Effects of a changing environment on the aboveground and belowground systems of yellow birch (*Betula alleghaniensis* Britton) and sugar maple (*Acer saccharum* Marsh)

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A Thesis

In

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada

May 2004

Your file Votre référence
ISBN: 0-612-96939-8
Our file Notre référence
ISBN: 0-612-96939-8

Abstract

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The environment is constantly changing. A change in one environmental factor will cause changes in many other environmental factors either simultaneously or sequentially. For example, an increase in available light, due to canopy opening by natural disturbances, may increase soil temperature and moisture on the forest floor. Therefore, a changing environment has interactive effects on the trees. In forest ecological studies, the effects of some single environmental factors on tree growth are known, but there is a lack of knowledge of the interactions between multiple factors. An understanding of the interactive effects of abiotic and biotic factors on trees is critical for an understanding of the growth and survival of understory saplings in a complex changing environment. The aboveground and belowground systems of a tree respond differently to the same abiotic factors, such as light, since these systems grow in differing environments. This thesis will focus on the interactive effects among available light (including canopy gap size), tree size, artificial shading, liming and plant competition on the aboveground and belowground systems of yellow birch and sugar maple growing in the understory in two field experiments and the interactive effects of light, elevated CO_2 and mycorrhizae on seedlings of both species in a phytotron experiment. The three investigations will address several key questions concerning the growth of these two species and the development of their mycorrhizae in a complex changing environment in the present and future.

Acknowledgements

The thesis is solemnly dedicated to my respected father, the late Mr. Cheng, Lei who is still always in my mind and my dear mother Mrs. Zhang, Yuqing for their great love, inspiration and encouragement to me during my studies. Particularly, my father's indomitable spirit of enquiry will motivate me forever. Although he can not see the day when I graduate from the University, he would smile to me with his deep willing and emotional greetings in paradise at that time.

During the more than four-year study, I have contributed all my enthusiasm and most of time to the projects and obtained an unforgettable experience of education and professional training in many aspects. I would particularly like to thank the following:

Supervisor: Dr. Paul Widden of Biology Department at Concordia University for his warm encouragement, guidance, suggestions, enlightenment and English correction at all stages of the study and his reference books.

Co-supervisor: Dr. Christian Messier of Département des Sciences Biologiques, Université du Québec for his guidance, valuable comments and suggestions in my proposal and thesis, and his enthusiastic encouragement to me for challenging academic questions.

Committee: Dr. Selvadurai Dayanandan of Biology Department at Concordia University for his kind academic support, helpful suggestions in my proposal and thesis and his warmth in caring and discussing my phytotron experiment.

I would also like to thank Concordia University and NSERC for sponsoring my Ph. D. program in the first three years.

My heartfelt thanks to my wife and my daughter for their love, moral and financial support and encouragement for me to complete the study and to attend conferences, particularly from my sweet daughter Miss Cheng, Jing who has been awarded a four-year scholarship in a private upper school for her excellent academic ability.

Finally, I sincerely thank all people who helped me during my three experiments and the data analyses during these studies. These persons will be listed at the end of the relevant chapters.

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Contributions of authors

The three chapters from 2 to 4 in the thesis have been all prepared for journal articles. My supervisor and co-supervisor are the co-authors of all these papers. They both contributed their academic knowledge and valuable suggestions to the subjects in the normal supervisory role; also to English correction for these papers, especially from Dr. Paul Widden in the later contribution.

Chapter 1. General introduction

Forests are an important natural resource, providing multiple benefits, such as timber, water and recreation for human life (Thompson 1994). In forest management, the method of harvesting is important, not only for obtaining the wood, but also for the renewal and regeneration of the forest. However, forest stands are often economically mature before they are biologically mature, and thus, are often logged before they acquire the characteristics of a mature community (Thompson 1994). The harvesting of forest products, especially clear cutting, directly affects the local and global environment, which can cause varying degrees of soil compaction, slope instability and the loss of organic matter and nutrients. On a broader scale, forests are crucial in the cycling of air and water. Atmospheric CO_2 uptake by plants is reduced due to the clearing of large forests (Thompson 1994). Timber harvesting can affect the movement of water into and through the soil, leading on some sites to either water-logging or soil erosion. Water quality can be adversely affected in areas with wet soils, high rainfall or snow-melt, unstable slopes, or areas where soil water movements are easily disrupted (Thompson 1994). The removal of large forested areas by clear cutting reduces biodiversity and causes the replacement of one dominant species by another. For these reasons, selective cutting methods have been developed and used in the forestry community of eastern North America (Majcen and Richard 1993). These are designed to provide better conditions for understory tree growth through the removal of mature or unwanted trees (e.g. diseased trees) and minimise changes in the environment. The system removes only 20-40 % of the trees by systematically cutting trees, singly or in groups, throughout the stand every 15 to 20 years instead of the earlier method of clear cutting.

Yellow birch (*Betula alleghaniensis* Britton) and sugar maple (*Acer saccharum* March) are commercially important species that grow together in the temperate forests of eastern North America (Burns and Honkala 1990). Natural regeneration of the species mainly occurs through the canopy gaps opened by windthrow, tree mortality and wildfire (Runkle and Yetter 1987, Environment Canada 1988, Lorimer 1989,, Peterken 1996). Seedlings of both species germinate abundantly in the gaps (Majcen and Richard 1993). The gaps created by the cuts create a higher light environment, promoting the growth of the seedlings of yellow birch, which is a mid-shade-tolerant species (Barker 1949), while that of sugar maple, a typical shade-tolerant species (Canham 1989) which can survive at very low light levels, is relatively unaffected. However, many of the yellow birch seedlings established after selective cutting die before the next cutting cycle (i.e. in 15-20 years), while the sugar maple seedlings have a much higher survival rate (C. Messier, personal communication).

Light directly affects the aboveground system of a tree. In both species, low light causes an increase in specific leaf area, leaf area ratio and leaf mass ratio (Beaudet and Messier 1998, Walters and Reich 2000), thus relatively increasing the photosynthetic surface to more efficiently capture light energy in a light-limited environment. On the other hand, low light decreases height growth (Canham 1988 and 1989, Beaudet and Messier 1998), total biomass, stem biomass ratio and relative growth rate (Walters et al. 1993, Walter and Reich 1996, 2000) to reduce the requirement for carbohydrates.

Light also affects the roots of saplings. Fitter et al (1998) reported that solar radiation flux controls root growth and the input of carbon to the soil. Although sugar maple saplings can form substantial root systems at low light levels, the root growth decreases greatly at 2% of full sunlight (Webb 1976). In a temperate forest, low light causes a decrease in the fine

root biomass (Wilczynski and Pickett 1993), the number of new fine roots ≥ 1 cm in length per tree and new root length (Noland et al. 1997), but, there is an increase in the ratio of root biomass to total plant biomass (Walter and Reich 1996, 2000). High light increases root biomass and modifies root metabolism and function by increasing the root diameters (Kolek and Kozinika. 1992). The tree root is an important organ for the uptake of mineral nutrients from the soil and for the storage of carbohydrates assimilated by photosynthesis. The absorptive capacity of the root and root respiration are positively related to the number of tips and branches. Generally, as root diameter increases, the number of tips and branches and the respiration rate decrease (McCully 1990) and the absorptive root gradually transforms into a storage root (McCully 1990, Kolek and Kozinika 1992). There have been many studies on the response of the aboveground system of trees to light, but, due to the technical difficulties involved in studying root systems, the belowground development of the tree still remains relatively unknown.

The belowground growth of a tree is correlated with the aboveground growth. Improved light conditions are generally associated with increased leaf carbohydrates and an increased total carbon allocation to the roots. When height increases as the tree grows, the light availability is also increased, resulting in increased root size. Whereas many researchers have looked at the effects of tree height on the aboveground traits, few have examined the effects of tree height on the belowground parameters.

