002) comparison between angiosperms and gymnosperms indicated that gymnosperms had lower fine root respiration rates, but this difference was largely explained by the lower fine root N concentrations in the gymnosperms. When N concentration was used as a covariate, root respiration rates were not significantly different between the angiosperms and gymnosperms.

While strong relationships were found between root respiration and root N when examined across study sites and species, Burton *et al.* (2002) found that for laboratory-measured root respiration rates, much of the within-site variation could not be accounted for by root N concentration. Root respiration rates for individual samples within sites were only occasionally correlated with root N concentration. A similar situation was seen in this study. Root respiration was positively correlated with root N when compared across both species and both nutrient treatments, but there were no significant

relationships between rN and root respiration within-species in a given nutrient treatment. Given the greater range of both rN and root respiration among species than within, it is not surprising that a stronger relationship between the two is also found on that basis. Preitzger *et al.* (1998) noted that the relationships between N concentration and root respiration only became apparent when a sufficiently large range of N concentrations was examined. Here, the range of root N concentrations within species and nutrient treatments was small (see rN means Appendix III). Root age, length, biomass, nutrients other than N and humidity variation in the measuring chamber may account for differences in root respiration, both within and among nutrient treatments, that were not explained by root N concentration.

Overall seedling growth characteristics and their relation to RGR

As evident in the above discussion sections, lodgepole pine and Stika spruce seedlings showed distinct differences in SRL, NUR and ULR, but minimal differences in biomass allocation in response to the nutrient environment. It has been reported that root and shoot morphology and structure are more important plant traits than biomass partitioning in regards to determining relative growth rates (Van der Werf *et al.* 1993; Reich *et al.* 1998a). Model simulations of Van der Werf *et al.* (1993) showed that at low nitrogen availability *Briza media*, a species characteristic of nutrient poor soils, and *Dactylis glomerata,* a species characteristic of nutrient rich soils, partitioned their biomass optimally with respect to RGR and NP. They found no evidence to state that *B. media* was better adapted to poor soils in terms of relative growth rate or utilization of nitrogen with respect to growth than was *D. glomerata.* As well, findings of Reich *et al.* (1998a) did not support the hypothesis that RGR was closely related to biomass allocation. These authors concluded that SLA, SRL, and ULR were closely associated with variation in life history traits such as seed size, and that they were more closely related to RGR than biomass allocation. Brown *et al.* (1996a) found that ULR made a significant contribution to RGR, and Poorter and Nagel (2000) found that increased growth rates caused by increased nutrient supply were caused by higher ULR as well as LMF and SLA. Garnier (1991) concluded that when different species were grown under productive conditions, the difference in growth rate between them was linked to

differences in the specific activities of their organs (leaves and roots) and much less to differences in biomass allocation between them. This study's findings; that biomass allocation did not differ significantly between lodgepole pine and Sitka spruce seedlings, that SRL was more closely related to NUR than root biomass, and that ULR was more closely related to photosynthesis than leaf biomass, support the contention that leaf and root structure and metabolism more strongly influence patterns of growth among species than does biomass partitioning.

Despite differences in growth parameters such as ULR and NUR lodgepole pine and Sitka spruce seedlings did not demonstrate significant differences in overall growth. In the 4% RAR treatment, RGR was controlled by RAR (recall from steady-state theory that RGR=RAR=RUR) therefore one would not expect to see differences in growth rates. In this case, the question of interest was whether or not the two species achieved the 4% RGR in different ways, reflecting adaptations to their native nutrient environments. In the FA treatment, however, the seedlings could have demonstrated different maximum growth rates but RGR's were not statistically different. However, the difference between a 7.9 % compound growth rate and an 8.1 % compound growth rate accumulates over time; thus statistically insignificant differences in growth rate may lead to significant differences in growth over the course of a tree's life. At H2 in the FA nutrient treatment, before the lodgepole pine seedlings developed secondary needles, the Sitka spruce seedlings had a higher mean RGR, higher NUR, SRL, and a greater rate of leaf mass accumulation. The development of secondary needles by H3 changed these relationships. Lodgepole pine seedlings had higher mean RGR and a significantly greater mean ULR than Sitka spruce seedlings. If this study was extended for a greater length of time after the development of secondary needles in pine, differences in overall seedling growth may become more apparent.

Comments on allometric analyses

Individual seedlings were not the same size when put into the Biotronic units at the start of each experiment. Thus, differences seen in the growth parameters measured at a common harvest may have reflected differences in seedling size rather than distinct species-specific responses to the nutrient treatments. To correct for seedling size,

allometric analyses were conducted. A disadvantage of allometric analysis is that growth (e.g. seedling size, measured here as total dry mass) and development (e.g. number of secondary needles, sexual maturity) are not necessarily the same thing. It is incorrect to assume that plants of a common size are necessarily at a common developmental stage. Another disadvantage of allometric analysis is that it is not always obvious which parameter should be used for plant size (Poorter and Nagel 2000). Plant dry mass is a common choice, but fresh mass or leaf area may be better parameters in some experiments. It has been shown in CO_2 enriched plants that increases in dry mass may be largely due to accumulations of non-structural carbohydrates (Poorter *et al.* 1988); dry mass would thus underestimate actual plant size. Finally, allometric relationships are often tested when the ranges of plant variables (e.g. total mass) only partially overlap between treatments. Any judgement based on the slope coefficients of the regression lines is then based on partial extrapolation of the data outside the actually size range measured. Poorter and Nagel (2000) suggest that in the case where size correction is required, plant parameters such as LMF should be plotted against a parameter of plant size, such as total mass, directly. I created plots such as these (data not shown) and overall trends were the same as those seen in allometric analyses.

I am confident that allometric analyses fostered a more accurate interpretation of the data collected in this study than common harvest comparison alone. Had I not accounted for ontogenetic drift, I would have misjudged the magnitude of the adjustments in biomass allocation made by these species in response to the high and low levels of N supply.

CONCLUSIONS

The analytical methods used in this study allowed changes in seedling biomass allocation, tissue N concentration, morphology and physiology which occurred in response to the nutrient environment to be distinguished from those which occurred as a normal consequence of plant growth. Both lodgepole pine and Sitka spruce seedlings showed distinct growth responses to the nutrient treatments, however, ontogenetic drift

obscured species differences. In general, data from this study are not consistent with theories and empirical data about habitat-related ecophysiological traits, which suggest that species adapted to high resource environments have high RGR's, high rates of resource capture and high tissue metabolic rates relative to species characteristic of low resource environments. Lodgpeole pine and Sitka spruce seedlings did not differ significantly in *overall* growth responses to high and low N environments. It would be incorrect to assume, however, that the different growth habits of lodgepole pine and Sitka spruce as seen in the field are due their adaptations to the nutrient environment alone. Moisture and temperature (to name two of many interacting environmental variables) are intricately linked to plant growth and they have important effects on nutrient availability and the general soil environment. For instance, rates of decomposition are higher in warm moist soils, and the mass flow of nutrients to roots via soil water is an important determinant of nutrient availability. Lodgepole pine had greater plasticity in SRL than Sitka spruce and a greater rate of photosynthesis compared to respiration at low N. While in this study these traits did not lead to growth differences between the two species, they may confer a growth advantage when coupled with the cold, high-elevation and frequently dry, nutrient-poor sites where lodgepole pine trees commonly grow (the lodgepole pine provenance originated from 1100 m elevation, while the Sitka spruce provenance originated from 31 m elevation). The selection pressures that have led to the adaptations that allow these two species to persist in their native ranges are likely varied and complex. It is not surprising that their morphological and physiological responses to nutrient availability are equally complex.

There is an overwhelming amount of literature published on photosynthesis and the response of the above ground portions of plants to limited nutrients (presumably because these are the parts we generally want to harvest). However, if we care to truly understand plant growth, more attention needs to be paid to below-ground plant parts and their influence on RGR, and researchers need to try and link above- and below-ground processes. There are many other root characteristics besides RMF and SRL, as presented here, that are likely to be important components of plant response to soil nutrient conditions; however, comparative studies of root morphology (both within species at different nutrient levels, and among species) are rare (see review by Hutchings and de

Kroon 1994). It is possible that root demography and the ability of plants to alter rates, timing and placement of root growth may have greater importance for plant success on a given site than shifts in biomass allocation between roots and shoots (Reynolds and D'Antonio 1996). Root turnover, for instance, may increase in response to nutrient supply even if relative root biomass allocation is unchanged (Hendricks *et al.* 1993). Bernston *et al.* (1995) found that root turnover increased with decreasing nutrient supply more in a slower-growing birch species compared to a faster growing birch species, and that the patterns of root production and loss in the faster growing birch provided it with a more expansive root system. As well, root physiology, specifically the density and efficiency of carriers in root cell membranes (and the plasticity of these characteristics) are other relatively unexplored plant traits which are critical to plant nutrition. A better understanding of below ground sources of phenotypic plasticity and their relationship to overall plant growth will enhance our ability to predict plant responses to novel environmental conditions.

Chapter 3. Flux characteristics of H^+, NH_4^+ and NO_3^- ions across individual roots of lodgepole pine and Sitka spruce seedlings grown at high and low levels of nitrogen supply

INTRODUCTION

It has long been known that plant species differ in their ability to obtain and utilize different forms of N. Relative to cereal and crop species, however, the nitrogen uptake characteristics of conifer roots have only recently been investigated. Adaptations to different forms of N may play a role in determining trees' spatial and/or temporal distribution on the landscape (Lavoie *et al.* 1992; Chapin *et al.* 1993; Kronzucker *et al.* 1997, 2003; Min *et al.* 1998, 1999, 2000). Kronzucker *et al.* (2003) argue that NH_4^+ transport inefficiency may provide a physiological basis for the elimination of pioneer tree species such as *Populus tremuloides* (trembling aspen) and *Pseudotsuga menzeisii* (Douglas-fir) in late successional soils, and discrimination against NO_3^- has been documented in late-successional species such *Picea glauca* (white spruce) (Kronzucker *et al.* 1995a; Kronzucker *et al.* 1995 b,c,d,e; 1996; 1997). In most late-successional communities of temperate and boreal forest ecosystems, NH_4^+ is the main form of available soil organic N as the ratio of NH_4^+ to NO_3^- generally increases over the course of forest succession (Rice and Pancholy 1972; Van Cleve *et al.* 1983; Chapin *et al.* 1986; Lavoie *et al.* 1992; Kronzucker *et al.* 1997, Britto and Kronzucker 2002, Kronzucker *et al.* 2003).

While species' adaptations to the successional stage of an ecosystem, and to the dominant N forms in their native habitats, may determine their nutrient uptake characteristics, the ability of plants to take up and process nutrients also responds to changes in their nutrient status over time (Britto *et al.* 2001). Cellular pools of nitrate and ammonium are known to change with external nutrient supply (Siddiqi *et al.* 1991; Wang *et al.* 1993; Kronzucker *et al.* 1995a,b). Furthermore, flux capacity measured at a given concentration is gradually up-regulated during a change to a nutrient limited condition, while it is down-regulated when nutrients are provided (Lee and Rudge 1986; Morgan and Jackson 1988 a,b; Kronzucker *et al.* 1998).

The aim of this study was to investigate the H^+, NH_4^+ and NO_3^- ion flux characteristics of *Pinus contorta* var. *latifolia* (lodgepole pine) and *Picea sitchensis* (Sitka spruce) grown in high and low nitrogen environments. Lodgepole pine is an early sucessional species that is tolerant of nutrient poor soil conditions, while Sitka spruce can be a dominant component of coastal climax forests and is generally restricted to moist, nutrient rich soils. The contrasting nutritional adaptations of these two species provide an excellent opportunity to investigate how ion flux characteristics relate both to these plants' native N environments and to their current nutrient status. The plants used were grown without mycorrhizal associations in aeroponic growth units. I predicted that both species would show higher fluxes of NH_4^+ relative to NO_3^-, but that lodgepole pine would have higher net influx of NO_3^- than Sitka spruce, reflecting its presence in early-successional, often fire dominated ecosystems. I also predicted that seedlings of both species grown with free access to nutrients (FA, the High N treatment) would show higher N fluxes than seedlings grown under conditions of 4% relative addition rate nutrient stress (4% RAR, the Low N treatment).

To address questions regarding the transport of mineral ions into and out of plant roots, various methods of chemical analysis are generally used. More recently, ^{13}N radiotracing has been used to characterize the kinetics of NH_4^+ and NO_3^- influx in tree species such as white spruce, trembling aspen and Douglas-fir (Kronzucker *et al.* 1995b, 1996; Min *et al.* 1998, 1999, 2000). As long as certain conditions are met (see Britto and Kronzucker 2001) this method is currently the most accurate way of estimating the kinetics of NH_4^+ and NO_3^- fluxes. The utility of this technique is limited, however, because the ^{13}N isotope is short lived (10 min half-life) requiring experiments to be carried out in close proximity to a cyclotron. In the last 15 years, an additional technique, the non-invasive measurement of ion fluxes using ion-selective microelectrodes, has contributed to our knowledge of ion fluxes across plant roots and to the functional characterization of ion transport systems (e.g. Henriksen *et al.* 1990, 1992; Kochian *et al.* 1992; Colmer and Bloom 1998; Taylor and Bloom 1998; Rubinigg *et al.* 2002). The microelectrode ion flux measurment system (MIFE), developed at the University of Tasmania, is a promising alternative method for investigating the concentration

dependence of NH_4^+ and NO_3^- fluxes (Newman *et al.* 1987; Shabala *et al.* 1997; Newman 2001; Shabala and Knowles 2002; Garnett *et al.* 2001, 2003).

An advantage of the MIFE system is that it allows fluxes of NH_4^+, NO_3^- and H^+ to be measured simultaneously. Both NH_4^+ and NO_3^- uptake by roots and assimilation within the plant are closely associated with H^+ fluxes. The main H^+ efflux system is the electrogenic proton extruding ATPase. This proton pump is the main determinant of the plasma membrane potential, which itself also regulates ATPase (Newman 2001). Many transport systems employ the resulting change in electrochemical potential of H^+ across the plasma membrane for co-transport of other ions or uncharged organic molecules. Based on the assumption that cytoplasmic NH_4^+ concentrations are generally very low, uptake of NH_4^+ by plant roots was thought to be energetically downhill via NH_4^+ uniport. However, recent estimates of cytoplasmic NH_4^+ concentrations in the milimolar range (Wang *et al.* 1993b; Kronzucker *et al.* 1995b; Britto *et al.* 2001) suggest that NH_4^+ uptake may be energetically uphill, perhaps by symport with H^+ (Wang *et al.* 1993a; Glass *et al.* 1997). Uptake of NO_3^- is thought to involve symport with two H^+ ions (Ullrich and Novacky 1981; Glass *et al.* 1992; Meharg and Blatt 1995; Miller and Smith 1996).

While N and H^+ fluxes have been mechanically linked, there are very few cases where they have been measured simultaneously, in intact root systems. By allowing concurrent flux measurements of NH_4^+, NO_3^- and H^+ the MIFE system will help further our understanding of the relationships between N and H^+ ions and plant nutrition. Unlike other methods of measuring nutrient uptake, the MIFE system measures fluxes over a very small area of the root surface (a few cells) instead of integrating fluxes over the whole root system, giving some insight on the association between fluxes.

In the first experiment of its kind, I used the MIFE system to compare the ion flux characteristics of two conifers at steady-state and transitional steady-state nutrient concentrations. The objectives of this study were to compare NH_4^+, NO_3^- and H^+ flux characteristics of intact lodgepole pine and Sitka spruce seedlings, to evaluate the relationships between H^+ flux and N uptake, and to analyse the extent to which plant nutrient status, as determined by the N concentration of culture solutions, affects ion flux.

MATERIALS AND METHODS

MIFE technique

The MIFE system (University of Tasmania, Hobart) was used with three microelectrodes, each selective for one of the three ions of interest: H^+, NH_4^+, and NO_3^-. The voltage (mV) of each ion-selective microelectrode was measured at two positions: 40 and 80 μm from the root surface. These voltages, V_1 and V_2, at radial distances R_1 and R_2 from the root, reflect the electrochemical potential of the given ion at those positions. The net flux of the ion was calculated using the equation:

$$J = cu\mathrm{F}(58/\mathrm{NernstSlope})(V_2-V_1)/\,[r\,\ln(R_2-R_1)],$$

where c is the concentration of the ion in solution, u is its mobility, F is the Faraday number and r is the root radius. The NernstSlope is the slope of the straight line fitted to the calibration data (mV against $\log_{10}$ concentration), and it incorporates the valence of the ions. The theory is discussed fully by Newman (2001).

The MIFE apparatus was built around a compound microscope positioned with its optic axis horizontal (Figure 3.1). The chamber that held the plant in solution was mounted onto a stainless steel holder that attached to a PatchMan NP2 micromanipulator (Eppendorf AG, Hamburg, Germany) by a steel rod. This allowed the root to be positioned into the microscope's plane of focus. Seedlings were positioned in the measurement chamber so that main root was apart from any lateral roots and easily accessible from above. The root was held in a horizontal position with pieces of nylon tubing and all measurements were made at least two centimetres from the growing tip in a white zone from which laterals were starting to emerge. The measurement chamber was 15 cm long × 0.5 cm wide × 1.5 cm high, fabricated from acrylic sheeting fastened with silicon sealant. The volume of the measurement chamber was 10 ml. Solution changes were made using a 10 ml pipet and took no longer than 2 min.