Light may influence the mycorrhizal system which aids the plant roots in the uptake of water and nutrients. Light has a positive effect on mycorrhizal colonization, fungal mycelium and spore production in the soil (Nakazato et al. 1999). Low light decreases mycorrhizal colonization in many ectomycorrhizal and arbuscular mycorrhizal plants

(Bethlenfalvay and Pacovsky 1983). Arbuscules are reduced by low light intensity although total vesicular-arbuscular mycorrhizae (VAM) colonization is unchanged in some crops (Franken and Gnadinger1994), while higher light induces a larger number of arbuscules in onion plant roots (Hayman 1974).

Canopy opening changes many abiotic and biotic factors in the soil. In the microenvironment of the forest floor, the small openings created by selective cutting not only minimize disturbance, but also increase the soil temperature and moisture from rain for the germination and growth of understory plants. These changes affect the growth and survival of understory saplings directly, for example, by increasing the photosynthetic rate (Pearcy and Smith 1994) and, indirectly, by enhancing mycorrhizal colonization, modifying microbial communities (Parke et al. 1984, Parsons et al. 1994), increasing fungal spore germination (Nakazato et al. 1999) and mineralization in the soil (Zak et al. 1993). In turn, nutrient and water uptake by the plants may be increased (Binkley and Richter 1986, Runkle and Yetter 1987, Staddon et al.1999).

Increasing soil nutrient availability increases plant growth. For plants, nutrients are important regulators of photosynthetic performance, growth and reproduction (Field and Mooney 1983). Greater nutrient availability in natural soils enhances net photosynthesis and stomatal conductance (Timmer et al. 1983). The addition of N (nitrogen) and P (phosphorus) can increase the stem diameter and volume of *Pinus radiata* by up to 130% (Sheriff et al. 1986). Optimum soil nutrients result in higher production of photosynthate, increasing the growth rate of the whole plant (Hunt et al. 1985) and the development of individual plant parts (e.g., roots) (Kozlowski et al. 1991). The availability of soil nutrients varies between forests (Timmer et al. 1983), causing different growth responses in different plant parts (Marschner

1995, Ericssion 1995). In nutrient-rich soils, shoot growth increases more rapidly than root growth, leading to a greater increase in shoot biomass than root biomass (Marschner 1995). In nutrient-poor soils, carbon allocation is increased to the roots. For example, annual root production in a low-fertility 40 - year - old Douglas-fir forest is 8,100 kg ha^{-1}, while it is only 4,100 kg ha^{-1} in a high-fertility site (Keyes and Grier 1981).

Liming ($CaCO_3$) affects the growth of tree roots by changing the soil chemical properties. The improvement of soil nutrients by the use of lime is an important silvicultural measure for improving tree regeneration. Lime has been applied to soil and it improves nutrient availability through reducing Al (aluminium) toxicity and increasing soil pH (Bakker 1998), thus, enhancing the growth and survival of seedlings in the understory. For example, the biomass of *Picea abies* roots is much lower in the humus at pH 3.3 than in the mineral soil at pH 4.1 (Nowotny et al. 1998). Al toxicity may also lead to a decrease in the branching and elongation of roots and in specific root length (Bakker 1999) and to an increase in fine root mortality (Helmisaari et al. 1999). Liming can reduce the Al toxicity (Bakker *el al.* 1999), but acts slowly (Bakker and Nys 1999). In acidic forest soils, liming increases the longevity (Majdi and Kangas 1997) and biomass of fine roots (Bakker 1999), total root length (Bakker *el al.* 1999) and growth rate (Hahn and Marschner 1998), and enhances the meristematic activity of the short root tips (Qian et al. 1998). However, Helmisaari and Hallbacken (1999) have reported contradictory results, suggesting that liming has a negative effect on fine roots. Helmisaari et al (1999) has stated that liming either increases (Hahn and Marschner 1998) or decreases (Helmisaari and Hallbaken 1999) the fine root biomass and growth of trees, depending on the availability of other nutrients (Helmisaari et al 1999), especially boron (Letho 1994).

Liming may also affect the mycorrhizal system. Ectomycorrhizae may be more sensitive to the soil environment than arbuscular mycorrhizae (Bakker and Nys 1999, Bakker et al. 2000). Liming greatly increases the total number of ectomycorrhizal tips in the root system of oak (Bakker et al. 2000), due to an increase in the pH value. Although a change in the soil pH from 4.5 to 7.5 by liming in some studies does not change the ectomycorrhizal colonization rate, the mycorrhizal community in the roots is changed (Abbott and Robson 1991). The change depends on the duration and amount of liming used. Long term and high doses (5,000 kg ha^{-1}) of lime cause changes in ectomycorrhizal communities (Anderson and Söderström 1995), but, at moderate doses (1.400 kg ha^{-1}), no changes in the communities occur (Karen and Nylun 1996). Plant competition from dense vegetation results in limitations to light aboveground and soil water and nutrients belowground for growth and mycorrhizal colonization of the saplings. Addition of fertilizer can release this competition. Mycorrhizal colonization may be effectively increased by liming when the resource limitation occurs as a result of competition. The effects of a low dose of lime (<1,000 kg ha^{-1}) on the growth of saplings and the development of arbuscular mycorrhizae are not well known.

Not only are the forests being subjected to changing management practices and natural disturbances, but it is also predicted that, due to human activities, the atmospheric CO_2 concentration will rise from 350 ppm today to 700 ppm by 2070 (Wegley 1999). Global climate changes resulting from increases in CO_2 and other greenhouse gases may comprehensively affect the regeneration of trees. Global climate changes will move the southern and northern boundaries of boreal forest 150 - 200 km northward (Stennes et al. 1998), expand the temperate forest area and gradually change the community composition

(Environment Canada 1988). In the temperate forested lands, vegetation density will progressively increase in the future (Environment Canada 1988).

Elevated CO_2 affects the growth and net photosynthesis of plants (Pospisilova and Catsky 1999). Plants grown in elevated CO_2 generally show an increase in biomass production (Curtis and Wang 1998). A doubling of the atmospheric CO_2 concentration increases tree biomass by about 40% (Lee and Jarvis 1996). Ecophysiological traits (e.g. photosynthesis, stomatal conductance, respiration, transpiration etc.) are important indicators that reflect the ability of a species to regenerate, survive and grow both directly and rapidly, when the environment changes. A doubling of CO_2 causes a 30%-40% reduction in stomatal conductance (g_s) in plants (Bowes 1993). The CO_2 concentration affects transpiration mainly through changes in g_s, causing a decrease in transpiration (Saralabai et al. 1997).

Elevated CO_2 affects ectomycorrhizae. Elevated CO_2 increases ectomycorrhizal colonization (Diaz et al 1993, Hodge 1996, Sadowsky and Schortemeyer 1997, Staddon and Fitter 1998), for example, in oak and yellow birch roots (Seegmuller and Rennenberg 1994, Berntson et al. 1997), and increases arbuscular mycorrhizal colonization in citrus trees (Koch and Johnson 1982), as more carbohydrates from the host-plant are available for the mycorrhizae (Tester 1986). In arbuscular mycorrhizae, elevated CO_2 increases the percentage of root length colonized (% RLC) for many plants (Monz 1994, Sanders 1996, Lovelock et al. 1996, Godbold et al. 1997) and mycorrhizal hyphal density in soil by up to 65% in *Plantago lanceolata* and over 200% in *Trifolium repens* (Staddon et al. 1999). However, the effects of elevated CO_2 on the intraradical structures of arbuscular mycorrhizae are poorly known (Rillig 1998, Staddon and Fitter 1998).

Environmental change involves changes in multiple environmental factors either simultaneously or sequentially. Those changes interactively affect the growth of understory saplings. However, the interactive effects of multiple variables, such as light and elevated CO_2, on many tree species, including yellow birch and sugar maple, have not been investigated (Pearcy and Smith 1994). An understanding of the responses of seedlings of these species to changing light and CO_2 are important to improve our knowledge of future forest regeneration. Yellow birch has a higher photosynthetic rate, stomatal conductance, respiration and transpiration rates than does sugar maple (Walter and Reich 2000). Thus, they both may behave very differently in the changed environment of the future.

The work described in this thesis was part of a larger study, designed to model the responses of yellow birch and sugar maple growing in the understory to the changing light regimes resulting from different sized gaps created by selective cutting and to the application of two common management practices (liming and the removal of competing vegetation) in northern mixed forests. This study is one of the first to examine the responses of both aboveground and belowground architecture and biomass allocation patterns in whole trees (saplings) to changing light regimes or management practices in a natural forest. The primary focus of this thesis is on the responses of the belowground systems of yellow birch and sugar maple, including their mycorrhizae, to these factors. In an attempt to understand how projected future changes in atmospheric CO_2 might change these respones, an experiment was also performed in the McGill Phytotron, to examine the interactive effects of elevated CO_2, light and mycorrhizal colonization on seedlings of these two species.

In this thesis, Chapters 2 and 3 will describe studies performed in the field and Chapter 4 will describe the Phytotron study. Chapter 2 will describe investigations on 1) the

effects of light and tree size on the belowground systems of the trees, 2) the comparative root architecture of the two species, 3) the dynamics of biomass allocation within the root system as the tree grows. Chapter 3 will principally investigate 1) the interactive effects of canopy gap size, liming and plant competition on the aboveground and belowground traits and mycorrhizae of both species, 2) the effects of a low dose of lime on these systems and on the mycorrhizae of both species in relation to the level of plant competition. Chapter 4 will investigate: 1) light thresholds for growth of the seedlings, 2) the effects of elevated CO_2 and mycorrhizae on these thresholds and, 3) the effects of elevated CO_2 on their mycorrhizae.

Chapter 2. Light, artificial shading and tree size influence belowground development in yellow birch and sugar maple

Abstract

The effects of light, artificial shading and tree size on the root architecture and mycorrhizae of yellow birch (*Betula alleghaniensis* Britton) and sugar maple (*Acer saccharum* Marsh) growing in the understory of deciduous forests in southern Québec, Canada were studied. At the study site, 50 small (<50 m^2), 37 medium (101-200 m^2) and 39 large (201-500 m^2) canopy gaps were investigated. Half of the gaps were covered by 8 m × 8 m shading cloths, reducing light by 50% for 4 growing seasons. From within these gaps, 43 yellow birch and 42 sugar maple saplings were sampled. In both species, root biomass and architectural traits were strongly correlated with tree size, but only weakly with light availability. Increased root biomass was primarily allocated to coarse roots and secondarily to fine roots. Yellow birch roots were longer, had a larger area, more endings and branches and grew more rapidly than sugar maple roots. Mycorrhizal colonization increased with available light and declined with tree age in sugar maple and was positively associated with tree size in yellow birch. Sudden artificial shading by 50% for the 4 years increased the ratio of root surface area to biomass in the small fine roots, of length to biomass in > 0.5-2.0 mm diameter roots and decreased that of root endings to biomass in >1.0-2.0 mm diameter roots. The study demonstrates that tree size is a very important determinant of how belowground systems acclimate to understory conditions.

Key words: Light, Mycorrhizal colonization, Roots, Sugar maple, Yellow birch.

Introduction

In most forests, about 70% of the sunlight is absorbed in the upper canopy layers and only 3-10% reaches the forest floor (Tang 1997). Few *in situ* studies have reported the effects of the light gradient on either the roots or, especially, the mycorrhizae of young trees. Light availability is however known to influence the belowground growth of understory saplings (Leverenz 1996). Fitter et al. (1998) reported that light controls the root growth of plants in grassland and that optimum root growth occurs at 45-50% of full sunlight. Improved light increases root biomass (Fitter et al. 1998) and new root length (Noland et al. 1997) but decreases the root biomass ratio (defined as total root biomass/total plant biomass) (Walters and Reich 1996, 2000).

A typical tree root system consists of roots of various diameters. Generally, the smaller roots have more tips and branches than the larger roots. The proportion of vascular and cortical tissues in roots decreases as the diameter decreases (McCully 1990). The changes in root architecture and anatomy associated with root development determine the ability of the roots to take up soil water and nutrients (McCully 1990, Kolek and Kozinika 1992). The aboveground tree size positively affects root development, sequentially increasing root diameters and modifying root metabolism and function (Kolek and Kozinika. 1992). How increased root biomass is allocated to the various sized roots of the taproot system of a tree and how it affects root traits in different sized roots are unknown.

Generally trees grow bigger with age. However, in some stressful environments, such as a very shaded understory, the tree size (height) is only weakly correlated with age. Recent studies have been concerned with the effects of tree size on changes in various tree traits. Messier and Nikinmmaa (2000) and Claveau et al. (2002) have shown that an increase

in tree size leads to important changes in numerous traits, which in turn affect growth and survival. For example, height growth cooperates with the canopy development in order to increase light capture. Tree size, therefore, has direct effects on the aboveground physiological and morphological traits of the tree (Walters et al. 1993, Lieffers et al. 1996 and Claveau et al. 2002). However, how tree size influences root biomass allocation, architectural traits and the development of mycorrhizae remains unknown.

Both yellow birch and sugar maple seedlings colonize gaps in the mixed forests of southern Québec, Canada. Yellow birch is an ectomycorrhizal (ECM) species that is more moderately shade-tolerant than the arbuscular mycorrhizal (AM) species, sugar maple. Generally, mycorrhizal colonization, mycelium and spore production in the soil are positively correlated with light (Bethlenfalvay and Pacovsky 1983). Increased light availability causes more photosynthate to be allocated to the roots (Fitter et al. 1998) for mycorrhizal formation. How this increased total root biomass affects mycorrhizal colonization is also unclear. As they age, some crop plants respond negatively to the AM association, as the resistance of roots to fungi may increase with age (Hayman 1974, Franken and Gnädinger 1994). There is, however, no information concerning this latter effect in trees.

This study, therefore, was undertaken to examine the responses of roots and mycorrhizae to changes in tree size and light created by selective cutting methods and by artificial shading treatments in both yellow birch and sugar maple saplings. The study attempted to test the hypotheses that: 1) both light and tree size affect root biomass allocation, root architecture and mycorrhizae; 2) the ECM mid-shade tolerant yellow birch is more affected by both light and tree size gradients and sudden increased shading than the

AM, shade tolerant sugar maple; 3) a sudden decrease in light availability in the understory more strongly affects the larger well established saplings that have spent many years adapting to a higher light availability than the smaller, younger ones.

Materials and methods

Study site

The study was conducted in the Duchesnay Experimental Forest Station 46°55' N, 71°40'W, near Québec City, Canada, located on a moderate slope at an elevation of 200 - 300 m. The soil was a moder with a humo-ferric podzol soil underlain by well-drained glacial till. The mean annual precipitation is 1200 mm and the mean daily temperature ranged from -13 to 28 °C from January to July (Environment Canada 1982). In the stands, the overstory was dominated by sugar maple (*Acer saccharum* Marsh.), beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula alleghaniensis* Britton) (60, 20 and 15% of merchantable volume, respectively) (Majcen and Richard 1993). American yew (*Taxus canadensis* Marsh), mooseberry (*Viburnum alnifolium* Marsh), and striped maple (*Acer pensylvanicum* L.) grew in the understory. Pin cherry (*Prunus pensylvanica* L. f.), red-berried elder (*Sambucus pubens* Michx.) and raspberry (*Rubus idaeus* L.) were sometimes found in canopy gaps.