The three ion selective electrodes were mounted vertically in a modified, adjustable, 3-electrode holder (model no. MMT-5, Narishige, Narishige, Tokyo, Japan)

(Figure 3.1b). A KCl/agar reference electrode was placed at the shoot-end of measurement chamber. During flux measurements the chamber oscillated such that the electrodes moved at 5 s intervals between 40 μm and 80 μm from the root surface.

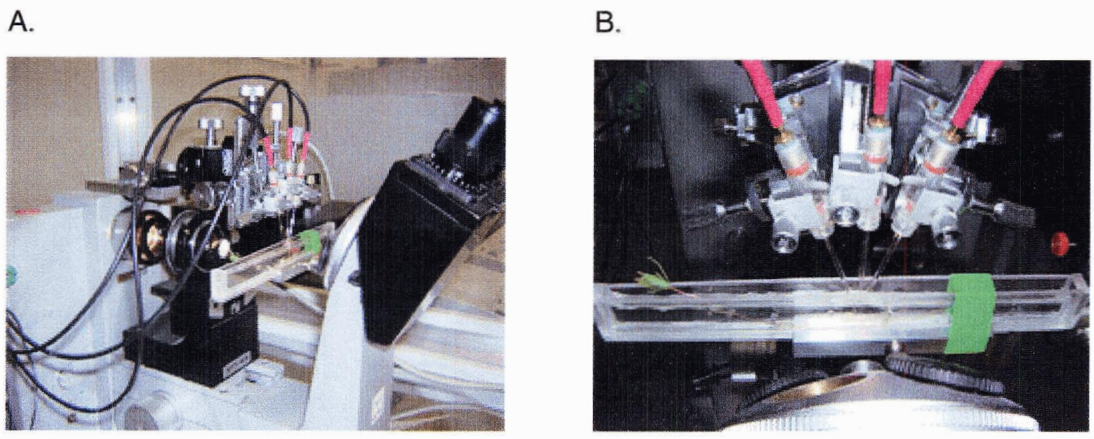


Figure 3.1. The MIFE apparatus. **A.** The compound microscope positioned with its optic axis horizontal and the vertically mounted three-electrode holder. The wires connected to the base of each electrode holder lead to a pre-amplifier that is connected to a PC. A specifically designed computer program records the mV electrical potential differences detected by the ion sensor in the tip of each microelectrode as the measurement chamber oscillates from 40 to 80 μm from the root surface every 5 s. **B.** A close up of the microelectrodes and the measurement chamber. The chamber holds 10 ml of solution and is mounted on to a stainless steel holder that is attached to a micromanipulator. The tips of the three microelectrodes (order from left to right: NO_3^-, H^+, NH_4^+) are positioned within 2 μm of one another.

Electrodes

Electrodes were made from 1.5 mm outer diameter, 0.86 mm inner diameter borosilicate glass capillaries (ref no. 30-0053, Harvard Apparatus Ltd. Edenbridge, Kent). The blanks were pulled to tips of <1μm diameter with a vertical pipet puller and silanised with tributylchlorosilane (Fluka, ref no. 90796). Tips of silanized electrodes were broken back to 2-3 μm and then backfilled. The backfilling solutions were, 200 mM NH_4Cl for NH_4^+, 500 mM KNO_3 + 100 mM KCl for NO_3^-, and 15 mM NaCl + 40 mM KH_2PO_4, adjusted to pH 6 with 0.1 M NaOH, for H^+. Electrodes were then front filled with their respective resins to a column length of approximately 200 μm.

The NH_4^+ and H^+ electrodes were front-filled using commercially available resins (NH_4^+ Sigma Aldrich ref no. 09879; H^+ Sigma Aldrich ref no. 95291) whereas the NO_3^- sensor was made according to Plassard *et al.* (2002) and contained 0.5% tridodeclymethylammonium nitrate (TDMA-NO_3^-) 0.084% methlytriphenylphosphonium bromide (MTPPB) and 99.4% 2-nitrophenyloctylether (NPOE). NH_4^+ and NO_3^- electrodes were calibrated using solutions of 50 μM, 500 μM, or 1000 μM NH_4NO_3 and H^+ electrodes were calibrated using solutions of NaH_2PO_4 adjusted to pH 5.75, 6.46 and 7.40. To make the reference electrode, 0.37 mm diameter silver wire (Warner Instruments Inc. ref no. AG15W) was electrolysed in 0.2 M HCl for 15 min. The chlorided silver wire was inserted into a 1.0 mm outer diameter, 0.58 mm inner diameter borosilicate glass capillary (ref no. 30-0053, Harvard Apparatus Ltd. Edenbridge, Kent) containing 1M KCl in 2% agar and fastened with parafilm. Due to the frequent solution changes and the distance from the measurement region, it was unlikely that K^+ leaking from the reference electrode would have reached the measurement region.

Plant Culture

Seedlings of lodgepole pine (provenance 29172, 53 ° 20' latitude, 123 ° 25' longitude, and 1100 m elevation) and Sitka spruce (provenance 09043, 52 ° 14' latitude, 127 ° 14' longitude and 31 m elevation) were imbibed for 24 h, surface sterilized with 3 % hydrogen peroxide for 5 min and then rinsed thoroughly in de-ionized water. Washed seeds were then placed onto moistened Kimpack in the base of petri dishes. The dishes

were covered, sealed with Parafilm and placed in the refrigerator at (4 °C) for 21 d. At the end of the stratification period seeds were evenly spaced in germination trays and placed in a germination chamber set for 8 h days at 30 °C and 16 h nights at 20 °C. When the roots of the germinants were approximately 3 cm long they were inserted into closed-cell foam collars (two seedlings per collar) and placed into the Biotronic units containing 5 L of nutrient solution that was continuously circulated and sprayed aeroponically onto the roots (see Table 2.1 for contents of pre-treatment nutrient solution). Once the seedlings reached approximately 0.03 g (1-2 weeks) they were weighed and each individual was assigned to a Biotronic treatment unit. Seedlings were subject to two nutrient treatments, free access to nutrients (FA), and 4% relative addition rate (4% RAR) nutrient stress (see Table 2.2 for nutrient solutions). A single Biotronic unit was used for each nutrient treatment. Each unit contained 5 L of either FA or 4% RAR nutrient solution, and 30 lodgepole pine and 30 Sitka spruce seedlings. The 60 seedlings were inserted singly into foam collars and distributed randomly within each Biotronic unit. The two units were arranged within a Conviron growth chamber set for 16 hr days with 500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and 8 hr nights with 0 PPFD. A relative humidity of 50 % and chamber temperatures of 20 °C was maintained throughout. Seedlings were grown in the Biotronic units for 3 weeks prior to flux measurements.

Flux measurements

The nutrient solution in the Biotronic units had a total N concentration of 71.23 μM for the 4% RAR treatment and 712.37 μM (Table 2.2) for the FA treatment, with conductivities of 12 and 72 μS respectively (data not shown). Measurement solutions were made with equivalent N concentrations, but contained only NH_4NO_3 (Low N solution = 71.23 μM NH_4NO_3, conductivity 9 μS; High N solution = 712.37 μM NH_4NO_3, conductivity 65 μS). All seedlings were measured at both nutrient concentrations and the measurement order (High N to Low N or vice versa) was completely randomized. Each day, seedlings were removed from the Biotronic units in the morning and placed in small plastic containers filled with either High N or Low N nutrient solution (Figure 2). Seedling roots were continually aerated by bubblers attached

to a small air pump. Seedling shoots were illuminated using a slide projector providing a PPFD of 700 μmol m^{-2} s^{-1}.

Seedlings were placed in the measurement chamber and electrodes were positioned 80 μm from the root surface. After fluxes were recorded for 10 min at the first N concentration, the solution was removed using a 10 ml pipet and the chamber was filled with the new N solution. The solution change took approximately 1 min and fluxes were measured immediately afterwards for another 10 min.

Experimental Design and Data analysis

Eighty seedlings were measured in total, 40 Sitka spruce and 40 lodgepole pine, over a period of 5 days (Table 3.1). Twenty of the measured seedlings per species were grown with free access to nutrients, and 20 were grown under conditions of 4% RAR nutrient stress. Flux measurements for 10 seedlings per species from each Biotronic treatment unit were made at High N followed by Low N solution concentrations, and 10 seedlings were measured first at Low N and then at High N solution concentrations. Measurement order was random. Seedling stem and root lengths were measured at the end of each flux measurement period, and all seedlings were individually weighed, dried and analysed for total N content at the end of the experimental period.

The simultaneous fluxes of H^+, NH_4^+ and NO_3^- at the surface of the main root of all seedlings, measured both at High N and Low N, were graphed over time. Average fluxes were calculated for a period when the fluxes were stable for a minimum of 2 min for all three ions (see Appendix IV for an example of raw flux data).

Means of each group of 10 seedlings subject to a given experimental nutrient treatment (e.g. High N to Low N, see Table 3.1) were compared via one-way analyses of variance (ANOVA) using SAS (SAS Institute Inc. 1988). When there were unequal number of observations, data were analysed using general linear models procedure (GLM). Correlation analyses were conducted using the PROC CORR procedure in SAS. T-tests were conducted with SigmaStat (Jandel Scientific Software 1995) and Bonferroni's adjustment was used to account for the number of comparisons.

Table 3.1. MIFE (microelectrode ion flux estimation) system experimental design. Ten lodgepole pine and Sitka spruce seedlings were analysed per treatment location. Seedlings were grown in Biotronic units with either free access to nutrients or under conditions of 4% RAR nutrient stress for a period of 3 weeks prior to measurement. Fluxes of H^+, NH_4^+ and NO_3^- at the surface of the main root of all seedlings were measured both at High N (712.37 μM) and Low N (71.23 μM). Measurement order was random.

Species	Biotronic nutrient treatment	Number of seedlings	Measurement solution 1	Measurement solution 2
lodgepole pine	Free access	10	High N	Low N
		10	Low N	High N
lodgepole pine	4% RAR	10	High N	Low N
		10	Low N	High N
Sitka spruce	Free access	10	High N	Low N
		10	Low N	High N
Sitka spruce	4% RAR	10	High N	Low N
		10	Low N	High N

RESULTS

H^+, NH_4^+ and NO_3^- fluxes

Changing solution caused considerable noise in the electrode readings and it generally took at least five minutes for fluxes to stabilize. These initial data have been excluded from Figure 3.2 (see appendix IV for an example of raw flux data). The stable period used to calculate average flux generally included the last few minutes of each 10 min measurement period (Figure 3.2). At this time, steady, diffusion-limited conditions were considered to be established in the region between the tissue and the electrode tips (see Newman 2001), and there was good agreement between the known solution concentrations and those measured by the microelectrodes ((Mean ± SD) 692.34 ± 34.29 μM NH_4NO_3, High N; 70.99 ± 14.78 μM NH_4NO_3, Low N, n=40).

Fluxes varied considerably for all three ions measured at each treatment combination, and there were no significant species or treatment differences between fluxes (Table 3.2). All seedlings showed net efflux when measurement at High N was followed by measurement at Low N (bold numbers Table 3.2), and there were significant

nutrient treatment (FA or 4% RAR) × experimental treatment (measurements made in order from High N to Low N or vice versa) interactions. Concentration dependent flux responses are only at steady state with respect to the solution in which plants are grown (Britto and Kronzucker 2001). I therefore felt that flux measurements at the N concentration in which seedlings were grown were the most representative. All subsequent analyses include only the data from FA seedlings measured at High N, and 4% RAR seedlings measured at Low N. Because all seedlings demonstrated efflux when the measurement solution was changed High to Low N, 4% RAR seedling measurements were only included when fluxes were first measured at the Low N solution concentration. There was no significant difference between FA seedling fluxes measured at High N regardless of measurement order ($p = 0.1717$) so all High N flux values were included. The resulting data set included ten 4% RAR seedlings per species, and twenty FA seedlings per species.

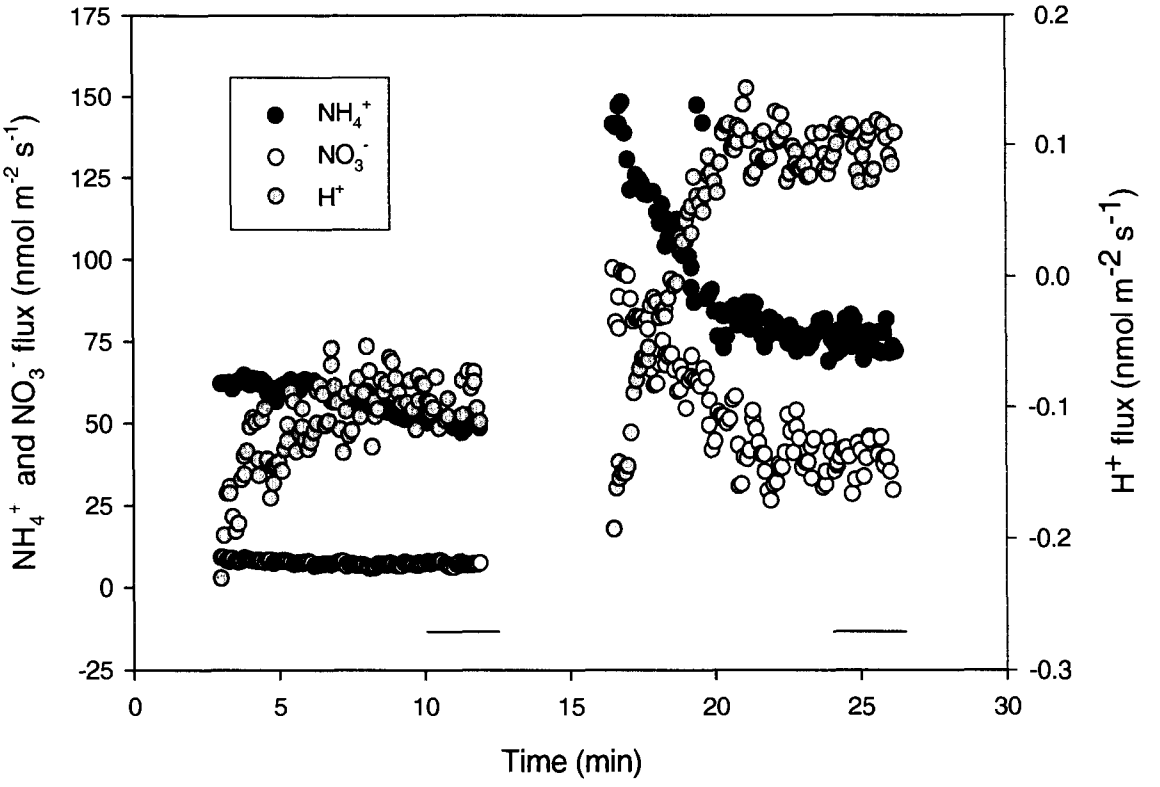


Figure 3.2. An example of net fluxes of H^+, NH_4^+ and NO_3^- measured across a Sitka spruce seedling root at 5 s intervals during a MIFE experiment. Measurements started at 0 min, the solution was changed from 71.23 μM NH_4NO_3 to 712.37 μM NH_4NO_3 at 12 min. The electrode readings from the initial measurement periods for both N concentrations were omitted because of considerable noise. Bars on the x-axis represent periods from which the mean flux values were calculated.

Table 3.2. Mean H^+, NH_4^+ and NO_3^- flux values (n=10) for lodgepole pine (LP) and Sitka spruce (SS) seedlings grown for 3 weeks with either free access (FA) to nutrients or under conditions of 4% relative addition rate nutrient stress (4%). Seedlings were measured at both High N (712.37 μM) and Low N (71.23 μM). Measurement order was random. Negative values indicate efflux. Bold values indicate that net efflux occurred for both NH_4^+ and NO_3^- when measurement order was from High N to Low N.