Experimental design

The experiment was established in 1995. Selective cuts that were made in 1988-89, 1993-94 and 1994-95 were selected to provide us with a gradient of gap sizes (ranging from <50 to 500 m^2), sapling sizes and ages. The gaps were classified as either small (less than 50 m^2), medium (101-200 m^2) or large (201-500 m^2). A series of between 20 and 35 gaps for

each gap size were investigated throughout the study for 4 full growing seasons. Shading cloths (8 m × 8 m) that reduced the light by 50% were installed in half of the gaps from 1996 to 2000. This was done to test the acclimation of the saplings to a simulated rapid canopy gap closure. Within these gaps, 43 yellow birch and 42 sugar maple saplings were randomly selected for harvesting out of a population of around 600 individuals that were previously marked. The saplings that were sampled were scattered among the different gap sizes and the different shading treatments in the three sites.

Light measurements

The light available to the saplings was measured during 4 homogenously overcast days during the month of July 2000, using the method of Messier and Puttonen (1995); three instantaneous light measurements were taken 5 cm above the top of the saplings using a LI-189 light radiometer (LI-COR, Lincoln, Nebraska, USA). At the same time, a quantum sensor linked to a LI-1000 datalogger (LI-COR, Lincoln, Nebraska, USA) recorded light every minute in an adjacent open area. To calculate the percentage of total overstory PPFD (photosynthetic photon flux density) available, the values made above the trees were divided by the reference value taken at the same time in the open area. These punctual light measurements have been shown to be highly correlated with the mean seasonal daily percent PPFD as measured under overcast and clear sky (Gendron et al. 1998).

Root sampling

A total of 43 marked yellow birch and 42 sugar maple saplings, selected to represent the range of different canopy gap sizes, shading, tree size and age and relative light conditions, were sampled. The entire root system of each selected tree was very carefully excavated by hand to avoid disrupting the finest roots. We collected as much fine root

biomass for each tree as possible by cautiously digging out each root by hand. To do so, a team of 4 to 6 persons took up to 40 h to dig out the root system of one large 4 m tall sapling. Before each root system was fully harvested, 4 to 6 sub-samples of at least 20 cm length were removed and placed in a Ziploc bag then in a cooler with ice, to minimize water loss and root respiration. These sub-samples, used for mycorrhizal and architectural measurements of the small fine roots (≤2 mm in diameter), were delivered to the field lab. Mycorrhizal samples were taken randomly from the root sub-samples of each root system and the fresh weight was measured. The biomass of these sub-sub-samples was calculated from the ratio of the fresh weight of the other sub-sample to its dry weight.

Parameters investigated

The root system was divided into five diameter classes: 0.0-0.5, >0.5-1.0, >1.0-2.0 (subclasses of small fine roots), >2.0-5.0 (coarse fine roots) and >5 mm (coarse roots). For each diameter class, the following parameters were measured: 1) surface area (dm^2), 2) length (m), 3) number of forks, 4) number of endings (for fine roots, the endings were root tips, whereas for coarse roots, the endings were partially small fibrous roots), and 5) volume (mm^3). To describe the root architecture, the following ratios for each size class were calculated: area/biomass ($dm^2\ g^{-1}$), length/biomass ($m\ g^{-1}$), endings/biomass (no. g^{-1}) and branches/biomass (no. g^{-1}). Root branches were calculated as forks/biomass (no. g^{-1}). Total root biomass (including sub-samples and sub-sub-samples), tree height (m) and tree age (year) were also recorded. The mycorrhizal colonization (percentage of root tips colonized for the ECM of yellow birch and percentages of intramatrical hyphae, coils, vesicles and arbuscules as well as total colonization for the AM of sugar maple) was also assessed.

Measurements

Measurements for the small fine roots were made within three hours of excavation so that shrinkage due to water loss was minimized. A McRhizo system (Regent Instruments Inc., Québec City, Québec, Canada) was used to scan the fresh sub-samples from each tree and to measure the surface area, volume, length and numbers of endings, separately, for the 0.0-0.5 mm, >0.5-1.0 mm and >1.0-2.0 mm root diameter classes and the total number of forks in each class. After fully harvesting the whole root system of each tree, the roots were sorted into small fine roots, coarse fine roots and coarse roots within five hours, using a digital caliper. The total biomass of each of the three diameter classes was measured using a digital balance after drying in an oven at 70 °C for 48 h. Five dry samples of the mid-diameter roots in each size category were selected to measure the morphological parameters using the McRhizo system. Each of the parameters (P) (root surface area or length or endings) in the three classes was calculated as:

$$P = \left(\frac{TDW}{SDW}\right) \times VSP$$

Where: *TDW* is the total dry weight in that diameter class; *SDW* is the dry weight of the dry samples in the same class; *VSP* is the value of the parameter in the dry samples. For the 0.0-0.5, >0.5 -1.0 and >1.0-2.0 mm diameter classes, root surface area, length and endings were calculated using the percentages obtained from the fresh sub-samples. The root forks and biomass for the 3 small fine root diameter classes were calculated separately as:

$$F = \left(\frac{SRV}{TRV}\right) \times TRF$$

Where: *F* is the fork number or the biomass for each of the small fine root classes; *SRV* is the root volume of the fresh sub-sample in the same class of small fine roots; *TRV* is the total root volume of the fresh sample in the ≤ 2 mm diameter classes; *TRF* is the total number of root forks or the biomass for each of the three small fine root classes, as volume has a strongly linear relationship with biomass (Ozier-Lafontaine et al. 1999). To estimate the age of the tree, the rings on the basal disk of each tree were counted using a microscope.

Arbuscular mycorrhizal colonization

The samples from sugar maples for mycorrhizal analyses were fixed in FAA (formalin 50 ml, acetic acid 50 ml and 900 ml of 50% ethanol) for at least 24 h. The roots were autoclaved for 60 min in 10% KOH at 15 psi to remove the phenolics, rinsed with water, placed in 35 % Hydrogen Peroxide (H_2O_2) for 1 h, rinsed in water and acidified in 15% HCl for 15 min. They were then stained in a solution of 85% lactic acid, 99.5% glycerin and Chlorazol Black E (4:4:1 v:v:v) at 90 °C for 45 min. After de-staining in a solution of 99.5% glycerol and 85% lactic acid (1:1 v:v), the roots were mounted on slides and squashed with a cover slip. The AM structures were examined using a Nikon Optiphot Differential Interference Contrast (DIC) Microscope at a magnification of either 200X or 400X. The AM colonization for each root sample was obtained using the magnified grid intersect method (McGonigle et al. 1990). One hundred intersects were evaluated for each sample, and the presence or absence of intramatrical hyphae, arbuscules, vesicles and coils was noted at each. The colonization rate for each fungus structure was determined as the percentage of intersects at which it was present. Total colonization levels were obtained by counting all intersects that had at least one of the structures present.

Ectomycorrhizal colonization

The grid line intersect method (Goodman and Trofymow 1998) was used to measure the frequency of ECM in yellow birch. Roots were cut into pieces of ~0.5-1.0 cm and laid out in an INTEGRID™ Petri Dish (Becton Dickson Labware, Lincoln Park, NJ). The quantification of the ECM colonization was performed using a dissecting microscope. The mycorrhizal colonization from 50 to 100 pieces of each tree was recorded as the % of the total number of root tips colonized by ECM fungi.

Data analyses

Analysis of covariance (ANCOVA) (Fu 1979, Huitema 1980) was used to investigate the effects of species, shading cloths, available light, total root biomass (as a measure of tree size) and their interactions on the architectural properties and mycorrhizal colonization of the roots. In the analysis, available light and total root biomass were used as covariates, as they both usually have a significant effect on many traits of a tree (Messier and Nikinmmaa 2000, Claveau et al. 2002). Regression analyses were used to examine the relationships between dependent and independent variables. Multiple regressions using forward stepwise analyses were used to examine the relationships between total root biomass and mycorrhizal colonization and light, tree height and tree age, respectively. All variables were graphically examined for the normality of their distribution, using histograms, and for homogeneity of variance using scatter plots. If necessary, variables were transformed logarithmically. After transformation, all the data were tested and satisfied the assumptions for ANCOVA and regression analyses. To test for the effects of a simulated rapid gap closure, the roots of the plants grown under shading cloths were compared to those of the non-shaded plants grown

under a similar range of light conditions. SPSS (version 10) statistical software (SPSS Inc. Chicago, USA) was used to perform the analyses.

Results

a) Trees under natural shade

Effects of light, tree height and age on root biomass

Light, tree age and height had a positive, nonlinear relationship with the total root biomass of both species (Fig 1). Stepwise regression analysis (Table 1) indicated that tree height was the most important predictor of total root biomass for both species and that light was only a marginally significant ($P = 0.044$) predictor of root biomass for sugar maple. For a given height, yellow birch had less root biomass than sugar maples (Fig 1c).

Biomass allocation within the root system

In root systems up to a total biomass of ~60 g, the proportion of coarse root biomass increased with increasing total root biomass for both species, while that of small fine root biomass decreased (Fig. 2). Above 60 g, the proportions of coarse roots, small and coarse fine roots tended to remain constant as total root biomass increased in yellow birch, whereas, in sugar maple, the proportion of coarse roots was still gradually increasing (Fig. 2).

Effects of total root biomass, light and species on root traits

Both tree species and total root biomass (tree size) had a significant or marginally significant effect on many architectural traits of the root, whereas light generally did not (Table 2). The significant interaction of species × tree size (root biomass) for the root area/biomass ratios and the effect of total root biomass on branches/biomass ratios indicated that, for both species, these ratios declined with increasing root biomass, but the effect of the

root area over biomass ratio was different between the two species (Fig. 3). The ratios for all the root categories showed some significant or near significant effects for many treatments and behaved in a similar way for both species (data not shown).

Root surface area, total length and number of endings increased with root diameter and biomass (Fig. 4) in the small fine-root diameter category (from 0-0.5 to 1-2 mm classes), whereas they decreased from the coarse fine-root to the coarse root classes in both species as their diameter and biomass increased. However, the decline was gradual from the smallest to the largest class for the number of branches (Fig. 4d). In most cases, the values for yellow birch were greater than for sugar maple. There were some significant differences in root architecture between species (Table 2). The ratios of root surface area, length, endings and branches to root biomass for many root diameter classes of yellow birch were generally higher than those of sugar maple (Fig. 5). The largest difference between the two species was found in the ratio of the numbers of endings/biomass (Fig. 5c). This ratio was the highest for the small fine roots up to 1.0 mm, and then declined for the coarse fine roots and the coarse roots (Fig. 5c). There were generally large differences in the ratios between the small (0-2 mm) and coarse fine (>2-5 mm) roots, except for branches over biomass ratio where the main differences occurred between the very small fine roots (0-0.5 mm) and the rest (Fig. 5).

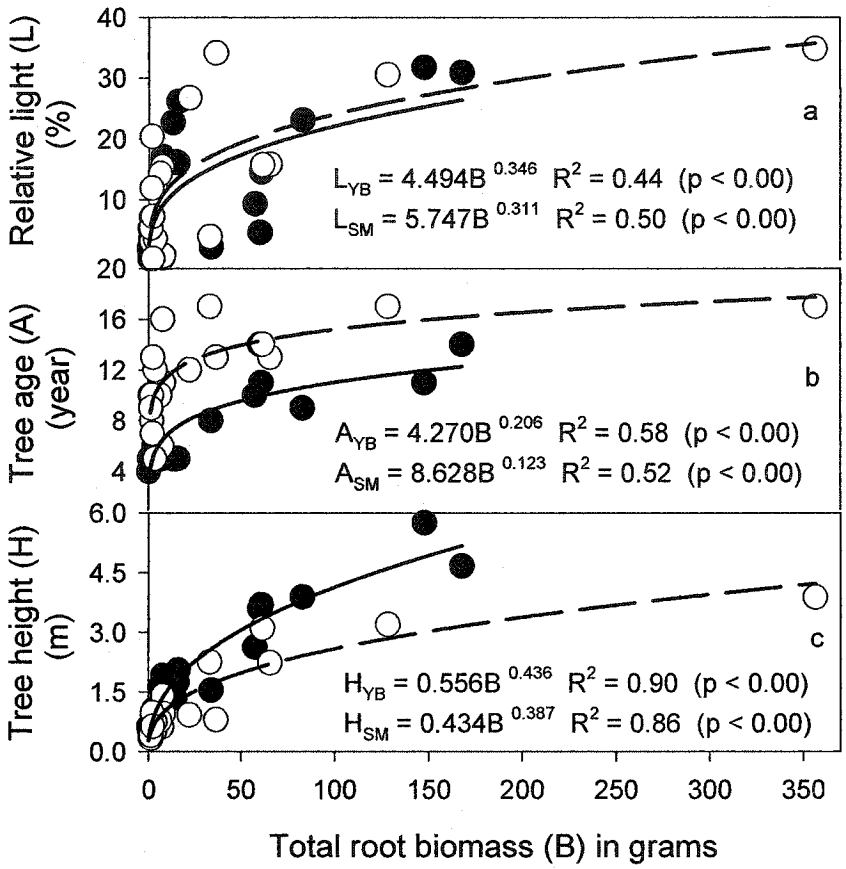
Mycorrhizal colonization in relation to light, tree size and age

Stepwise regression analysis showed that tree height had a significant, positive effect on mycorrhizal colonization in yellow birch, but the R^2 value was low (0.26) (Table 3; Fig. 6a). For sugar maple, light had a significant positive effect, whereas the relationship with

Table 1. Stepwise regression analysis for the effects of light (%), tree age (year) and height (m) on total root biomass (g) of yellow birch (YB) and sugar maple (SM) grown without shading cloths. ** = significant at 99%. The bolded numbers indicate a significant relationship.

Dependent variable	Independent variable		R^2
Total root biomass (YB)	= -30.773 + 31.235 (Height)		**0.86****
(Probability)	(0.000)		(0.00)
Total root biomass (SM)	= -52.282 + 49.940(Height) + 2.193 (Light)		**0.73****
(Probability)	(0.000)	(0.044)	(0.00)

Fig. 1. The relationships between total root biomass (g), the relative light (%), tree age (year) and height (m) in yellow birch (YB = ●, solid line) and sugar maple (SM = ○, dash line) grown without shading cloths. The number of YB and SM sampled were 17 and 21, respectively.


Relative light (L) (%)
40
30
20
10
20
a
$L_{YB} = 4.494B^{0.346}$ $R^2 = 0.44$ ($p < 0.00$)
$L_{SM} = 5.747B^{0.311}$ $R^2 = 0.50$ ($p < 0.00$)
Tree age (A) (year)
16
12
8
4
b
$A_{YB} = 4.270B^{0.206}$ $R^2 = 0.58$ ($p < 0.00$)
$A_{SM} = 8.628B^{0.123}$ $R^2 = 0.52$ ($p < 0.00$)
Tree height (H) (m)
6.0
4.5
3.0
1.5
0.0
c
$H_{YB} = 0.556B^{0.436}$ $R^2 = 0.90$ ($p < 0.00$)
$H_{SM} = 0.434B^{0.387}$ $R^2 = 0.86$ ($p < 0.00$)
0
50
100
150
200
250
300
350
Total root biomass (B) in grams

age was negative (Table 3; Fig. 6b). In sugar maple, intramatrical hyphae increased as light increased, while the arbuscules, coils and vesicles (of which very few were seen in this study) did not respond to light.

b) Effects of shading cloths

Overall, the shaded and non-shaded trees had grown under a similar range of light (from 0.4 to 11.4% for yellow birch, and 0.5 to 15.9% for sugar maple) for the 4 years previous to sampling. The overall patterns of change in the biomass allocation and root traits in relation to tree size and species were similar in both shaded and non-shaded trees in accordance with the data presented in Table 2 for the non-shaded trees. The effect of a sudden reduction in light availability by 50%, however, interacted with tree size to influence many architectural traits in the roots (Table 4). As there was no significant interaction between the presence of shading cloth and species (Table 4), data for both species have been combined to represent the effects of the shading cloths. In the shaded trees, the area/biomass ratio for all small fine roots and the length/biomass ratio for roots from >0.5 to 2.0 mm diameter significantly decreased with increasing total root biomass, while that of the non-shaded tree roots did not respond (Fig. 7).