		High N to Low N					
		Mean ion flux at high N (nmol m^{-2} s^{-1}) (SE)			Mean ion flux at low N (nmol m^{-2} s^{-1}) (SE)		
		H^+	NH_4^+	NO_3^-	H^+	NH_4^+	NO_3^-
4%	LP	-1.00 (1.40)	-0.23 (17.65)	17.74 (10.01)	7.20 (5.68)	**-33.89** (10.63)	**-18.31** (10.92)
	SS	2.18 (1.43)	22.17 (4.19)	36.12 (10.71)	23.92 (22.00)	**-2.50** (9.10)	**-13.36** (8.23)
FA	LP	21.08 (11.28)	23.61 (7.29)	16.75 (21.37)	5.43 (5.17)	**-13.91** (9.00)	**-8.00** (6.43)
	SS	12.23 (13.27)	25.07 (6.14)	18.50 (12.83)	-0.15 (6.94)	**-13.19** (7.13)	**-2.84** (5.94)

		Low N to High N					
		Mean ion flux at low N (nmol m^{-2} s^{-1}) (SE)			Mean ion flux at high N (nmol m^{-2} s^{-1}) (SE)		
		H^+	NH_4^+	NO_3^-	H^+	NH_4^+	NO_3^-
4%	LP	-0.36 (1.42)	13.69 (5.36)	13.63 (11.69)	-2.18 (2.17)	30.18 (6.05)	79.58 (31.60)
	SS	6.51 (6.65)	17.87 (7.97)	13.95 (13.79)	-0.87 (4.13)	99.55 (85.88)	35.59 (18.57)
FA	LP	1.80 (4.73)	9.65 (4.54)	9.66 (6.25)	-14.3 (8.94)	22.07 (6.46)	53.24 (15.76)
	SS	2.31 (1.50)	23.59 (9.35)	3.21 (4.65)	-1.69 (4.78)	48.26 (9.17)	25.60 (12.28)

GLM analysis of the condensed data set revealed a significant nutrient treatment effect on NH_4^+ flux (Table 3.3). Regardless of species, seedlings grown under FA conditions had higher net influx of NH_4^+ than seedlings grown under 4% RAR nutrient stress conditions. Mean flux values for NO_3^- were also higher for seedlings grown at FA, but not significantly so. The large variation in the data (note the large standard errors in Table 3.3, Figure 3.3) requires all trends to be interpreted with caution. H^+ fluxes were the most variable of the three ions (Figure 3.3a) and there were no significant species or nutrient treatment differences in net proton movement. There were also no significant species differences in net fluxes of NH_4^+ or NO_3^-, and no clear species preferences for either ion.

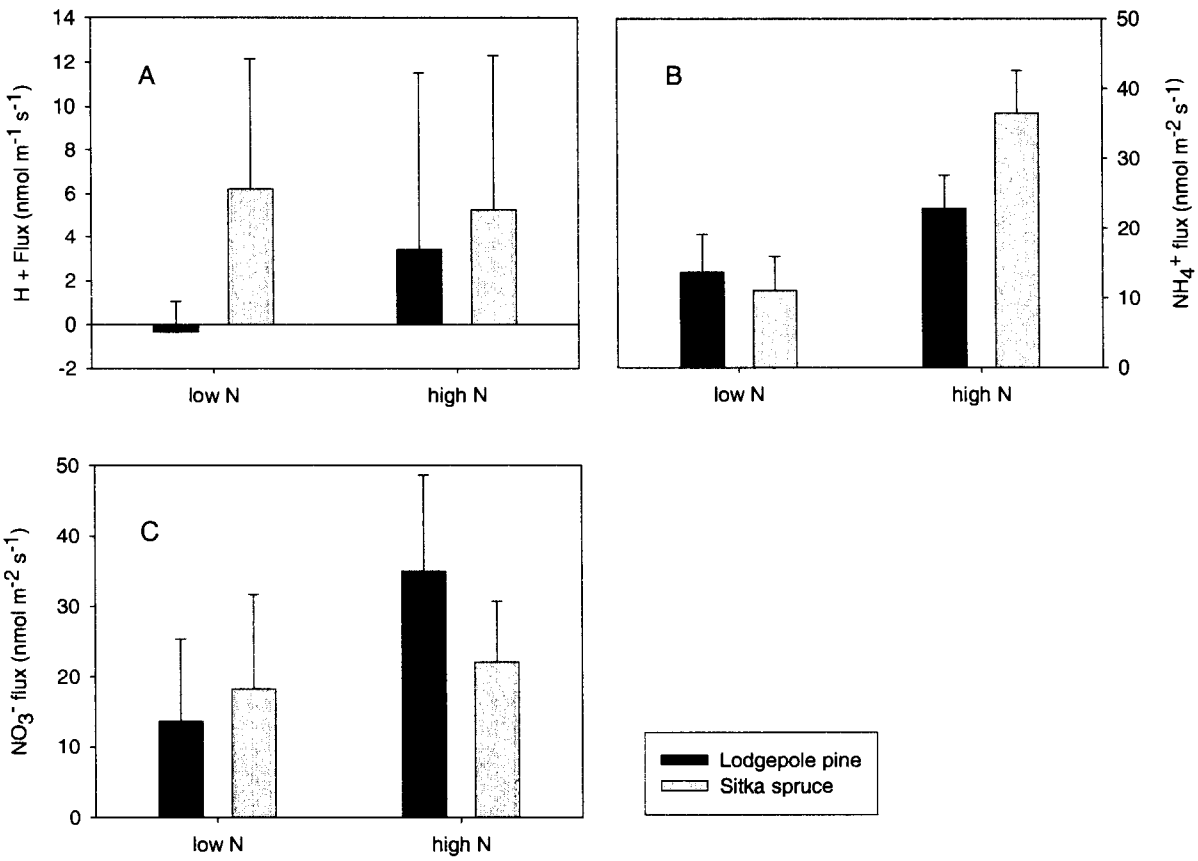


Figure 3.3. Fluxes of **A.** H^+ **B.** NH_4^+ and **C.** NO_3^- (bars represent standard errors) measured across individual roots of lodgepole pine (black bars) and Sitka spruce (grey bars) seedlings grown at FA and 4% RAR for three weeks prior to measurement. Note the smaller scale of graph A. FA seedlings were measured at high N (712.37 μM NH_4NO_3) and 4% RAR seedlings were measured at low N (71.23 μM NH_4NO_3). Measurement N concentrations were equivalent to N concentrations in the Biotronic unit culture solutions.

Table 3.3. Significance values and mean H^+, NH_4^+ and NO_3^- fluxes across the roots of lodgepole pine and Sitka spruce seedlings, measured at the N concentration in which they were cultured. Low N = 71.23 μM NH_4NO_3, High N = 712.37 μM NH_4NO_3. Because all seedlings demonstrated net efflux when measured from high to low N they were excluded from the analyses, thus n = 10 for Low N, n = 20 for High N. P values were calculated using type three sums of squares from the general linear models procedure (GLM). * = significant at the 0.05 level.

	Low N			High N		
Mean flux (nmol m^{-2} s^{-1}) ± SE	H^+	NH_4^+	NO_3^-	H^+	NH_4^+	NO_3^-
lodgepole pine	-0.36 ± 1.42	13.69 ± 5.37	13.63 ± 11.70	3.40 ± 8.09	22.84 ± 4.75	34.99 ± 13.59
Sitka spruce	6.21 ± 5.96	11.04 ± 4.82	18.23 ± 13.50	5.27 ± 7.05	36.67 ± 5.99	22.05 ± 8.68

GLM P- value	H^+	NH_4^+	NO_3^-
Nutrient treatment	0.8594	0.0054*	0.3392
Species	0.5979	0.3506	0.7506
Nutrient treatment x species	0.7686	0.1754	0.5049

Ion flux in relation to seedling size and whole-plant N concentration

GLM analysis of plant growth parameters (Table 3.4) revealed that there were significant species differences in root length. Lodgepole pine had significantly longer roots than Sitka spruce ($p<0.001$) in both FA and 4% RAR nutrient treatments. Within species, there were no significant nutrient treatment differences in root length. All other plant growth parameters had significant nutrient treatment x species interactions when analysed using the overall model. T-tests were used to compare all growth parameters between species within nutrient treatments, and between nutrient treatments within species. All comparisons were statistically significant ($p<0.001$) except for stem length in FA Sitka spruce vs. 4% RAR Sitka spruce seedlings. At both FA and 4% RAR lodgepole pine seedlings were larger than Sitka spruce seedlings in terms of stem length, root fresh mass, stem fresh mass, and the change in total plant fresh mass from the beginning to the end of the experimental period (Table 3.4). For both Sitka spruce and lodgepole pine all FA seedlings were significantly larger than 4% RAR seedlings in terms of root fresh mass, stem fresh mass and the change in fresh mass throughout the experimental period. FA

lodgepole pine seedlings had longer stems than 4% RAR seedlings, but there was no difference in Sitka spruce seedling stem length between nutrient treatments.

Despite the significant differences in seedling size between species and between nutrient treatments, no significant correlations were found between any of the measured growth parameters and either H^+, NH_4^+ or NO_3^- fluxes.

Table 3.4. Size characteristics of seedlings used in MIFE experiments. Data shown are means ± standard error (n=10 for 4% RAR, n=20 for FA seedlings)

Species	Nutrient treatment	Root length (cm)	Stem length (cm)	Root fresh mass (g)	Stem fresh mass (g)	Change in total fresh mass (g)
lodgepole pine	FA	21.50 ± 1.44	5.14 ± 0.16	0.22 ± 0.018	0.19 ± 0.013	0.39 ± 0.030
	4% RAR	17.81 ± 1.94	4.07 ± 0.21	0.048 ± 0.0044	0.040 ± 0.0035	0.069 ± 0.0054
Sitka spruce	FA	12.12 ± 0.99	3.64 ± 0.16	0.098 ± 0.024	0.080 ± 0.0076	0.16 ± 0.028
	4% RAR	11.32 ± 0.83	3.39 ± 0.22	0.028 ± 0.0026	0.039 ± 0.0065	0.052 ± 0.0082

GLM analysis indicated that there were no significant species or nutrient treatment differences in whole-plant N concentrations ((percent N ± SE) lodgepole pine, 4% RAR: 1.54 ± 0.065; FA: 1.64 ● 0.094; Sitka spruce 4% RAR: 1.57 ± 0.096; FA: 1.54 ± 0.083).

Nitrogen and H^+ flux relationships

Mean net H^+ fluxes were lower than N fluxes but the differences were only significant for NH_4^+ in lodgepole pine at Low N ($p = 0.021$). Mean ratios of both NO_3^- and NH_4^+ to H^+ for lodgepole pine at Low N were 1: -0.026, while at High N the mean ratio of NO_3^- : H^+ flux was 1:0.097, and of NH_4^+: H^+ flux was 1: 0.15. For Sitka spruce at Low N the mean ratio of NO_3^- : H^+ flux was 1:0.34, and of NH_4^+: H^+ flux was 1: 0.56. For Sitka spruce at High N the mean ratio of NO_3^- : H^+ flux was 1:0.239, and the mean ratio of NH_4^+: H^+ flux was 1: 0.14. Due to the high variability in flux relationships between H^+ and both N ions

(Figure 3.4), these ratios were significantly different from each other. The overall mean flux ratio of N: H^+ was 1:0.18. Fluxes were more commonly positive for both NH_4^+ and NO_3^- (indicating net influx), and concurrent H^+ influx was more commonly seen than was efflux.

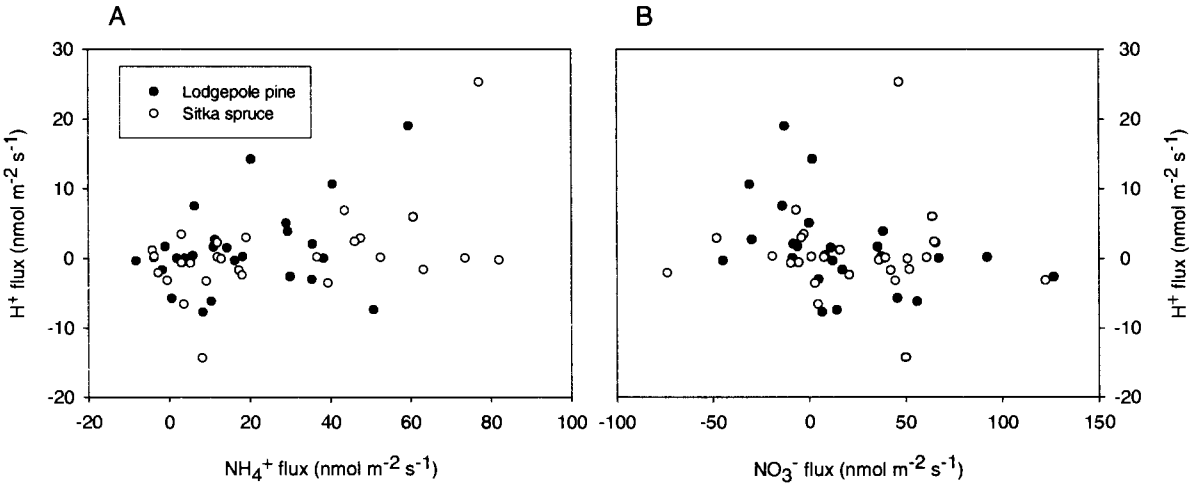


Figure 3.4. Proton fluxes in relation to **A.** NH_4^+ and **B.** NO_3^-.

DISCUSSION

Measurement of H^+, NH_4^+ and NO_3^- fluxes

The requirement that there is no convection, mixing or other bulk solution flow around the root is central to the theory of the MIFE measurements. Measurements of the net diffusive flux of the ions in solution close to the tissue give the net flux of the ions across the tissue surface only if ionic movement is solely by diffusion (Newman 2001). At the start of the measurement period, or after a change in solution it may take several minutes for steady, diffusion-limited conditions to be established in the region between the tissue and the electrode tips (Newman 2001). In my experiment, flux measurements generally

took from 3-5 min to stabilize. Initial values were not considered valid because the assumption of diffusion-limited conditions had been violated. Mean flux values were calculated over a period during which fluxes for all three ions were stable for at least 2 minutes (Figure 3.2, Ryan *et al.* 1992; Shabala and Newman 1998).

In a MIFE experiment directed at characterizing the NH_4^+ and NO_3^- uptake kinetics of *Eucalyptus nitens*, Garnett *et al.* (2003) measured ion fluxes across individual *E. nitens* seedling roots at 8 different N concentrations (0, 25, 50, 75, 100, 125, 150, and 250 uM) of NH_4^+ or NO_3^-, supplied as either $(HN_4)_2SO_4$ or $Ca(NO_3)_2$. Each solution change took 50 s and fluxes were measured immediately afterwards for 150 s before the next solution change. The authors claim that fluxes stabilized (diffusion limited conditions were reached) after 50 s, thus their flux values were averaged over the final 100 s at each concentration. Despite my similar experimental approach, I found that fluxes generally took several minutes to stabilize. I omitted the data from the first 100 s of each measurement for all flux calculations, contrary to Garnett *et al.* (2003) where 100 s made up the entire flux measurement at each N concentration. If the erratic initial fluxes at the beginning of each measurement period in my experiments were included, the scale of the y-axis would be so large as to cause all the data following this period to seem erroneously stable (see Appendix IV). Stable NH_4^+ and NO_3^- flux values covered an average range of 8 nmol m^{-2} s^{-1} (note that the scale of the left y-axis in Figure 3.2 is large, even with initial flux values excluded). This variation is of the same magnitude as that seen in Figure 1 of Garnett *et al.* (2003), although the y-axis increments make it less evident.

A common procedure used in the exploration of ion uptake kinetics is to expose seedling roots to increasing concentrations of a given ion and to use this data to create influx isotherms. Such data have been used to make statements about the affinity and saturation levels of various ion transport systems (e.g. Kronzucker *et al.* 1995b, 1996; Min *et al* 2000; Garnett *et al.* 2003). However, Britto and Kronzucker (2001) warn that ion influx measured when plant roots are perturbed by exposure to solutions of new ion concentration cannot be interpreted easily. Because influx isotherms frequently fit Michaelis-Menten kinetic patterns, it is often taken for granted that they can yield direct information about the kinetic properties of transport systems. When external ion

concentrations are changed, plant cells may undergo large and rapid changes in parameters such as membrane electrical potential and cytosolic pH (Ulrich *et al.* 1984; Ayling 1993; Wang *et al.* 1994; Kosegarten *et al.* 1997; Crawford and Glass 1998). These changes occur rapidly over a time scale from seconds to minutes and inevitably affect the thermodynamic conditions that influence ion fluxes across the plasma membrane (Britto and Kronzucker 2001). Furthermore, changes in membrane potentials and substrate gradients may also change the spatial configuration and substrate affinity of transport proteins (Britto and Kronzucker 2001). Thus, it cannot be assumed that patterns seen in influx isotherms result solely from a change in external solution concentration. By measuring the net H^+, NH_4^+ and NO_3^- fluxes of seedlings grown at FA or 4% RAR nutrient stress at their steady-state N ion concentrations I avoided the problems associated with rapid changes in external ion concentration used to create kinetic profiles of ion flux. The extreme variation in the fluxes of H^+, NH_4^+ and NO_3^- seen in the total data set (Table 3.2) likely points to the variety of cellular and membrane level changes that can occur as plant roots are exposed to different external ion concentrations.