Discussion

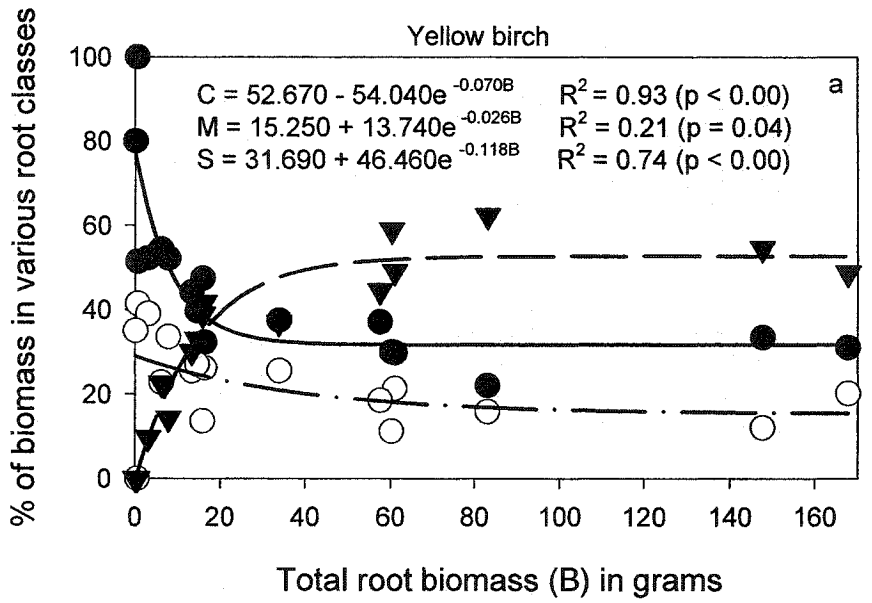
Effects of light and tree size on root traits

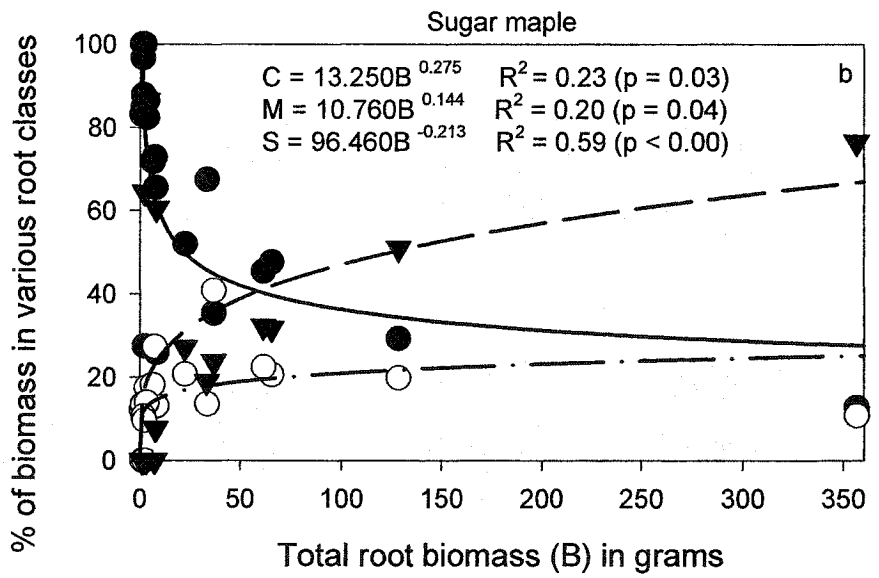
In both yellow birch and sugar maple, total root biomass was found to be the major determinant of root traits (Table 2). Root biomass is positively related to tree height, and to a lesser extent to light availability (Table 1). Tree size aboveground (height) affects the ability

Table 2. Analysis of covariance for the effects of tree species on the root surface area/biomass ($dm^2\ g^{-1}$), length/biomass ($m\ g^{-1}$), endings/biomass (no. g^{-1}) and branches/biomass (no. g^{-1}) ratios in the five root classes for trees grown without shading cloths. A light gradient between 0.3 and 35% of full sunlight and the total root biomass are the covariates. F is the mean square ratio. * = significant at 95%. ** = significant at 99%. The bolded numbers indicate a significant difference. Data for non-significant interactions (species × light, light × total root biomass and species × light × total root biomass) are not shown.

Source	Root diameter class (mm)	Area/Biomass F	Length/Biomass F	Endings/Biomass F	Branches/Biomass F
Species (S)	0.0-0.5	2.084	0.045	**18.43****	0.435
	>0.5-1.0	3.609	0.180	**22.84****	0.947
	>1.0-2.0	**4.319***	0.218	**25.15****	**6.138***
	>2.0-5.0	**4.592***	0.053	**8.402****	0.518
	>5.0	**15.98****	**14.81****	0.253	**5.343***
Light (L)	0.0-0.5	0.708	0.155	0.928	2.224
	>0.5-1.0	0.674	0.184	1.430	0.838
	>1.0-2.0	0.193	0.159	**7.878***	0.000
	>2.0-5.0	0.548	0.001	0.034	0.696
	>5.0	0.760	3.032	0.073	3.749
Total root biomass (B)	0.0-0.5	**5.380***	0.398	0.080	**46.99****
	>0.5-1.0	**8.435****	0.937	0.029	**62.82****
	>1.0-2.0	**6.649***	1.010	**6.426***	**181.6****
	>2.0-5.0	**6.406***	1.173	4.113	**63.89****
	>5.0	**15.49****	3.686	0.611	**19.79****
S × B	0.0-0.5	3.724	0.821	0.972	0.044
	>0.5-1.0	**5.254***	1.301	2.162	0.168
	>1.0-2.0	**5.472***	1.387	0.002	0.058
	>2.0-5.0	**5.676***	0.518	1.791	2.118
	>5.0	**12.54****	1.186	1.978	4.374

Fig. 2. Changes in proportions (%) of coarse root (= ▼, dash line), coarse fine root (= ○, dash-dot line) and small fine root (= ●, solid line) biomass (g) of yellow birch and sugar maple with increasing total biomass (g) of the roots grown without shading cloths. C = ▼, M = ○, S = ●.


Yellow birch
C = 52.670 - 54.040e -0.070B
M = 15.250 + 13.740e -0.026B
S = 31.690 + 46.460e -0.118B
R2 = 0.93 (p < 0.00)
R2 = 0.21 (p = 0.04)
R2 = 0.74 (p < 0.00)
a
% of biomass in various root classes
Total root biomass (B) in grams
0
20
40
60
80
100
120
140
160


Sugar maple
C = 13.250B 0.275
M = 10.760B 0.144
S = 96.460B -0.213
R2 = 0.23 (p = 0.03)
R2 = 0.20 (p = 0.04)
R2 = 0.59 (p < 0.00)
b
% of biomass in various root classes
Total root biomass (B) in grams
0
50
100
150
200
250
300
350

of the tree to survive in low light (Messier et al. 1999), as the size determines changes in many aboveground traits of the tree (Walters et al, 1993, Lieffers et al. 1996, Claveau et al. 2002, Delagrange et al. 2004) which enable it to capture more light (Claveau et al. 2002), and indirectly affects nutrient acquisition by its association with total root biomass. As the root develops, the main function of the accumulating biomass changes from traits associated with soil exploration and nutrient acquisition to building structural tissues for support and storage.