Ion flux comparisons between species

Even when data were only included for seedlings measured at the N concentration in which they were grown, a high degree of flux variation remained. Because many conifers exhibit better growth on ammonium than nitrate (van den Dreissche 1971; Rice and Pancholy 1972; van Cleve *et al.* 1983; Chapin *et al.* 1986; Lavoie et al. 1992; Kronzucker *et al.* 1997; Britto *et al.* 2002; Kronzucker *et al.* 2003), I predicted that both lodgepole pine and Sitka spruce would show higher net fluxes of NH_4^+ than NO_3^-. Lodgepole pine, which is often associated with fire disturbed sites where NO_3^- is high (Brayshaw 1996), can have high NO_3^- uptake rates at high external NO_3^- concentrations (i.e. 1.5 mol m^{-3}) (Min *et al.* 1999), and the late successional species white spruce shows a clear preference for NH_4^+ over NO_3^- (Kronzucker *et al.* 1995a; Kronzucker *et al.* 1995b, c, d; 1996; 1997). Thus, I also predicted that lodgepole pine would show higher fluxes of NO_3^- relative to those seen for Sitka spruce. When measured at High N, the mean NH_4^+ flux was higher for Sitka spruce than lodgepole pine, and the mean NO_3^- flux was higher for lodgepole

pine than Sitka spruce, but not significantly so. The measurement techniques used in this study did not reveal *any* statistically significant species differences in ion fluxes.

While the lack of statistically significant flux patterns may be due to limitations of the MIFE technique (see below), it may also reflect cell level variability that is an inherent property of ion transport systems. In 1992, Henrikson *et al.* measured the general uptake patterns of NH_4^+ and NO_3^- uptake over the apical 50 mm of barley roots in unbuffered low salt medium with added NH_4^+ or NO_3^-. They found time variability and no particular flux trend for either ion across the measurement region. Plassard *et al.* (1999), in their report of H^+ flux profiles along the apical 90 mm of corn roots in 1 mM NH_4^+ or 4 mM NO_3^- at pH 6.5, also found high variability in fluxes between roots. Garnett *et al.* (2003) measured fluxes of H^+, NO_3^- and NH_4^+ across the sub-apical region (20-50 mm from the tip) of *Eucalyptus nitens* seedlings roots exposed to increasing N concentrations. While they fit their mean flux data to a Michaelis-Menten function, they found high levels of variation in NH_4^+ fluxes (see Garnett *et al.* 2003, Figure 2). Garnett *et al.* found that for NO_3^- measured in the presence of ammonium there were no significant differences in fluxes measured over the whole range of concentrations from 10-250 μM $Ca(NO_3)_2$. For NO_3^- measured in the absence of ammonium, only the fluxes measured at 10 μM $Ca(NO_3)_2$ were different from those at all other NO_3^- concentrations (see Garnett *et al.* 2003, Figure 3). H^+ fluxes measured simultaneously with each N ion showed a much variability, only differing significantly (if at all) in the first concentration shifts (see Garnett *et al.* 2003, Figures 4-6). In my study, N concentrations were higher than the range for which Garnett *et al.* (2003) found significant flux differences, and measured fluxes demonstrated a similarly high degree of variation.

The highly variable nature of ion transport systems in plant roots is further evident in Plassard *et al.*'s (2002) study of the NO_3^- and K^+ fluxes along ectomycorrhizal roots of *Pinus pinaster* (maritime pine). They found that local NO_3^- fluxes varied greatly in relation to root zone. NO_3^- uptake occurred mainly in the subapical zone of main roots and NO_3^- influxes decreased strongly when the root was brown. In their study of NH_4^+ and NO_3^- uptake in *Eucalyptus nitens*, however, Garnett *et al.* (2001) found little variation in NH_4^+, NO_3^- and H^+ fluxes outside the zone of elongation. Previous work in our lab, measuring NH_4^+ and H^+ fluxes across roots of white spruce seedlings, indicated

that fluxes at the growing tips of roots were considerably more variable than those in the sub-apical region. For this reason flux measurements were all localized to a root position at least 2 cm from the growing tip in a white zone from which laterals were starting to emerge. It is likely that the diverse patterns of flux data reported in the literature are, in part, due to differences in experimental design (intact vs. excised roots, soil vs. solution culture, mycorrhizal associations or a lack thereof) and plant species studied. Barley and eucalyptus may very well have N-uptake systems with very different characteristics than pine or spruce. It is also possible that the ion uptake characteristics vary so greatly over the surface of the roots of any individual plant, that ion flux measurements at one position do not reflect the uptake characteristics of the whole root. Given the complex nature of the rhizosphere and ability of plant roots to proliferate in nutrient rich soil patches (Coutts and Philipson 1976; Coutts and Philipson 1977; Chapin 1980; Friend *et al.* 1990, Fitter 1994; Hildebrand 1994; George *et al.* 1997), it seems unlikely that plant roots would have uniform ion transport along their length.

The consensus in the literature is that net NH_4^+ influx to roots is greater that the net influx of NO_3^- from equimolar solutions, and that the presence of NH_4^+ tends to inhibit the uptake of NO_3^- (Glass *et al.* 1985; Lee and Drew 1989; Ayling 1993; King *et al.* 1993; Aslam *et al.* 1994, 1996, 1997; Taylor and Bloom 1998; Kronzucker *et al.* 1999). Lee and Drew (1989) suggest that, depending on nutritional background, this effect will be exhibited by all plant species. In this study, both Sitka spruce and lodgepole pine show no significant differences in NO_3^- uptake relative to NH_4^+ uptake. While this may be due to the high individual seedling variation masking true species differences, these two species may take up both ions equally well at the measurement concentrations used in this study. Many of the above mentioned studies involved seedlings grown without NH_4^+ or subject to various levels of induction by NO_3^- prior to flux measurements. Different results may reflect variation in N transporter function, with high affinity and low affinity transport systems (HATS and LATS, respectively) up- or down-regulated to different degrees as a result of differing experimental treatments. For instance, Min *et al.* (2000) found that induced lodgepole pine seedlings, grown at 0.1 mol m^{-3} external NO_3^- concentration, had HATS V_{max} values for NO_3^- influxes that were 8-fold higher that those of non-induced plants (plants grown without NO_3^-). In contrast,

NH_4^+ influx of lodgepole pine was not inducible and both the V_{max} and K_m of HATS for NH_4^+ influx were similar between plants pre-treated with NH_4^+ and untreated plants (Min *et al.* 2000). Further, Min *et al.* (2000) found the LATS NO_3^- uptake capacity of lodgepole pine to be substantial, and they suggest that this may represent an important adaptation for colonizing sites that have high soil NO_3^- (note the high mean NO_3^- flux in Figure 3.2). Both the induction of NO_3^- HATS uptake and the high LATS capacity for NO_3^- may be part of the reason why, in this study, there are no clear differences between NO_3^- and NH_4^+ uptake in lodgepole pine.

Given the substantially higher influx of NH_4^+ compared to NO_3^- documented for white spruce (Kronzucker *et al.* 1996), the lack of ion preference seen here for Sitka spruce is harder to explain. In contrast to the cool, boreal soils where white spruce grows, the calcium rich soils in which Sitka spruce thrive can provide a relatively favourable medium for nitrification (Klinka *et al.* 1998). Sitka spruce is known to be an early pioneer on immature soils recently exposed by glacial retreat or uplift from the sea (Harris 1990). On highly disturbed sites it frequently establishes concurrently with *Alnus rubra* (red alder) or *Alnus sinuata* (Sitka alder) (Harris 1990), and in Oregon and Washington, spruce follows lodgepole pine in succession on coastal sand dunes as they become stabilized by vegetation. Its presence in both early and late successional stages of forest ecosystems may therefore be responsible for a lack of discrimination against NO_3^-.

Ion fluxes in relation to nutrient pre-treatment

This study did not reveal any within or between species differences in ion fluxes that could be related to the distinct differences in seedling size that resulted from growth at High vs. Low N (Tables 3.3 and 3.4). If there are no real differences in ion transport at the cellular level between Sitka spruce and lodgepole pine, the greater length and biomass of lodgepole pine seedling roots may translate into increased nutrient uptake rates at the whole root level.

The only statistically significant result evident from the data was that, regardless of species, seedlings grown at FA and measured at High N demonstrated higher NH_4^+ fluxes than seedlings grown at 4% RAR nutrient stress and measured at Low N. It is of

interest that NH_4^+ fluxes were the least variable of all three ions measured and this may reflect the well-documented preference that many conifers have for NH_4^+ as an N source. Kinetic analyses have revealed that influxes of NH_4^+ in lodgepole pine and white spruce are mediated by distinct high- and low- affinity transport systems. At external [NH_4^+] below 500 μM, saturable NH_4^+ influx has been shown to occur via HATS. At [NH_4^+] above 500 μM, influx occurs via LATS, following a linear pattern with increasing [N] and showing no indication of saturation even at external concentrations as high as 50 mM NH_4^+ (Kronzucker *et al.* 1996; Min *et al.* 2000). The reason for the NH_4^+ flux difference between 4% RAR and FA seedlings may result from 4% RAR seedlings having uptake restricted to HATS, while FA seedlings utilized LATS.

Mycorrhizae

An important consideration in the context of MIFE experiments is whether or not the presence of mycorrhizae changes ion flux characteristics at the root surface. The extent of the extracellular mycelium varies much between ectomycorrhizal plant species, as does the thickness of the fungal sheath and its hydraulic resistance to solute flow (Marschner 1995). While Ashford *et al.* (1988) found that in *Eucalyptus* with the ectomycorrhizal fungus *Pisolithus tinctorius,* the fungal sheath may be more or less sealed and prevent an apoplastic route of solute and water flux to the root cortex, Behrmann and Heyser (1992) have shown that the fungal sheath does not restrict movement in the *Pinus sylvestris* - *Suillus bovimus* association. It is unlikely that N penetration to the surface of the root membrane would be prevented by the mycorrhizal associations of the two coniferous species used in this study (see Eltrop and Marschner 1996; Plassard *et al.* 2000; Constable *et al.* 2001). Ion fluxes measured at the surface of root cell membranes should reflect actual uptake characteristics of seedlings in the field.

Ion flux measurement solution

An aspect of the flux measurement method used in this study which may confound ion flux results is the composition of the measurement solution. Plants were grown in full nutrient solution (Table 2.2), but measurement solutions contained only NH_4NO_3. This

was done to minimize the possible interference of other ions on the ion selective microelectrodes and to simplify calibrations. Importantly, the N concentrations of both the growth and measurement solution were kept the same (4% RAR and Low N solution = 71.23 μM N, FA and High N solution = 712.37 μM N) and the conductivities of the two solutions were similar (4% RAR 12 μS, Low N 9 μS; FA 72 μS, High N 65 μS). In this way, the plants were measured at steady-state external N concentrations. As well, after they were removed from the Biotronic solutions, all plants were kept in containers of well-aerated (either High N or Low N) measurement solution for at least one hour prior to measurements. There were no flux differences between seedlings measured early in the day and those measured later, thus it can be assumed that this period was long enough for plants to reach steady state. The concern, then, is that the lack of the other ions present in the Biotronic unit growth solution may be altering the observed flux patterns. No researchers using microelectrode flux measurement systems have made comparisons of ion fluxes measured in full nutrient solution vs. simplified nutrient solutions. This is an important topic for future research.

Stoichiometry between N and H+ fluxes

Similar to Ryan *et al.* (1990) who found no relationship between H^+, Ca^{2+} and K^+ fluxes, and Glass and Siddiqi (1982) who found variable stoichiometry between H^+ and K^+ fluxes, there were no consistent relationships between N and H^+ fluxes across the roots of lodgepole pine and Sitka spruce seedlings (see Figure 3.3). Kochian *et al.* (1989), also using a microelectrode system and intact roots, found no correlation between K^+ and H^+ fluxes, while Behl and Raschke (1987) found a consistent ratio between K^+ and H^+ fluxes in excised barley roots over a range of K^+ concentrations. Garnett *et al.* (2003) found that proton fluxes were highly correlated with NH_4^+ and NO_3^- fluxes. The relationships between H^+ and the fluxes of other ions are complex and further work is needed to clarify flux relationships due both to uptake and to assimilation.

The influx of the negative NO_3^- ion into the negative plant cytosol, requires secondary active transport, and H^+ co-transport has been implicated in NO_3^- uptake (McClure *et al.* 1990; Plassard *et al.* 1999). Glass *et al.* (1992) related H^+ fluxes in barley

to root cell membrane potential in 0.2 mM $CaSO_4$, and to previously observed (Siddiq *et al.* 1990) kinetics of $^{13}NO_3^-$ uptake. They suggested that NO_3^- is taken up in both low and high affinity transport via the symport of two H^+ ions. More recent research supports this finding (Meharg and Blatt 1995; Miller and Smith 1996) and it is generally accepted that two H^+ ions move inward to facilitate the inward transport of one NO_3^- ion.

The transport of NH_4^+, on the other hand, is not as well resolved. NH_4^+ uniport has long been suggested because the low cytosolic NH_4^+ concentrations commonly found in root cells would make its transport into roots electrochemically downhill (Wang *et al.* 1993a; Glass *et al.* 1997; Britto *et al.* 2001a). However, whether or not NH_4^+ transport is electrochemically uphill or downhill is likely dependent upon previous ammonium nutrition (Wang *et al.* 1993a). Recent estimates of cytoplasmic NH_4^+ concentrations in the mM range suggest that NH_4^+ uptake may be energetically uphill, perhaps via symport with H^+ (Wang *et al.* 1993b; Kronzucker *et al.* 1995b; Britto *et al.* 2001). Using ion selective electrodes to simultaneously measure external N fluxes and cytoplasmic N concentrations could help answer some of these questions.

There are many possible explanations as to why this study did not detect any of the above-mentioned relationships between N and H^+ fluxes (and why the ion flux stoichiometries reported in the literature are so variable). One of these relates to the surface geometry of plant roots. The flux that emerges into solution from the root surface, has come not only from the membrane immediately adjacent to the electrodes but also from inner membranes and cell layers through the apoplast (Newman 2000). The relative contributions of these inner membrane fluxes to the observed flux have not been quantified. When Shabala and Newman (2000), measured protoplast fluxes they were smaller than the fluxes from the tissue from which the protoplasts had been derived. The observed flux from the tissue surface is likely greater than the flux through any single cell membrane.

Another reason for variable relationships between H^+ and the flux of other ions relates to the involvement of H^+ in many aspects of plant cell biochemistry. Internal pH is closely regulated by homeostatic mechanisms, including internal buffering and the transport of H^+ across the plasma membrane to maintain a slightly alkaline cytosolic pH (Marschner 1995). Apoplastic pH is also regulated because the action of enzymes and

processes such as wall loosening are pH dependent (Taiz and Zeiger 1997). The efflux of respiratory products and metabolically generated organic acids and their anions further contributes to the changing H^+ concentration of the wall and the external medium (Felle 1998; Peters and Felle 1999). Finally, H^+ exchanges with other ions in the cell wall's complex Donnan system whose strength itself is pH dependent (Ryan *et al.* 1992a; Metraux *et al.* 1980; Rayle and Cleland 1992). The effect of known buffers can be taken into account in flux calculations (Arif *et al.* 1995), but not the effect of unknown extrusion of unknown buffers through the plasma membrane. With all these complex interactions it is very difficult to control all the relevant variables, and it is not surprising that an externally measured H^+ flux, particularly over a short time interval, may be different from the plasma membrane H^+ flux. Shabala *et al.* (1998) measured fluxes simultaneously at opposite ends of 60 um-diameter protoplasts isolated from corn coleoptiles and deduced that the plasma membrane H^+ fluxes are a complex mosaic that changes with time (sometimes showing oscillations). This observation is further warning that stoichiometries involving H^+ at the transporter level may be masked when H^+ fluxes are viewed at the level of whole cells. Researchers should exercise caution in making inferences about uptake kinetics, ion flux relationships, and the mechanistic properties of transport proteins from measurements of net fluxes.

CONCLUSIONS

There was a high degree of individual tree variability in the simultaneously measured fluxes of NH_4^+, NO_3^- and H^+ across the roots of lodgepole pine and Sitka spruce seedlings using the MIFE system. No ion flux differences between lodgepole pine and Sitka spruce were evident and neither species showed a significant preference for NH_4^+ over NO_3^-, though NH_4^+ fluxes were the least variable of the three ions. The only statistically significant flux result was that, regardless of species, seedlings grown at FA demonstrated higher NH_4^+ fluxes than seedlings grown under 4% RAR nutrient stress. This may be due to the up-regulation of LATS in plants grown under high N conditions. Lodgepole pine and Sitka spruce may have relatively plastic nutrient transport

mechanisms, rendering previous N nutrition the most important determinant of ion flux characteristics.

While the MIFE system provides the unique opportunity to measure the simultaneous fluxes of N and H^+ I was unable to detect clear stoichiometries between H^+ and either NH_4^+ or NO_3^- and thus could shed no light on the potential uptake mechanisms (symport or uniport) of these ions.

It is important to note that the MIFE system as used here can only measure net flux and not unidirectional influx. Further work is needed to quantify the degree to which inner membranes contribute to the observed tissue-surface flux. Future work should also focus on investigating differences between fluxes measured at steady-state and non steady-state external nutrient concentrations. This will involve investigating the effects that ionically complex nutrient solutions have on the measurement properties of ion selective microelectrodes. Also, fluxes measured in complete solutions (i.e. those of chemical composition equal to culture solutions) should be compared to those in simplified nutrient solutions such as NH_4NO_3.

With the wide range of ionophores becoming available I see the MIFE system as being most effectively used in conjunction with other techniques in cell and molecular biology. For instance, progress in molecular genetics is allowing expression of transporters controlling nutrient acquisition in plant roots. Ion flux characteristics of *Arabidopsis* mutants with or without a particular transporter, for instance, could be compared using the MIFE to screen, identify or confirm the transporters function *in planta* (Newman 2000).

As Britto and Kronzucker (2001) suggest, measurements of steady-state ion fluxes are undoubtedly useful for comparative purposes in an ecophysiological context. With trends towards high production forestry, intensively managed plantations, and increasing concerns over environmental impacts of forest fertilization and atmospheric N deposition, it is important to better understand the N uptake of conifers. In concert with other experiments on the physiology of nutrient uptake, further, and more refined, MIFE experiments will improve our understanding of conifer nutrition and enable more informed, ecosystem-specific forest management practices.

OVERALL CONCLUSIONS

All multicellular organisms undergo changes in external shape (morphology) internal structure (anatomy) and process (metabolism) as they grow and develop (Niklas 1994). These changes appear to unfold in an organized manner during ontogeny and, without serious disturbance, tend to produce the proportions and characteristics of the species to which each individual belongs. The tendency is for growth and development to achieve species norms, yet the variation that exists among individuals indicates that, in addition to genetic variation, all levels of plant organization are affected by the physical environment. A major focus in plant ecology is how plant growth is altered by limited resources, and developmental constraints on phenotypic plasticity are often overlooked. Models that attempt to predict the ecological outcome of natural or anthropogenically induced environmental changes are often based on optimal partitioning theories (Sharpe and Rykiel 1991) that do not adequately incorporate developmental constraints (McConnaughay and Coleman 1999). A basic assumption of optimal partitioning is that plants can and do shift partitioning in response to the environment, and these shifts alter growth rates. If this assumption does not hold for the species of interest, or is limited to a brief period in plant development, then the predictions of such models need to be re-evaluated (McConnaughay and Coleman 1999). This study highlights the variability of tissue N relationships over time; however, many models that investigate possible N limitations when atmospheric CO_2 concentrations increase, assume that terrestrial plant C/N ratios are constant (Rastetter *et al.* 1991; Melillo *et al.* 1993; Hudson *et al.* 1994; Comins and McMurtrie 1993). Because tree C/N ratios can vary with plant size in addition to nutrient availability (Gifford 1994; Kirschbaum 1994; Lloyd and Farquar 1996), allometric carbon and nitrogen interrelationships need to be taken into account when attempting to link carbon and nitrogen cycles.

It is not yet clear how photosynthetic nitrogen use and whole-plant nitrogen use are related (Coleman 1993). Both lodgepole pine and Sitka spruce had higher wpN's, photosynthetic rates and growth rates in the FA as opposed to the 4% RAR nutrient treatments. I was unable, however, to tease apart the complex relationships between photosynthesis, leaf nitrogen concentrations and specific leaf area in such a way as to

identify how the species-specific physiological and morphological differences led to similar overall seedling growth rates. The growth of lodgepole pine seedlings was affected by the development of secondary needles. Studies investigating species differences in seedling response to nutrient supply should span the duration of secondary needle development in pine seedlings before making conclusions regarding overall seedling growth.

While the reductionist approach of isolating nutrients as an experimental variable allows one to comment on growth responses due to nutrients alone, it is not surprising that species differences do not reveal themselves to the extent that one might expect from the ecological characteristics of their native ranges. Nutritional adaptations of lodgepole pine and Sitka spruce are likely intricately related to variables such as soil moisture and temperature. If we care to predict species responses to changing N supply, interactions between these variables need to be investigated.

A preference for ammonium over nitrate as an inorganic nitrogen source has been observed in many coniferous tree species (Marschner *et al.* 1991; Peuke and Tischner 1991; Kronzucker *et al.* 1997; Malagoli *et al.* 2000) and has been attributed to a lower capacity of the NO_3^- transport system, due to the adaptation of these species to soil with low pH and higher NH_4^+ availability (Stadler and Gebauer 1992). My research indicates, however, that both lodgepole pine and Sitka can acquire both NH_4^+ and NO_3^- ions equally well at the concentrations used in this study. While, the high degree of variability in fluxes of NH_4^+, NO_3^- and H^+ seen in all MIFE measurements underlies the lack of significant differences, Sitka spruce and lodgepole pine may have similar nutrient uptake capacities. Both species are found in northern forest soils where NH_4^+ is the main form of available soil inorganic N. However, the calcium rich soils in which Sitka spruce thrive can provide a relatively favourable medium for nitrification (Klinka *et al.* 1998), and Sitka spruce is known to be an early pioneer on immature soils recently exposed by glacial retreat or uplift from the sea (Harris 1990). Lodgepole pine seedlings commonly regenerate after fire on sites with high soil NO_3^-. Therefore, both species may have efficient uptake mechanisms for both NH_4^+ and NO_3^-.

To my knowledge, this is the first time that comparisons of biomass allocation, morphology and physiology have been made between two coniferous tree species grown

at steady state nutrition, using allometric analyses. Growing plants at constant relative growth rates within Biotronic units eliminated the possibility that differences in biomass allocation within a nutrient treatment were only due to differences in growth rate, and allometric analyses accounted for allocational changes that resulted as a normal consequence of increasing plant size. Because nutrients were added exponentially, concurrent with plant growth, changes in relative growth rate and internal nutrient concentration during the experiments were minimized, making it possible to directly examine the effects of environmental variables on the morphology and physiology of lodgepole pine and Sitka spruce seedlings. By growing plants with steady-state nutrition, one can compare growth and nutrient allocation to multiple plant compartments over time. The wider application of this technique will aid researchers interested in addressing the physiological mechanisms that underlie the diverse nutritional adaptations of plants.

This is the first time the MIFE (microelectrode ion flux measurement) technique has been used to compare ion fluxes between two conifers subject to high and low N growth conditions. Further work is needed to establish standardized experimental protocols, to quantify the contribution of inner membranes to measured ion fluxes, and to investigate the differences between fluxes measured at steady-state and non steady-state external nutrient concentrations. Nonetheless, this technique is a promising new alternative for the characterization and comparison of plant nutrient flux. Importantly, the non-destructive nature of MIFE measurements means that plants can be measured multiple times and used in further chemical or molecular studies.

Currently, our limited understanding of the fundamental relationships between the dynamics of soil fertility, tree nutrition and tree growth undermines our ability to combine forestry production goals with environmental considerations. As research improves our understanding of the nutritional physiology of forest trees species, we will be better equipped to predict consequences of future change.

LITERATURE CITED

Aerts R, Berendse F, de Caluwe H, Schmitz M. 1990. Competition in healthland along an experimental gradient of nutrient availability. Okios 57: 310-318.

Albaugh TJ, Allen HL, Dougherty PM, Kress LW, King JS. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. For Sci. 44: 317-328.

Alexander RR, Edminster CB. 1980. Lodgepole pine management in the central Rocky Mountains. J. Forestry 78: 196-201.

Arif I, Newman IA, Keenlyside N. 1995. Proton flux measurements from tissues in buffered solution. Plant Cell Environ. 18: 1319-1324.

Ashford AE, Allaway WG, Peterson CA, Cairney JWG. 1988. Nutrient transfer and the fungus-root interface. Aust. J. Plant Physiol. 91: 1152-1156.

Aslam M, Huffaker RC, Rains W, Rao P. 1979. Influence of light and ambient carbon dioxide concentration on nitrate assimilation by intact barley seedlings. Plant Physiol. 63: 1205-1209.

Aslam M, Travis RL, Huffaker RC. 1994. Stimulation of nitrate and nitrite efflux by ammonium in barley (*Hordeum vulgare* L.) seedlings. Plant physiol. 106: 1293-1301.

Aslam M, Travis RL, Rains DW, Huffaker RC. 1996. Effect of ammonium on the regulation of nitrate and nitrite transport systems in roots of intact barley (*Hordeum vulgare* L.) seedlings. Planta 200: 58-63.

Aslam M, Travis RL, Rains DW, Huffaker RC. 1997. Differential effect of ammonium on the induction of nitrate and nitrate reductase activities in the roots of barley (*Hordeum vulgare* L.) seedlings. Physiol. Plant. 101: 612-619.

Ayling SM. 1993. The effect of ammonium ions on membrane potential and anion flux in roots of barley and tomato. Plant Cell Environ. 16: 297-303.

Ågren GI. 1985. Theory for growth of plants derived from the nitrogen productivity concept. Physiol. Plant. 64: 17-28.

Bassirirad H, Griffin KL, Reynolds JF, Strain BR. 1997. Changes in root NH_4^+ and NO_3^- absorption rates of loblolly and ponderosa pine in response to CO_2 enrichment. Plant Soil. 190: 1-9.

Bazzaz FA, Garbutt K, Reekie EG, Williams WE. 1989. Using growth analysis to interpret competition between a C_3 and a C_4 annual under ambient and elevated CO_2. Oecologia 79: 223-235.

Bazzaz FA, Maio SL. 1993. Successional status, seed size, and responses of tree seedlings to CO_2, light and nutrients. Ecology 74: 104-112.

Behl R, Raschke K. 1987. Close coupling between extrusion of protons and uptake of potassium by barley roots. Planta 172: 531-538.

Behrmann P, Heyser W. 1992. Apoplastic transport through the fungal sheath of *Pinus sylvestrus/Suillus bovimus* ectomycorrhizae. Bot. Acta. 105: 427-434.

Bernardo J. 1993. Determinants of maturation in animals. Trends Ecol. Evol. 8: 166-173.

Bijlsma RJ, Lambers H, Kooijman SALM. 2000. A dynamic whole-plant model of integrated metabolism of nitrogen and carbon. 1. Comparative ecological implications of ammonium-nitrate interactions. Plant Soil. 220: 49-69.

Billes G, Routher H, Bottner P. 1993. Modifications of the carbon and nitrogen allocations in the plant (*Triticum aestivium* L.) soil system in response to increased atmospheric CO_2 concentration. Plant Soil. 157: 215-225.

Blew RD, Parkinson D. 1993. Nitrification and denitrification in a white spruce forest in southwest Alberta, Canada. Can. J. For. Res. 23: 1715-1719.

Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants – an economic analogy. Ann. Rev. Ecol. Syst. 16: 363-392.

Boot RGA. 1990. The significance of size and morphology of root systems for nutrient acquisition and competition. *In* 'Variation in growth rate and productivity.' H Lambers, ML Cambridge, H Konings, TL Pons (eds.) pp. 299-311. SPB Academic Publishing, The Hague

Boot RGA, Mensink M. 1990. The effect of nitrogen supply on the size and morphology of root systems of perennial grasses from contrasting habitats. Plant Soil. 129: 291-299.

Bormann FH, Likens GE, Fisher DW, Pierce RS. 1968. Nutrient loss accelerated by clearcutting of a forest ecosystem. Science 159: 882-884.

Bowman WD, Connant RT. 1994. Shoot growth dynamics and photosynthetic response to increased nitrogen availability in alpine willow *Salix glauca.* Oecologia 97: 93-99.

Brayshaw TC. 1996. Trees and Shrubs of British Columbia. UBC Press, Vancouver.

Brenchley WE. 1916. The effect of concentration of the nutrient solution on the growth of barely and wheat in water cultures. Ann. Bot. 3: 77-91.

Britto DT, and Kronzucker HJ. 2001. Can unidirectional influx be measured in higher plants? A mathematical approach using parameters from efflux analysis. New Phytol. 150: 37-47.

Britto DT, Kronzucker HJ. 2002. NH_4^+ toxicity in higher plants: a critical review. J. Plant Physiol. 159: 567-584.

Brouwer R. 1962. Nutritive influences on the distribution of dry matter in the plant. Neth. J. Agric. Sci. 10: 399-408.

Brown JK. 1975. Fire cycles and community dynamics in lodgepole pine forests. *In* 'Proceedings, Symposium on Management of Lodgepole Pine Ecosystems.' pp. 429-456. October 9-11, 1973. Washington State University, Pullman.

Brown KR. 1991. Carbon dioxide enrichment accelerates the decline in nutrient status and relative growth rate of *Populus tremuloides* Michx. seedlings. Tree Physiol. 8: 161-173.

Brown KR, Thompson WA, Weetman GF. 1996a. Effects of N addition rates on the productivity of *Picea sitchensis*, *Thuja plicata* and *Tsuga heterophylla* seedlings. Trees 10: 189-197.

Burns IG, Walker RL, Moorby J. 1997. How do nutrients drive growth? Plant Soil. 196: 321-325.

Burntson GM, Farnsworth EJ, Bazzaz FA. 1995. Allocation, within and between organs, and the dynamics of root length changes in two birch species. Oecologia 101: 439-447.

Burton AJ, Pregitzer KS, Zogg GP, Zak DR. 1996. Latitudinal variation in sugar maple fine root respiration. Can. J. For. Res. 26: 1761-1768.

Burton AJ, Pregitzer KS, Ruess RW, Hendrick RL, Allen MF. 2002. Root respiration in North American forests: effects of nitrogen concentration and temperature across biomes. Oecologia 131: 559-568.

Caldwell MM. 1994. Exploiting nutrients in fertile soil microsites. *In* 'Exploitation of soil heterogeneity by plants: Ecophysiological processes above- and belowground.' MM Caldwell, RW Pearcy (eds.) pp. 325-347. Academic Press, San Deigo, USA.

Cannell MGR, Dewar RC. 1994. Carbon allocation in trees: a review of concepts for modelling. Adv. Ecol. Res. 25: 59-104.

Chalot M, Brun A. 1998. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. FEMS Microbiol. Rev. 22: 21-44.

Chapin III FS. 1980. The mineral nutrition of wild plants. Annu. Rev. Ecol. System. 11: 223-260.

Chapin III FS, Bloom AJ, Field CB, Waring RH. 1987. Plant responses to multiple environmental factors. BioScience 37: 49-57.

Chapin FS III, Moilanen L. Kielland K. 1993. Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature. 361: 150-153.

Chapin FS III, van Cleve K, Tyron PR. 1986. Relationship of ion absorption to growth rate in taiga trees. Oecologia 69: 238-242.

Chu CC, Coleman JS, Mooney HA. 1992. Examining the controls on the partitioning of biomass between roots and shoots: effects of elevated levels of CO_2 on growth and resource use of California coastal wild radish. Oecologia 89: 580-587.

Clarkson DT. 1985. Factors affecting mineral nutrient acquisition by plants. Annu. Rev. Plant. Physiol. 36: 77-115.

Cochran PH. 1969. Thermal properties and surface temperatures of seedbeds: a guide for foresters. USDA Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, OR.

Cochran PH. 1975. Response of pole-size lodgepole pine to fertilization. USDA Forest Service, Research Note PNW-247. Pacific Northwest Forest and Range Experiment Station, Portland, OR.

Coleman JS, McConnaughy KDM, Ackerly DD. 1994. Interpreting phenotypic variation in plants. Trends Ecol. Evol. 9: 187-191.

Coleman JS, McConnaughy KDM, Bazzaz FA. 1993. Elevated CO_2 and plant nitrogen use: is reduced tissue nitrogen size dependent? Oecologia 93: 195-200.

Coleman MD, Dickson RE, Isebrands JG. 1998. Growth and physiology of aspen supplied with different fertilizer addition rates. Physiol. Plant. 103: 513-526.

Colmer TD, and Bloom AJ. 1998. A comparison of NH_4^+ and NO_3^- net fluxes along roots of rice and maize. Plant Cell Environ. 21: 240-246.

Comins HN, McMurtrie RE. 1993. Long-term biotic response of nutrient limited forest ecosystems to CO_2-enrichment: equilibrium behaviour of integrated plant soil models. Ecol. App. 3: 661-681.

Conroy JP. 1992. Influence of elevated atmospheric CO_2 concentrations on plant nutrition. Aust. J. Bot. 40: 445-456.

Constable JVH, Bassirirad H, Lussenhop J, Zerihun A. 2001. Influence of elevated CO_2 and mycorrhizae on nitrogen acquisition: contrasting responses in *Pinus taeda* and *Liquidambar styraciflua*. New Phytol. 128: 629-637.

Corre WJ. 1983. Growth and morphogenesis of sun and shade plants. I. The influence of light intensity. Acta Bot. Neerland. 32: 49-62.

Crawford NM, Glass ADM. 1998. Molecular and physiological aspects of nitrate uptake in plants. Trends. Plant Sci. 3: 389-395.

Crick JC, Grime JP. 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology. New Phytol. 107: 403-414.

Cromer RN, and Jarvis PG. 1989. Allocation of dry matter in *Eucalyptus grandis* seedlings in response to nitrogen supply. Annales des Sciences Forestieres. 46 (supplement): 680-683.

Cromer RN, Jarvis PG. 1990. Growth and biomass partitioning in *Eucalyptus grandis* seedlings in response to nitrogen supply. Aus. J. Plant Physiol. 17: 503-515.