Light was not found to affect root architecture (Table 2). Although Noland et al. (1997) reported that light increases root length in jack pine seedlings, our data show that the length/biomass ratios of the various size classes of roots do not respond to light (Table 2), thus the increase in root length is directly proportional to the increase in root biomass as light increases. As reported elsewhere, it is possible that the reported relationship between light and many root traits is only indirect, as trees receiving more light are often found to be larger in size (Claveau et al. 2002 and Delagrange et al. 2004). Thus, light probably affects root architecture only indirectly through its effects on tree size. As the tree size increases, more carbohydrates are assimilated at the tree level and more reserves are available to the tree, resulting in increased carbohydrate transport to the root system (Ericsson et al. 1996). Light may also indirectly increase root biomass by increasing soil temperature, moisture and nutrients (Zak et al. 1993), by stimulating microbial activities (Nakazato et al. 1999) and by enhancing mycorrhizal colonization (Parke et al. 1984, Parsons et al. 1994, this study). However, DeBellis (2000) found no significant differences in the soil temperature, water, pH and nutrients under the different sized canopy gaps in an adjacent forest. Mycorrhizae affect the carbon allocation to roots in a complex fashion. Mycorrhizae increase the photosynthetic rate and stomatal conductance (Read 1999) by increasing the phosphorus and nitrogen

Fig. 3. Effects of species and total root biomass (g) on the root surface area/biomass ($dm^2\ g^{-1}$) and branches/biomass (no. g^{-1}) ratio in <1.0-2.0 mm diameter root class in yellow birch (YB = ●, solid line) and sugar maple (SM = ○, dash line) grown without shading cloths. These affected ratios show similar patterns in the other diameter classes.

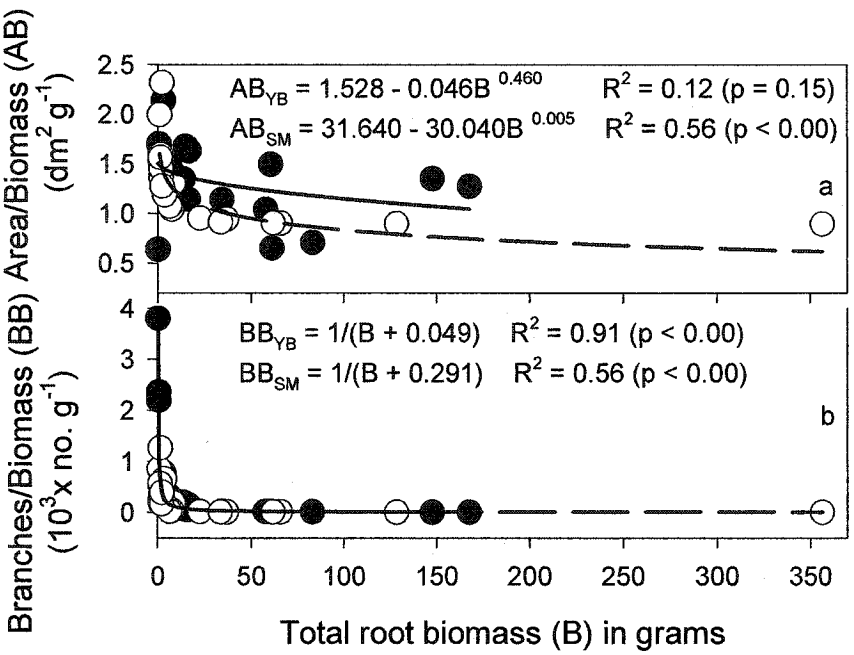

Branches/Biomass (BB) (10^3 x no. g^-1)
Area/Biomass (AB) (dm^2 g^-1)
AB_YB = 1.528 - 0.046B 0.460
R^2 = 0.12 (p = 0.15)
AB_SM = 31.640 - 30.040B 0.005
R^2 = 0.56 (p < 0.00)
a
BB_YB = 1/(B + 0.049)
R^2 = 0.91 (p < 0.00)
BB_SM = 1/(B + 0.291)
R^2 = 0.56 (p < 0.00)
b
Total root biomass (B) in grams

Fig. 4. Root surface area (dm^2), length (m), endings (no.), branches (no.) and biomass (g) in different sized roots of yellow birch (= ●, solid line) and sugar maple (= ○, dash line). Error bars present the standard error (SE) of the mean. Each plot is the mean of each of the root traits of 17 yellow birch or 21 sugar maples, respectively.