Curtis PS, Wang X. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form and physiology. Oecologia 113: 299-313.

Ceulemans R, Mousseau M. 1994. Effects of elevated atmospheric CO_2 on woody plants: a review. Tansley Review No.71. New Phytol. 125: 425-446.

Davidson RL. 1969. Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover. Ann. Bot. 33: 561-569.

Davidson RL. 1969. Effects of soil nutrients and moisture on root/shoot ratios in *Lolium perenne* L. and *Trifolium repens* L. Ann. Bot. 33: 571-577.

DeJong TM. 1983. CO_2 assimilation characteristics of five *Prunus* tree fruit species. Am. J. Soc. Hortic. Sci. 108: 303-307.

Dewar RC. 1993. A root-shoot partitioning model based on carbon-nitrogen-water interactions and Munch phloem flow. Funct. Ecol. 7: 356-368.

Dijk E, Eck N. 1995. Ammonium toxicity and nitrate response of axenically grown *Dactylorhiza incarnata* seedlings. New Phytol. 131: 361-367.

Doley D. 1975. Effect of shade on gas exchange and growth in seedlings of *Eucalyptus grandis* Hill ex Maiden. Aust. J. Plant. Physiol. 5: 723-738.

Eamus D, and Jarvis PG. 1989. The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. Adv. Ecol. Res. 19: 1-55.

Eltrop L, Marschner H. 1996. Growth and mineral nutrition of non-mycorrhizal Norway spruce (*Picea abies*) seedlings growth in semi-hydroponic sand-cultures. New Phytol. 110: 59-66.

Erickson RG, Michelini FJ. 1957. The plastochron index. Am. J. Bot. 44: 297-305.

Ericsson T. 1981. Growth and nutrition of three *Salix* clones in low conductivity solutions. Physiol. Plant. 52: 239-244.

Etter HM. 1969. Growth, metabolic components and drought survival of lodgepole pine seedlings at three nitrate levels. Can. J. Plant Sci. 49: 393-402.

Evans GC. 1972. The quantitative analysis of plant growth. Oxford: Blackwell Scientific Publications.

Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78: 9-19.

Evans JR, Seemann JR. 1989. The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences, and control. *In* 'Photosynthesis.' WR Briggs (ed.) pp. 183-205, Plant Biology, Vol 8. Alan R. Liss Inc., New York, USA.

Eviner VT, Chapin FS III. 1997. Plant-microbial interactions. Nature 385: 26-27.

Eyre FH. 1980. Forest cover types of the United States and Canada. Society of American Foresters, Washington, DC.

Farnsworth EJ, Ellison AM, Gong WK. 1996. Elevated CO_2 alters anatomy, physiology, growth and reproduction of red mangrove (*Rhizophora mangle* L.). Oecologia 108: 599-509.

Farrar JL. 1995. Trees in Canada. Fitzhenry and Whiteside Ltd., Markham, Canada.

Felle H.H. 1998. The apoplastic pH of the *Zea mays* root cortex as measured with pH-sensitive microelectrodes – aspects of regulation. J. Exp. Bot. 49:987-995.

Field C, Mooney HA. 1986. The photosynthesis-nitrogen relationship in wild plants. *In* 'The Economy of Plant Form and Function.' TJ Givinish (ed.) pp 25-55. Cambridge University Press, Cambridge, UK.

Fowells HA. 1965. Silvics of forest trees of the United States. U.S. Department of Agriculture, Agriculture Handbook 271. Washington, DC.

Freijsen AHJ, Otten H. 1984. The effect of nitrate concentration in flowing solution system on growth and nitrate uptake of two *Plantago* species. Plant Soil. 77: 159-169.

Garnett TP, Shabala SN, Smethurst PJ, Newman IA. 2001. Simultaneous measurement of ammonium, nitrate and proton fluxes along the length of eucalypt roots. Plant Soil. 236: 55-62.

Garnett TP, Shabala SN, Smethurst PJ, Newman IA. 2003. Kinetics of ammonium and nitrate uptake by eucalypt roots and associated proton fluxes measured using ion selective electrodes. Funct. Plant. Biol. 30: 1-12.

Garnier E. 1991. Resource capture, biomass allocation and growth in herbaceous plants. Tree 6: 126-131.

Gebaur RLE, Reynolds JF, Strain BR. 1996. Allometric relations and growth in *Pinus taeda*: the effect of elevated CO_2 and changing N availability. New Phytol. 134: 85-93.

Gedroc JJ, McConnaughay KDM, Coleman JS. 1996. Plasticity in root/shoot partitioning: optimal, ontogenetic, or both? Funct. Ecol. 10: 44-50.

George E, Seith B, Schaeffer C, Marschner H. 1999. Responses of *Picea*, *Pinus* and *Pseudotsuga* root to heterogeneous nutrient distribution in the soil. Tree Physiol. 17: 39-45.

Gifford RM. 1994. The global carbon cycle: a viewpoint on the missing sink. Aust. J. Plant Physiol. 21: 1-15.

Glass ADM, Siddiqi MY. 1982. Cation-stimulated hydrogen ion efflux by intact roots of barley (*Hordeum vulgare*). Plant Cell Environ. 5: 385-394.

Glass ADM, Thompson RG, Bordeleau L. 1985. Regulation of NO_3^- influx in barley. Studies using $^{13}NO_3^-$. Plant Physiol. 77: 397-381.

Glass ADM, Schaff JE, Kochian LV. 1992. Studies of the uptake of nitrate in barley. IV. Electrophysiology. Plant Physiol. 99: 456-463.

Gobert A, Plassard C. 2002. Differential NO_3^- -dependent patterns of NO_3^- uptake in *Pinus pinaster, Rhizopogon roseolus* and their ectomycorrhizal association. New Phytol. 154: 509-516.

Goodchild JA, Givan CV. 1990. Influence of ammonium and extracellular pH on the amino and organic acid contents of suspension culture cells of *Acer pseudoplatanus*. Physiol. Plant. 78: 29-37.

Gosz JR, White CS. 1986. Seasonal and annual variation in nitrogen mineralization and nitrification along and elevational gradient in New Mexico. Biogeochemistry 2: 281-297.

de Graff MCC, Bobbink R, Roelofs JGM, Verbeek PJM. 1998. Differential effects of ammonium and nitrate on three heathland species. Plant Ecol. 135: 185-196.

Gulmon SL, Chu CC. 1981. The effects of light and nitrogen on photosynthesis, leaf characteristics and dry matter allocation in the Chaparral shrub *Diplacus aurantiacus*. Oecologia 49: 207-212.

Gunn S, Farrar JF. 1999. Effects of a 4 °C increase in temperature on partitioning of leaf area and dry mass, root respiration and carbohydrates. Funct. Ecol. 13: 12-20.

Harris AS. 1978. Distribution, genetics, and silvical characteristics of Sitka spruce. *In* 'International Union of Forest Research Organizations Joint Meeting Working Parties.' pp. 95-122. Vol. 1. British Columbia Ministry of Forests, Victoria, BC.

Harris AS, Ruth RH. 1970. Sitka spruce: a bibliography with abstracts. USDA Forest Service, Research Paper PNW-105. Pacific Northwest Forest and Range Experiment Station, Portland, OR.

Harris AS. 1990. Picea sitchensis. *In* 'Silvics of North America.' RM. Burns and BH. Honkala (technical coordinators) pp. 260-267, Vol. 1. Agri. Handbook 654, USDA For. Serv., Washington, DC.

Hartvigsen G, McNaughton SJ. 1995. Tradeoff between height and relative growth rate in a dominant grass from the Serengeti ecosystem. Oecologia 102: 273-276.

Haynes RJ, Goh KM. 1978. Ammonium and nitrate nutrition of plants. Biol Rev. 53: 465-510.

Heilman PE, Xie FG. 1994. Effects of nitrogen fertilization on leaf area, light interception and productivity of short rotation *Populus trichocarpa* x *Populus deltoides* hybrids. Can. J. For. Res. 24: 166-173.

Hendricks JJ, Nadelhoffer KJ, Aber JD. 1993. Assessing the role of fine roots in carbon and nutrient cycling. Trends. Ecol. Evol. 8: 174-178.

Henrikson GH, Bloom AJ, Spanswick RM. 1990. Measurement of net fluxes of ammonium and nitrate at the surface of barley roots using ion-selective microelectrodes. Plant Physiol. 93: 271-280.

Henrikson GH, Raj Raman D, Walker LP, Spanswick RM. 1992. Measurement of net fluxes of ammonium and nitrate at the surface of barley roots using ion-selective microelectrodes. II. Patterns of uptake along the root axis and evaluation of the microelectrode flux estimation technique. Plant Physiol. 99: 734-747.

Hilbert DW. 1990. Optimization of plant root:shoot ratios and internal nitrogen concentrations. Ann. Bot. 66: 91-99.

Hirose T. 1987. A vegetative plant growth model: adaptive significance of phenotypic plasticity in matter partitioning. Funct. Ecol. 1: 195-202.

Hirose T, Werger MJA. 1987. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. Physiol. Plant. 70: 215-222.

Houghton JT, Meiro-Fillao LG, Callander BA, Harris N, Kattenberg A, Maskell K. 1996. Climate Change 1995 - the science of climate change. Second assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK.

Hudson RJM, Gherini SA, Goldstein RA. 1994. Modelling the global carbon cycle: nitrogen fertilization of the terrestrial biosphere and the 'missing' CO_2 sink. Global Biogeochem. Cycles. 8: 307-333.

Huges AC, Evans GC. 1962. Plant growth and the aerial environment. II. Effects of light intensity on *Impatiens parviflora*. New Phytol. 61: 154-174.

Hunt R. 1990. Basic growth analysis. Unwin Hyman, London.

Hutchings MJ, de Kroon H. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Adv. Ecol. Res. 159-238.

Ibrahim L, Proe MF, Cameron AD. 1997. Main effects of nitrogen supply and drought stress upon whole-plant carbon allocation in poplar. Can. J. For. Res. 27: 1413-1419.

Idso KE, Idso SB. 1994. Plant responses to atmospheric CO_2 enrichment in the face of environmental constraints: a review of the past 10 years. Agric. For. Meteorol. 69: 153-203.

Ibrahim L, Proe MF, Cameron AD. 1997. Main effects of nitrogen supply and drought stress upon whole-plant carbon allocation in poplar. Can. J. For. Res. 27: 1413-1419.

Ingestad T. 1982. Relative addition rate and external concentration: Driving variables used in plant nutrition research. Plant Cell Environ. 5: 443-453.

Ingestad T. 1991. Nutrition and growth of forest trees. Tappi J. Jan. 55-62.

Ingestad T, Kähr M. 1985. Nutrition and growth of coniferous seedlings at varied relative nitrogen addition rate. Physiol. Plant. 65: 109-116.

Ingestad T, Lund AB. 1979. Nitrogen stress in Birch seedlings. I. Growth technique and growth. Physiol. Plant. 45: 137-148.

Ingestad T, Lund AB. 1986. Theory and techniques for steady state mineral nutrition and growth of plants. Scand. J. For. Res. 1: 439-453.

Ingestad T, McDonald AJS. 1989. Interaction between nitrogen and photon flux density in birch seedlings at steady-state nutrition. Physiol. Plant. 77: 1-11.

Ingestad T, Ågren GI. 1988. Nutrient uptake and allocation at steady state nutrition. Physiol. Plant. 72: 450-459.

Ingestad T, Ågren GI. 1991. The influence of plant nutrition on biomass allocation. Ecol. Appl. 1: 168-174.

Ingestad T, Ågren GI. 1995. Plant nutrition and growth: Basic principles. Plant Soil. 168/189: 15-20.

Jackson RB, Sala OE, Field CB, Mooney HA. 1994. CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. Oecologia 98: 257-262.

Jasienski M, Bazzaz FA. 1999. The fallacy of ratios and the testability of models in biology. Okios 84: 321-326.

Jasper DA, Abbott LK, Robson AD. 1989. Soil disturbance reduced the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. New Phytol. 112 (1): 93-100.

Jia H, Ingestad T. 1984. Nutrient requirements and stress response of *Populus simonii* and *Paulownia tomentosa*. Physiol. Plant. 67: 117-124.

Johnson IR. 1985. A model of the partitioning of growth between the shoots and roots of vegetative plants. Ann. Bot. 55: 421-431.

Johnson IR, Thornley JHM. 1987. A model of root:shoot partitioning with optimal growth. Ann. Bot. 60: 133-142.

Johnstone WD. 1975. Variable stand density yields of natural lodgepole pine stands in Alberta. *In* 'Proceedings, Symposium on Management of Lodgepole Pine Ecosystems, October 9-11, 1973.' pp. 186-207. Washington State University, Pullman, WA, USA.

Kamminga-van Wijk C, Prins HBA.1993. The kinetics of NH_4^+ and NO_3^- uptake by Douglas-fir from single N solutions and from solutions containing both NH_4^+ and NO_3^-. Plant Soil. 151: 91-96.

Kattenberg A, Giorgi F, Grassl H, Meehl GA, Mitchell JFB, Stoufer RJ, Tokioka T, Weaver AJ, Wigley TML. 1996. Climate models - projections of future climate. *In* 'Climate Change 1995 - the science of climate change. Second assessment report of the intergovernmental panel on climate change.' JT Houghton, LG Meiro-Fillao, BA Callander, N Harris, A Kattenberg, K Maskell (eds.) pp. 285-358. Cambridge University Press, Cambridge, UK.

King JS, Albaugh TJ, Allen HL, Kress LW. 1999. Stand-level allometry in *Pinus taeda* as affected by irrigation and fertilization. Tree Physiol. 19: 769-778.

King JS, Thomas RB, Strain BR. 1996. Growth and carbon accumulation in root systems of pinus taeda and Pinus ponderosa seedlings as affected by varying CO_2, temperature and nitrogen. Tree Physiol. 16: 635-642.

Kinerson RS, Higginbothham KO, Chapman RC. 1974. The dynamics of foliage distribution within a forest canopy. J. Appl. Ecol. 11: 347-352.

Kirkby EA. 1968. Influence of ammonium and nitrate nutrition on the cation-anion balance and nitrogen and carbohydrate metabolism of white mustard plants grown in dilute nutrient solutions. Soil Sci. 105: 133-141.

Kirschbaum MUF. 1994. The sensitivity of C_3 photosynthesis to increasing CO_2 concentration: a theoretical analysis of its dependence on temperature and background CO_2 concentration. Plant Cell Environ. 17: 747-754.

Kirschbaum MUF, King DA, Commins HN, McMurtrie RE, Medlyn BE, Pongracic S, Murty D, Keith H, Raison RJ, Khanna PK and Sheriff DW. 1994. Modelling forest response to increasing CO_2 concentration under nutrient-limited conditions. Plant Cell Environ. 17: 1081-1099.

Klinka K, Worral J, Skoda L, Varga P, Krajina VJ. 1998. The distribution and synopsis of ecological and silvical characteristics of tree species of British Columbia forests. Scientia Silvica: extension series number 10. Forest Sciences Department, University of British Columbia, Vancouver, BC, Canada.

Kochian LV, Shaff JE, Lucas WJ. 1989. High affinity potassium uptake in maize roots: a lack of coupling with proton efflux. Plant Physiol. 91: 1202-1211.

Kochian LV, Shaff JE, Kuhtreiber WM, Jaffe LF, Lucas WJ. 1992. Use of an extracellular, ion-selective, vibrating microelectrode system for quantification of K^+, H^+ and Ca^{++} fluxes in maize roots and maize suspension cells. Planta 188: 601-610.

Körner C. 1994. Biomass fractionation in plants: a reconsideration of definitions based on plant functions. *In* 'A whole plant perspective on carbon and nitrogen interactions.' J Roy, E Garnier (eds.) pp 173-185. SPB Academic Publishing: The Hague.

Körner C, Renhardt U. 1987. Dry matter partitioning and root/leaf area ratios in herbaceous perennial plants with diverse altitudinal distribution. Oecologia 74: 411-418.

Kosegarten H, Grolig F, Wieneke J, Wilson G, Hoffman B. 1997. Differential ammonia-elicited changes of cytosolic pH in root hair cells of rice and maize as monitored by 2',7'-bis-(2-carboxyethly)-5 (and-6)-carboxyfluorescein-florescence ratio. Plant Physiol. 113: 451-461.

Krajina VJ. 1969. Ecology of forest trees in British Columbia. Ecol. Western North America. 2: 1-146.

Krajina VJ, Madoc-Jones S, Mellor G. 1973. Ammonium and nitrate in the nitrogen economy of some conifers growing in Douglas-fir communities of the Pacific North-West of America. Soil Biol. Bioch. 5: 143-147.

Kronzucker HJ, Britto DT, Davenport R, and Tester M. 2001. Ammonium toxicity and the real cost of transport. Trends Plant Sci. 6: 335-337.

Kronzucker HJ, Schjoerring JK, Erner Y, Kirk GJD, Siddiqi MY, Glass ADM. 1998. Dynaminc interactions between root NH4+ influx and long distance N translocation in rice: Insights into feedback processes. Plant Cell Physiol. 39: 1287-1293.

Kronzucker HJ, Siddiqi MY, Glass ADM, Britto DT. 2003. Root ammonium transport efficiency as a determinant in forest colonization patterns: an hypothesis. Physiol. Plant. 117: 164-170.

Kronzucker HJ, Siddiqi MY, and Glass ADM. 1995a. Compartmentation and flux characteristics of nitrate in spruce. Planta 196: 674-682.

Kronzucker HJ, Siddiqi MY, and Glass ADM. 1995b. Compartmentation and flux characteristics of ammonium in spruce. Planta 196: 691-698.

Kronzucker HJ, Siddiqi MY, Glass ADM. 1995c. Compartmentation and flux characteristics of nitrate in spruce. Planta 196: 674-682.

Kronzucker HJ, Siddiqi MY, Glass ADM. 1995d. Compartmentation and flux characteristics of ammonium in spruce. Planta 196: 691-698.

Kronzucker HJ, Siddiqi MY,Glass ADM. 1995e. Analysis of $^{13}NH_4^+$ efflux in spruce roots: a test case for phase identification in compartmental analysis. Plant Physiol. 109: 481-490.

Kronzucker HJ, Siddiqi MY, Glass ADM. 1996. Kinetics of NH_4^+ influx in spruce. Plant Physiol. 110: 773-779.

Kronzucker HJ, Siddiqi MY, and Glass ADM. 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385: 59-61.

Kronzucker HJ, Siddiqi MY, Glass ADM, Britto DT. 2003. Root ammonium transport efficiency as a determinant in forest colonization patterns: an hypothesis. Physiol. Plant. 117: 164-170.

Lambers H. 1985. Respiration in intact plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms. *In* 'Encyclopedia of plant physiology: new series. Vol. 18.' R Douce, DA Day (eds.) pp. 418-473. Springer-Verlag, Berlin, Germany.

Lambers H, Poorter H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Adv. Ecol. Res. 23: 187-261.

Larsson S, Wiren A, Lundgren L, Ericsson T. 1986. Effects of light and nutrient stress on leaf phenolic chemistry in Salix dasyclados and susceptibility to *Galerucella lineola* (Coleoptera). Okios 47: 205-210.

Lavoie N, Vezina LP, Margolis HA. 1992. Apsorption and assimilation of nitrate and ammonium ions by Jack pine seedlings. Tree Physiol. 11: 171-183.

Ledig FT, Bormann FH, Wenger KF. 1970. The distribution of dry matter growth between shoot and roots in loblolly pines. Bot. Gaz. 13: 349-359.

Ledig FT, Perry TO. 1965. Physiological genetics of the root-shoot ratio. Proc. Soc. Am. Foresters. pp. 39-43.

Ledig FT, Perry TO. 1966. Physiological genetic of the shoot-root ratio. *In* 'Proceedings of the society of American foresters, October 1965.' pp. 39-43. Society of American Foresters, Washington, DC, USA.

Levin SA, Mooney HA, Field CB. 1989. The dependence of plant root:shoot ratios on internal nitrogen concentration. Ann. Bot. 64: 71-76.

L'Hirondelle SJ, Jacobson JS, Lassoie JP. 1992. Acidic mist and nitrogen fertilization effects on growth nitrate reductase activity, gas exchange and frost hardiness of red spruce seedlings. New Phytol. 121: 611-622.

Li B, Allen HL, McKeand SC. 1991. Nitrogen and family effects on biomass allocation of loblolly pine seedlings. For. Sci. 37: 271-283.

Likens GE, Bormann FH, and Johnson NM. 1969. Nitrification: importance to nutrient losses from a cutover forested ecosystem. Science 163: 1205-1206.

Lloyd J, Farquhar GD. 1996. The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. Funct. Ecol. 10: 4-32.

Linder S, Rook DA. 1984. Effects of mineral nutrition on carbon dioxide exchange and partitioning of carbon in trees. *In* 'Nutrition of plantation forests.' GD Bowen and EKS Nambiar (eds.) pp. 211-236. Academic press, London.

Linder S, Troeng E. 1980. Photosynthesis and transpiration of 20-year-old Scots pine. Ecol. Bull. 44: 178-190.

Little, Freund, Spector. 1991. SAS systems for linear models. Third Edition. Chapter 6.5 p. 243- 246. SAS Institute Inc., Cary, NC, USA.

Lloyd J, Farquhar GD. 1996. The CO_2 dependence of photosynthesis, plant growth responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. Funct. Ecol. 10: 4-32.

Linder S, Rook DA. 1984. Effects of mineral nutrition on carbon dioxide exchange and partitioning of carbon in trees. *In* 'Nutrition of plantation forests.' GD Bowen and EKS Nambiar (eds.) pp. 211-236. Academic press, London, UK.

Lodhi MAK. 1978. Inhibition of nitrifying bacteria, nitrification, and mineralization of spoil soils as related to their successional stages. Bull. Torry Bot. Club. 106: 284-289.

Lotan JE. 1976. Cone serotiny-fire relationships in lodgepole pine. *In* 'Proceedings, Fourteenth Annual Tall Timbers Fire Ecology Conference.' pp. 267-278. Tall Timbers Research Station, Tallahassee, FL, USA.

Lotan JE, Perry DA. 1983. Ecology and regeneration of lodgepole pine. U.S. Department of Agriculture, Agriculture Handbook 606. Washington, DC.

Luo Y, Field CB, Mooney HA. 1994. Predicting responses of photosynthesis and root fraction to elevated [CO2]: interactions among carbon, nitrogen and growth. Plant Cell Environ. 17: 1195-1204.

MacDuff JH, Jarvis SC, Larsson CM, Oscarson P. 1993. Plant growth in relation to the supply and uptake of NO_3^-: A comparison between relative addition rate and external concentration as driving variables. J. Exp. Bot. 44: 1475-1484.

Maier CA, Zarnoch SJ, Dougherty PM. 1998. Effects of temperature and tissue nitrogen on dormant season stem and branch maintenance respiration in a young loblolly pine (*Pinus teada*) plantation. Tree Physiol. 18: 11-20.

Malagoli M, Canal A, Quaggiotti S, Pegoraro P, Botacin A. 2000. Differences in nitrate and ammonium uptake between Scots pine and European larch. Plant Soil. 221 (1): 1-3.

Marschner H. 1995. Mineral Nutrition of Higher Plants, Second Edition. Academic Press. London. UK.

Marschner H, Häussling M, George E. 1991. Ammonium and nitrate uptake rates and rhizosphere pH in non-mycorrhizal roots of Norway spruce [*Picea abies* (L.) Karst]. Trees 5: 14-21.

Marschner H, Kirkby EA, Cakmak I. 1996. Effect of mineral nutrition status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. J. Exp. Bot. 47: 1255-1263.

McConnaughay KDM, Coleman JS. 1999. Biomass allocation in plants: Ontogeny or optimality? A test along three resource gradients. Ecology 80: 2581-2593.

McGilvray JM, Barnett JP. 1982. Relating seedling morphology to field performance of containerized southern pines. *In* 'Proc. Southern Containerized Forest Tree Seedling Conference.' RW Gudin, JP Barnett (eds.) pp. 39-46. Gen. Tech. Rep. SO-37 USDA Forest Service, Southern For. Exp. Sta.

Meharg AA, Blatt MR. 1995. NO_3^- transport across the plasma membrane of *Arabidospis thaliana* root hairs – kinetic control by pH and membrane voltage. J. Membrane Biol. 145: 4-66.

Melillo JM, McGuire AD, Kicklighter DW, Moore B III, Vorosmary CJ, Schloss AL. 1993. Global climate change and terrestrial net primary production. Nature 363: 234-240.

Metraux JP, Richmond P, Taiz L. 1980. Control of cell elongation in *Nitella* by endogeneous cell wall pH gradients. Multiaxial extensibility and growth studies. Plant Physiol. 65: 204-210.

Millard P. 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. Z. Pflanz. Bodenkunde. 159: 1-10.

Miller AJ, Smith SJ. 1996. Nitrate transport and compartmentation in cereal root cells. J. Exp. Bot. 47: 843-854.

Miller MH, McGonigle TP. 1992. Soil disturbance and the effectiveness of arbuscular mycorrhizas in an agricultural ecosystem. *In* 'Mycorrhizas in Ecosystems'. DJ Read, DH Lewis, AH Fitter and IJ Alexander (eds.) pp. 156-163. CAB International, Wallingford, UK.

Min XJ, Siddiqi MY, Guy RD, Glass ADM, Kronzucker HJ. 1998. Induction of nitrate uptake and nitrate reductase activity in trembling aspen and lodgepole pine. Plant Cell Environ. 21: 1039-1046.

Min XJ, Siddiqi MY, Glass ADM, Guy RD, Kronzucker HJ. 1999. A comparative study of fluxes and compartmentation of nitrate and ammonium in early-successional tree species. Plant Cell Environ. 22: 821-830.

Min XJ, Siddiqi MY, Guy RD, Glass ADM, Kronzucker HJ. 2000. A comparative kinetic analysis of nitrate and ammonium influx in two early-successional tree species of temperate and boreal forest ecosystems. Plant Cell Environ. 23: 321-328.

Mooney HA. 1972. Carbon balance of plants. Ann. Rev. Ecol. Syst. 3: 315-346.

Mooney HA, Winner WE. 1991. *In* 'Responses of plants to multiple stresses.' HA Mooney, WE Winner, EJ Pell (eds.) pp. 129-142. Academic Press, San Deigo, California, USA.

Morgan MA, Jackson WA. 1988a. Inward and outward movement of ammonium in root systems: transient responses during recovery from nitrogen deprivation in the presence of ammonium. J. Exp. Bot. 39: 179-191.

Morgan MA, Jackson WA. 1988b. Supression of ammonium uptake by nitrogen supply and its relief during nitrogen limitation. Physiol. Plant. 73: 38-45.

Murray MB, Leith ID, Jarvis PG. 1996. The effect of long term CO_2 enrichment on the growth, biomass partitioning and mineral nutrition of Sitka spuce (*Picea stichensis* (Bong.) Carr.). Trees 10: 393-402.

Murray MB, Smith RI, Friend A, Jarvis PG. 2000. Effect of [CO_2] and varying nutrient application rates on physiology and biomass accumulation of Sitka spruce (*Picea sitchensis*). Tree Physiol. 20: 421-434.

Mustard J, Hawkins C, Kooistra C. Secondary needles lodgepole pine: What do we get? *In:* 'Proceedings of the 1995, 1996 and 1997 annual meetings of the Forest Nursery Association of BC, Canada.' CM Kooistra (ed.)

Muzika RM, Pregitzer KS, Hanover JW. 1989. Changes in terpene production following nitrogen fertilization of grand fir [*Abies grandis* (Dougl.) Lindl.] seedlings. Oecologia 80: 485-489.

Newman IA, Kochian LV, Grusak MA, Lucas WJ. 1987. Fluxes of H+ and K+ in corn roots. Characterization and stoichiometries using ion-selective microelectrodes. Plant Physiol. 84: 1177-1184.

Newman IA. 2001. Ion transport in roots: measurements of fluxes using ion-selective microelectrodes to characterize transporter function. Plant Cell Environ. 24: 1-14.

Niklas KJ. 1994. Plant Allometry. The scaling of form and process. The University of Chicago Press, Chicago, USA.

Norby RJ, Gunderson CA, Wullschleger SD, O'Neill EG, McCracken MK. 1992. Productivity and compensatory responses o f yellow poplar trees in elevated CO_2. Nature. 354: 322-324.

Norby RJ, O'Niell EG, Wullschleger SD, Gunderson CA, Nietch CT. 1993. Growth enhancement of Quercus alba saplings by CO_2 enrichment under field conditions. Bull. Ecol. Soc. Am. 74 (Suppl.): 375.

Nye PH, Tinker PB. 1977. Solute movement in the soil root system. Blackwell, Oxford.

Obvington JD. 1957. Dry-matter production by *Pinus sylvestrus* L. Ann. Bot. 21: 287-314.

Omi SK, Eggleston KL, Marshall JD, Wenny DL. 1992. Primary vs. secondary needle development in lodgepole pine: update on current investigations from Coeur D'Alene nursery. pp. 35-42. Paper presented at the 12th Annual Meeting of the Forest Nursery Association of BC, September 28- October 1, 1992.

Olff H, Huisman J, van Tooren BF. 1993. Species dynamics and nutrient accumulation during early primary succession in coastal sand dunes. J. Ecol. 81: 693-706.

Olsen C. 1953. The significance of concentration on the rate of ion absorption by higher plants in water culture. III. The importance of stirring. Physiol. Plant. 6: 844-847.

Pace GM, Volk RJ, Jackson WA. 1990. Nitrate reduction in response to CO_2 limited photosynthesis. Plant Physiol. 92: 288-292.

Packard GC, Boardman TJ. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. Physiol. Zool. 61: 1-9.

Persson J, Nasholm T. 2001. Amino acid uptake: a widespread ability among boreal forest plants. Ecol. Lett. 4: 434-438.

Peters WS, Felle HH. 1999. The correlation profiles of surface pH and elongation growth in maize roots. Plant Physiol. 121: 905-912.

Pettersson R, McDonald AJS. 1994. Effects of nitrogen supply on acclimation of photosynthesis to elevated CO_2. Photosynth. Res. 39: 389-400.

Peuke AD, Tischner R. 1991. Nitrate uptake and utilization of aseptically cultivated spruce seedlings, *Picea abies* (L.) Karst. J. Exp. Bot. 42: 723-728.

Plassard C, Bonafos B, Touraine B. 2000. Differential effects of mineral and organic N sources, and of ectomycorrhizal infection by *Hebeloma cylindrosporum*, on growth and N utilization in *Pinus pinaster*. Plant Cell Environ. 23: 1195-1205.

Plassard C, Guérin-Laguette A, Véry AA, Casarin V, Thibaud JB. 2002. Local measurements of nitrate and potassium fluxes along the roots of maritime pine. Effects of ectomycorrhizal symbiosis. Plant Cell Environ. 25: 75-84.

Plassard C, Meslem M, Souche G, Jaillard B. 1999. Localization and quantification of net fluxes of H^+ along maize roots by combined use of videodensitometry and H^+ selective microelectrodes. Plant Soil. 211: 29-39.

Plassard C, Scheromm P, Mousain D, Salsac L. 1991. Assimilation of mineral nutrition and ion balance in the two partners of ectomycorrhizal symbiosis: Data and hypothesis. Experimentia 47: 340-349.

Perry DA, Lotan JE. 1977. Opening temperatures in serotinous cones of lodgepole pine. USDA Forest Service, Research Note INT-228. Intermountain Forest and Range Experiment Station, Ogden, UT.

Pfister RD, Daubenmire JR. 1975. Ecology of lodgepole pine (*Pinus contorta* Douglas). *In* 'Proceedings, Symposium on Management of Lodgepole Pine Ecosystems, October 9-11, 1973.' pp. 27-46. Washington State University, Pullman, WA, USA.

Poorter H, Evans JR. 1998. Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 116: 26-37.

Poorter H, Nagel O. 2000. The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Aust. J. Plant. Physiol. 27: 595-607.

Poorter H, Pot CS, Lambers H. 1988. The effect of elevated atmospheric CO_2 concentration on growth, photosynthesis and respiration of *Plantago major*. Physiol. Plant. 73: 553-559.

Poorter H, Pothmann P. 1992. Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on ontogeny. New Phytol. 120: 159-166.

Pregitzer KS, Laskowksi MJ, Burton AJ, Lessard VC, Zak DR. 1998. Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiol. 18: 665-670.

Proe MF, Millard P. 1994. Relationships between nutrient supply, nitrogen partitioning and growth in young Stika spruce (*Picea sitchensis*). Tree Physiol. 14: 75-88.

Rastetter EB, Ryan MG, Shaver GR, Melillo JM, Nadelhoffer KJ, Hobbie JE, Abet JE. 1991. A general biochemical model describing the response of the C and N cycles in terrestrial ecosystems to changes in CO_2, climate and N deposition. Tree Physiol. 9: 101-126.

Raven JA, Smith FA. 1976. Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol. 76: 415-431.

Raven JA, Wollenweber B, Handley LL. 1992. A comparison of ammonium and nitrate sources for photolithotrophs. New Phytol. 121: 19-32.

Rayle DL, Cleland RE. 1992. The acid growth theory of auxin-induced cell elongation is alive and well. Plant Physiol. 99: 1271-1274.

Read DJ. 1991. Mycorrhizas in ecosystems. Experimentia 47: 376-391.

Reiche PB, Oleksyn J, Tjoelker MG. 1996. Needle respiration and nitrogen concentration in Scots pine populations from a broad latitudinal range: a common garden test with field grown trees. Funct. Ecol. 10: 768-776.

Reiche PB, Schoettle AW. 1988. Role of phosphorous and nitrogen in photosynthetic and whole plant carbon gain and nutrient use efficiency in eastern white pine. Oecologia 77: 25-33.

Reich PB, Tjoelker MG, Walters MB, van der Klein D, Buschena C. 1998a. Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species growth in high and low light. Funct. Ecol. 12: 327-338.

Reich PB, Walter MB. 1994. Photosynthesis-nitrogen relations in Amazonian tree species. II. Variation in nitrogen vis-à-vis specific leaf area influences mass- and area-based expression. Oecologia 97: 73-81.

Reich PB, Walters MB, Ellsworth. 1997. From tropics to tundra: global convergence in plant functioning. Proc. Natl. Acad. Sci. 94: 13,730-13,734.

Reich PB, Walters MB, Tjoelker MG, van der Klein D, Buschena C. 1998b. Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. Funct. Ecol. 12: 395-405.

Reynolds HL, D'Antonio C. 1996. The ecological significance of plasticity in root weight ratio in response to nitrogen: opinion. Plant Soil. 185: 75-97.

Rice SA, Bazzaz FA. 1989. Quantification of plasticity of plant traits in response to light intensity: comparing phenotypes at a common weight. Oecologia 78: 502-507.

Rice EL, Pancholy SK. 1972. Inhibition of nitrification by climax ecosystems. Am. J. Bot. 59: 1033-1040.

Robertson GP. 1982. Nitrification in forested ecosystems. Phil. Trans. Royal Soc. London, Series B. Biol. Sci. 296: 445-457.

Robinson D. 1986. Compensatory changes in the partitioning of dry matter in relation to nitrogen uptake and optimal variations in growth. Ann. Bot. 58: 841-848.

Robinson D, Rorison IH. 1988. Plasticity in grass species in relation to nitrogen supply. Funct. Ecol. 2: 249-257.

Rook DA. 1991. Seedling development and physiology in relation to mineral nutrition. *In* 'Mineral nutrition of conifer seedlings.' van den Dreissche R (ed.) pp. 85-111. CRC Press, Boca Raton, USA.

Rosati A, Esparza G, DeJong TM, Pearcy RW. 1999. Influence of canopy light environment and nitrogen availability on leaf photosynthetic characteristics and photosynthetic nitrogen-use efficiency of field-grown nectarine trees. Plant. Physiol. 19: 173-180.

Rubinigg M, Stulen I, Elzenga JTM, Colmer TD. 2002. Spatial patterns of radial oxygen loss and nitrate net flux along adventitious roots of rice raised in aerated or stagnant solution. Funct. Plant Biol. 29: 1475-1481.

Runge M. 1983. Physiology and ecology of nitrogen nutrition. *In* 'Encyclopedia of Plant Physiology.' O Lange, PS Nobel, CB Osmond, H Ziegler (eds.) pp. 163-200. Vol. 12, Part C. Springer-Verlag, Berlin, Germany.

Ruth RH. 1965. Sitka spruce *(Picea sitchensis* (Bong.) Carr.). *In* 'Silvics of forest trees of the United States.' pp. 311-317. HA Fowells (comp.) U.S. Department of Agriculture, Agriculture Handbook 271. Washington, DC, USA.

Ruth RH, Harris AS. 1979. Management of western hemlock-Sitka spruce forests for timber production. USDA Forest Service, General Technical Report PNW-88. Pacific Northwest Forest and Range Experiment Station, Portland, OR.

Ryan MG. 1991. Effects of climate change on plant respiration. Ecol. Appl. 1: 57-167.

Ryan MG. 1995. Foliar maintenance respiration of sub-alpine and boreal trees and shrubs in relation to nitrogen content. Plant Cell Environ. 18: 765-772.

Ryan PR, Newman IA, Arif I. 1992a. Rapid calcium exchange for protons and potassium in cells walls of *Chara*. Plant Cell Environ. 15: 675-683.

Ryan PR, Newman IA, Shields B. 1990. Ion flux in corn roots measured by microelectrodes with ion-specific liquid membranes. J. Membrane Sci. 53: 59-69.

SAS Institute Inc. 1988. SAS/STAT Guide for personal computers, version 6 edition. SAS Institute Inc. Cary, NC, USA.

Satterlund DR. 1975. Climatic factors and lodgepole pine. *In* 'Proceedings, Symposium on Management of Lodgepole Pine Ecosystems, October 9-11, 1973.' pp. 297-309. Washington State University, Pullman, WA, USA.

Saxe H, Cannell MGR, Johnsen O, Ryan MG, Vourlitis G. 2001. Tree and forest functioning in response to global warming. New Phytol. 149: 369-400.

Scheromm P, Plassard C. 1988. Nitrogen nutrition of non-mycorrhized maritime pine (*Pinus pinaster*) grown on nitrate or ammonium. Plant Physiol. Biochem 26: 261-269.

Schmid B. 1992. Phenotypic variation in plants. Evol. Trends in Plants. 6: 45-60.

Shabala SN, Newman IA, Morris J. 1997. Oscillations in H^+ and Ca^{2+} ion fluxes around the elongation region of corn roots and effects of external pH. Plant Physiol. 113: 111-118.

Shabala SN, Newman IA, Whittington J, Juswono U. 1998. Protoplast ion fluxes: their measurement and variation with time, position and osmoticum. Planta 204: 146-152.

Shabala SN, Knowles A. 2002. Rhythmic patterns of nutrient acquisition by wheat roots. Funct. Plant. Biol. 29: 595-605.

Sharp PJH, Rykiel Jr. EJ. 1991. Modelling integrated responses of plants to stress. *In* 'Responses of plants to multiple stresses.' HA Mooney, WE Winner, EJ Pell (eds.) pp. 206-226. Academic Press, San Diego, California, USA.

Sheriff DW, Nambiar EKS, Fife DN. 1986. The effect of nitrogen content on the photosynthesis of Scots pine needles and shoots. Ann. Sci. For. 46: 473-475.

Shinano T, Osaki M, Kato M. 2001. Differences in nitrogen economy of temperate trees. Tree Physiol. 21: 617-624.

Siddiqi MY, Glass ADM, Ruth TJ. 1991. Studies of the uptake of nitrate in barley. III. Compartmentation of NO_3^-. J. Exp. Bot. 42: 1455-1463.

Sinclair TR. 1992. Mineral nutrition and plant growth response to climate change. J. Exp. Bot. 43: 1141-1146.

Smithers LA. 1961. Lodgepole pine in Alberta. Canadian Department of Forestry, Bulletin 127. Ottawa, ON. 153 p.

Smolander H, Oker-Blom P. 1989. The effect of nitrogen content on the photosynthesis of Scots pine needles and shoots. Ann. Sci. For. 46: 473-475.

Stadler J, Gebauer G. 1992. Nitrate reduction and nitrate content in ash trees (*Fraxinus excelsior* L.): Distribution between compartments, site comparison and seasonal variation. Trees 6 (4): 236-240.

Stark JM, Hart SC. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature 385: 61-64.

Strand M. 1997. Effect of mineral nutrient content on oxygen exchange and chlorophyll *a* fluorescence in needles of Norway spruce. Tree Physiol. 17: 221-230.

Szaniawski RK. 1987. Plant stress and homeostasis: a hypothesis. Plant Physiol. Biochem. 25: 63-72.

Tamm CO. 1964. Determination of nutrient requirements of forest stands. Int. Rev. For. Res. 1: 115-170.

Tamm CO. 1968. An attempt to assess the optimum nitrogen level in Norway spruce under field conditions. Stud. For. Seuc. 61: 1-67.

Taylor AR, Bloon AJ. 1998. Ammonium, nitrate, and proton fluxes along the maize root. Plant Cell Environ. 21: 1255-1263.

Taylor HM. 1974. Root behaviour as affected by soil structure and strength. *In* 'The plant root and its environment.' E.W. Carson (ed.) pp. 271-291. University Press of Charlottesville, VA, USA.

Terry N. 1968. Developmental physiology of sugar beet I. The influence of light and temperature on growth. Ann. Bot. 33: 419-430.

Teskey RO, Gholz HL, Cropper WPJ. 1994a. Influence of climate and fertilization on net photosynthesis of mature slash pine. Tree Physiol. 14: 1215-1227.

Teskey RO, Whitehead D, Linder S. 1994b. Photosynthesis and carbon gain by pines. Ecol. Bull. 43: 35-49.

Thompson S. 1976. Some observations on the shoot growth of pine seedlings. Can J. For. Res. 6: 341-347.

Thompson S. 1981. Shoot morphology and shoot growth potential in 1-year-old Scots pine seedlings. Can. J. For. Res. 11: 789-795.

Thornley JM. 1969. A model to describe the partitioning of photosynthate during vegetative plant growth. Ann. Bot. 33: 419-430.

Thornley JM. 1972. A balanced quantitative model for root:shoot ratios in vegetative plants. Ann. Bot. 36: 431-441.

Tilman D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, NJ, USA.

Tissue DT, Thomas RB, Strain BR. 1993. Long-term effects of elevated CO_2 and nutrients on photosynthesis and Rubisco in loblolly pine seedlings. Plant Cell Environ. 16: 859-865.

Tjoelker MG, Luxmore RJ. 1991. Soil nitrogen and chronic ozone stress influence physiology, growth and nutrient status of *Pinus taeda* L. and *Liriodendron tulpifera* L. seedlings. New Phytol. 119: 69-81.

Ullrich WR, Larsson M, Larsson CM, Lesch S, Novacky A. 1984. Ammonium uptake in *Lemna gibba* G1, related membrane potential changes, and inhibition of anion uptake. Physiol. Plant. 61: 369-376.

Ullrich WR, Novacky A. 1981. Nitrate-dependent membrane potential changes and their induction in *Lemna gibba* G1. Plant Sci. Let. 22: 211-217.

van Cleve K, Oliver L, Schlentner R, Viereck LA, Dryness CT. 1983. Productivity and nutrient cycling in taiga forest ecosystems. Can. J. For. Res. 13: 747-766.

van den Driessche R. 1971. Response of conifer seedlings to nitrate and ammonium sources of nitrogen. Plant Soil. 34: 421-439.

van Veen JA, Merckx R, van de Geijn. 1989. Plant and soil related controls of the flow of carbon from roots through the soil microbial biomass. Plant Soil. 115: 179-188.

van de Vijver CADM, Boot RGA, Poorter H, Lambers H. 1993. Phenotypic plasticity in response to nitrate supply of an inherently fast-growing species from a fertile habitat and an inherently slow-growing species from an infertile habitat. Oecologia 96: 548-554.

van der Werf A, Viser AJ, Schieving F, Lambers H. 1993. Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast- and a slow-growing species. Funct. Ecol. 7: 63-74.

Vapaavouri EM, Vuorinen EM, Aphalo PJ, Smolander. 1995. Relationship between net photosynthesis and nitrogen in Scots pine: seasonal variation in seedlings and shoots. Plant Soil. 169: 263-270.

Vincentz M, Moureaux T, Leydecker MT, Vaucheret H, Caboche M. 1993. Regulation of nitrate and nitrite reductase expression in *Nicotania plumbaginofolia* leaves by nitrogen and carbon metabolites. The Plant J. 3: 315-324.

Vitousek PM, Gosz JR, Grier C, Melillo JM, Reiners WA. 1982. A comparitive analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecol. Mon. 52: 155-177.

Wang MY, Siddiqi MY, Ruth TJ, Glass ADM. 1993. Ammonium uptake by rice roots. II. Kinetics of $^{13}NH_4^+$ influx across the plasmalemma. Plant Physiol. 103: 1259-1267.

Wilcox HE. 1991. Mycorrhizae. *In* 'Plant Roots: the Hidden Half.' Y Waisel, A Eshel and U. Kafkafi (eds.) pp. 731-765. Marcel Dekker, New York, USA.

Wilson JB. 1988. A review of evidence on the control of root:shoot ratio, in relation to models. Ann. Bot. 61: 433-449.

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL. 1993. Elevated atmospheric CO2 and feedback between carbon and nitrogen cycles. Plant Soil. 151: 105-117.

Zogg GP, Zak DR, Burton AJ, Pregitzer KS. 1996. Fine root respiration in northern hardwood forests in relation to temperature and nitrogen availability. Tree Physiol. 16: 719-725.

APPENDICES

APPENDIX I

Further comments on how CO_2 and temperature affect the efficiency of ribulose 1,5 bisphosphate carboxylase-oxygenase (RubisCO)

Since the concentration of O_2 in air is 21% and that of CO_2 only 0.035%, the velocity of the oxygenase reaction of RubisCO is very high (even though the CO_2 concentration required for the half saturation of the enzyme RubisCO ($Km[CO_2]$=9 μmol L^{-1}) is much lower than that of O_2 ($Km[O_2]$=535 μmol L^{-1})) (Woodrow and Berry 1988). For this reason, the ratio of oxygenation to carboxylation during photosynthesis of a leaf at 25°C is in the range of 1:4 and 1:2. Increased CO_2 will increase the carboxylation reaction. However, as temperatures rise, the CO_2/O_2 specificity of RubisCO decreases and, as a consequence, the ratio of oxygenation to carboxylation increases (Heldt 1999). Thus increased CO_2 benefits may be offset by temperature increases.

Literature Cited

Heldt HW. 1999. Plant biochemistry and molecular biology. Oxford University Press, New York, USA.

Woodrow IE, Berry JA. 1988. Enzymatic regulation of photosynthetic CO_2 fixation in C_3 plants. Annu. Rev. Plant Physiol. Molec. Biol. 39: 533-94.

APPENDIX II

Raw biomass data for lodgepole pine and Sitka spruce seedlings grown with free access to nutrients (FA) or under conditions of 4% relative addition rate nutrient stress (4% RAR) at harvests two and three. Values are means ± SE, n=24.

Nutrient Treatment	Species	Harvest	Total dry mass (g)	Leaf dry mass (g)	Stem dry mass (g)	Root dry mass (g)
FA	Lodgepole pine	2	0.117 ± 0.007	0.066 ± 0.004	0.011 ± 0.001	0.040 ± 0.003
		3	0.419 ± 0.016	0.229 ± 0.010	0.033 ± 0.001	0.157 ± 0.006
	Sitka spruce	2	0.083 ± 0.008	0.055 ± 0.005	0.010 ± 0.001	0.018 ± 0.002
		3	0.199 ± 0.031	0.135 ± 0.023	0.021 ± 0.003	0.044 ± 0.006
4% RAR	Lodgepole pine	2	0.197 ± 0.015	0.077 ± 0.005	0.012 ± 0.001	0.108 ± 0.045
		3	0.464 ± 0.051	0.198 ± 0.025	0.040 ± 0.005	0.226 ± 0.022
	Sitka Spruce	2	0.144 ± 0.010	0.070 ± 0.005	0.012 ± 0.001	0.062 ± 0.004
		3	0.543 ± 0.038	0.282 ± 0.020	0.049 ± 0.004	0.212 ± 0.015

APPENDIX III

Mean component N concentrations for lodgepole pine and Sitka spruce seedlings grown with free access to nutrients (FA) or under conditions of 4% relative addition rate (4% RAR) nutrient stress. There were significant harvest x species interactions in the overall model, thus means are given for harvests 2 and 3 within each nutrient treatment.

	FA, Harvest 2		FA, Harvest 3	
	(Mean N concentration (%) ± SE)		(Mean N concentration (%) ± SE)	
	Lodgepole pine	Sitka spruce	Lodgepole pine	Stika spruce
Leaf N	2.024 ± 0.060	2.028 ± 0.071	2.372 ± 0.058	2.182 ± 0.120
Stem N	2.246 ± 0.022	2.100 ± 0.034	2.067 ± 0.035	2.023 ± 0.013
Root N	2.503 ± 0.019	3.179 ± 0.036	2.230 ± 0.053	2.979 ± 0.017
Total N	2.120 ± 0.043	2.293 ± 0.057	2.318 ± 0.045	2.334 ± 0.086
	4% RAR, Harvest 2		4% RAR, Harvest 3	
	(Mean N concentration (%) ± SE)		(Mean N concentration (%) ± SE)	
	Lodgepole pine	Sitka spruce	Lodgepole pine	Stika spruce
Leaf N	1.364 ± 0.095	1.911 ± 0.081	1.931 ± 0.035	1.289 ± 0.032
Stem N	1.565 ± 0.078	1.636 ± 0.064	1.666 ± 0.051	1.148 ± 0.026
Root N	1.776 ± 0.073	2.063 ± 0.082	1.580 ± 0.042	1.539 ± 0.024
Total N	1.600 ± 0.064	1.49 ± 0.077	1.731 ± 0.033	1.370 ± 0.025

APPENDIX IV

Example of raw flux data

Net fluxes of H^+, NH_4^+ and NO_3^- measured at 5 s intervals across a Sitka spruce seedling root grown for 3 weeks with low N supply (71.23μM NH_4NO_3). Measurements started at 0 min, the solution was changed from 71.23μM NH_4NO_3 to 712.37 μM NH_4NO_3 at 12 min. Considerable noise in the electrode readings is evident from the initial measurement periods for both N concentrations. These values were omitted from final flux calculations. Positive values indicate influx of a given ion, negative values indicate efflux.