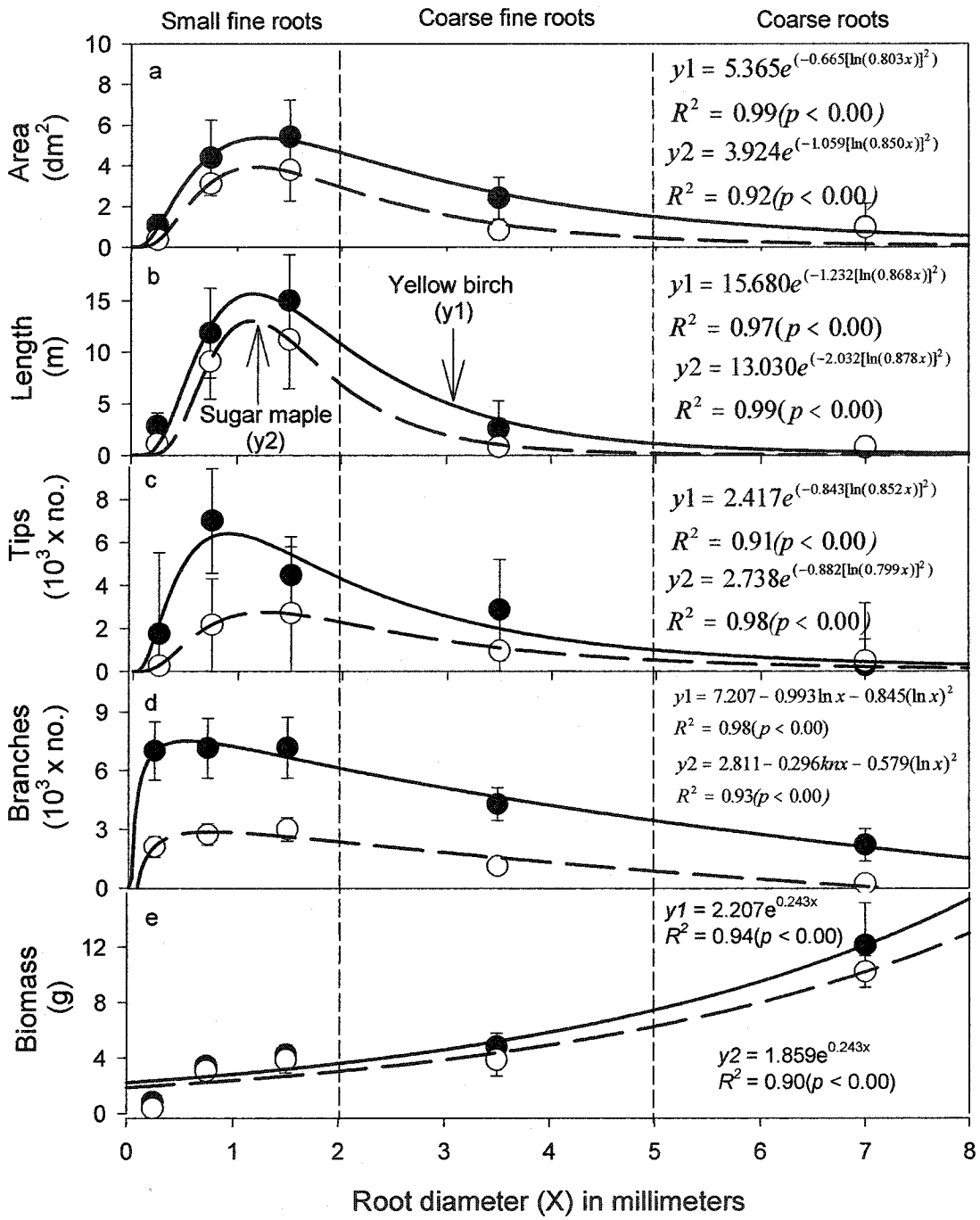

Small fine roots
Coarse fine roots
Coarse roots
a
Area (dm²)
y1 = 5.365e^(−0.665[ln(0.803x)]²)
R² = 0.99(p < 0.00)
y2 = 3.924e^(−1.059[ln(0.850x)]²)
R² = 0.92(p < 0.00)
b
Length (m)
Yellow birch (y1)
Sugar maple (y2)
y1 = 15.680e^(−1.232[ln(0.868x)]²)
R² = 0.97(p < 0.00)
y2 = 13.030e^(−2.032[ln(0.878x)]²)
R² = 0.99(p < 0.00)
c
Tips (10³ x no.)
y1 = 2.417e^(−0.843[ln(0.852x)]²)
R² = 0.91(p < 0.00)
y2 = 2.738e^(−0.882[ln(0.799x)]²)
R² = 0.98(p < 0.00)
d
Branches (10³ x no.)
y1 = 7.207 − 0.993ln x − 0.845(ln x)²
R² = 0.98(p < 0.00)
y2 = 2.811 − 0.296knx − 0.579(ln x)²
R² = 0.93(p < 0.00)
e
Biomass (g)
y1 = 2.207e^0.243x
R² = 0.94(p < 0.00)
y2 = 1.859e^0.243x
R² = 0.90(p < 0.00)
Root diameter (X) in millimeters

Fig. 5. Root surface area/biomass ($dm^2\ g^{-1}$) length/biomass ($m\ g^{-1}$), endings/biomass ($no.\ g^{-1}$), branches/biomass ($no.\ g^{-1}$) ratios and root biomass (g) in different sized roots of yellow birch (YB = ■) and sugar maple (SM = □) grown without shading cloths. Error bars present the standard error (SE) of the mean. Means with the same letter do not differ significantly from each other ($p > 0.05$).

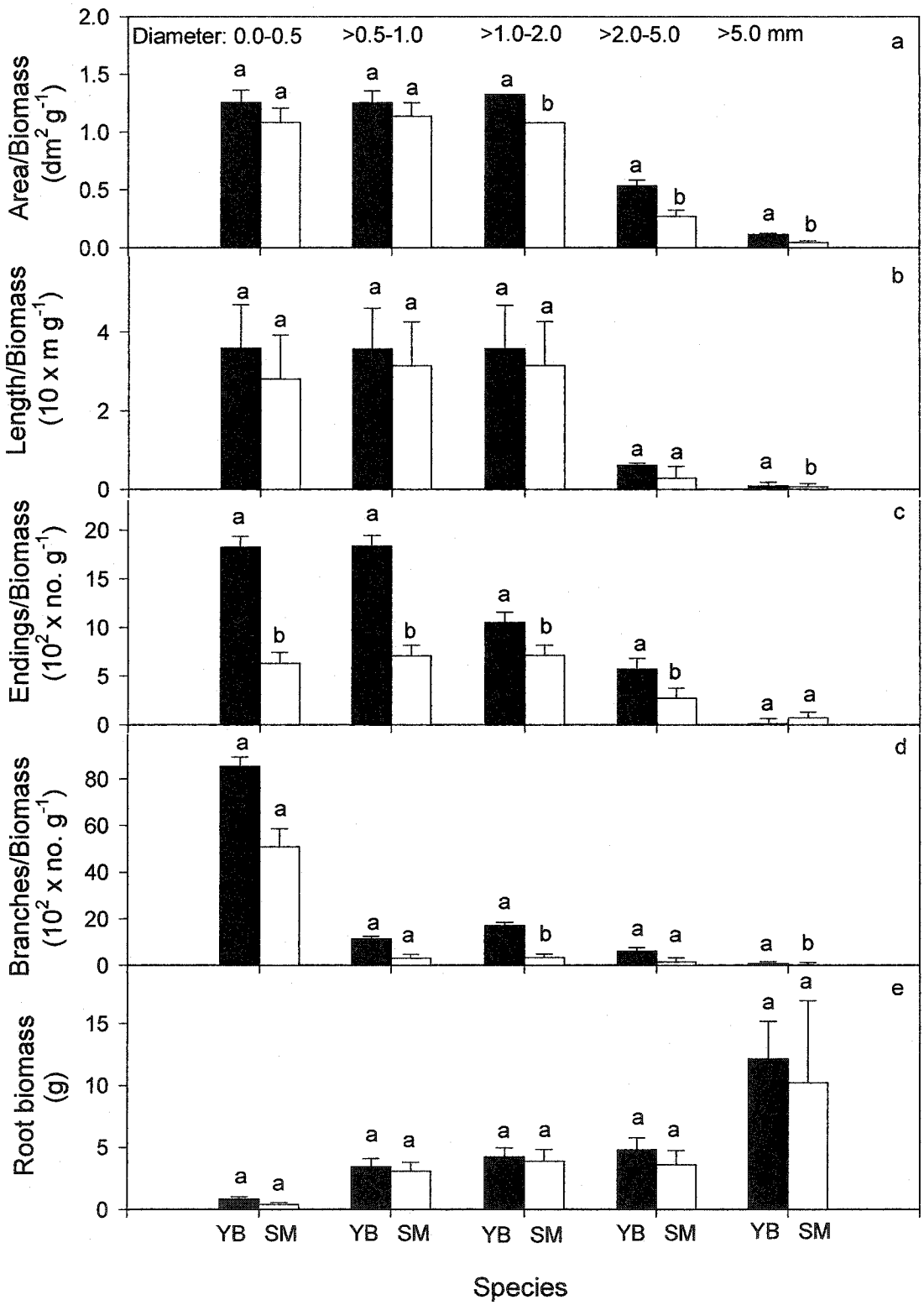

Diameter: 0.0-0.5
>0.5-1.0
>1.0-2.0
>2.0-5.0
>5.0 mm
a
b
c
d
e
Area/Biomass (dm^2 g^{-1})
Length/Biomass (10 x m g^{-1})
Endings/Biomass (10^2 x no. g^{-1})
Branches/Biomass (10^2 x no. g^{-1})
Root biomass (g)
YB SM
Species

Table 3. Stepwise regression analysis for the effects of light (%), tree age (year), height (m) and total root biomass (g) on ectomycorrhizal colonization (%) in yellow birch (YB) and arbuscular mycorrhizal (AM)colonization in sugar maple (SM) grown without shading cloths. * = significant at 95%, ** = significant at 99%. The bolded numbers indicate a significant relationship.

Dependant variable	Independent variable		R^2
Ectomycorrhizal colonization (YB)	= 67.506 + 3.279 (Height)		**0.26***
(Probability)	(0.037)		(0.04)
AM colonization (SM)	= 78.617 + 1.049 (Light) - 2.343 (Age)		**0.60****
(Probability)	(0.000)	(0.003)	(0.00)

Fig. 6. The relationships between mycorrhizal colonization (%) of small fine roots, tree height (m), light (%) and tree age (year) in yellow birch (YB = ●, solid line) and sugar maple (SM = ○, dash line in graph c) grown without shading cloths. In graph b, arbuscular mycorrhizalcolonization (Y) = ●, solid line; intramatrical hyphae (Y_1) = ○, dotted line; arbuscules (Y_2) = ▼, dash line; coils (Y_3) = ▽, dash-dot line.

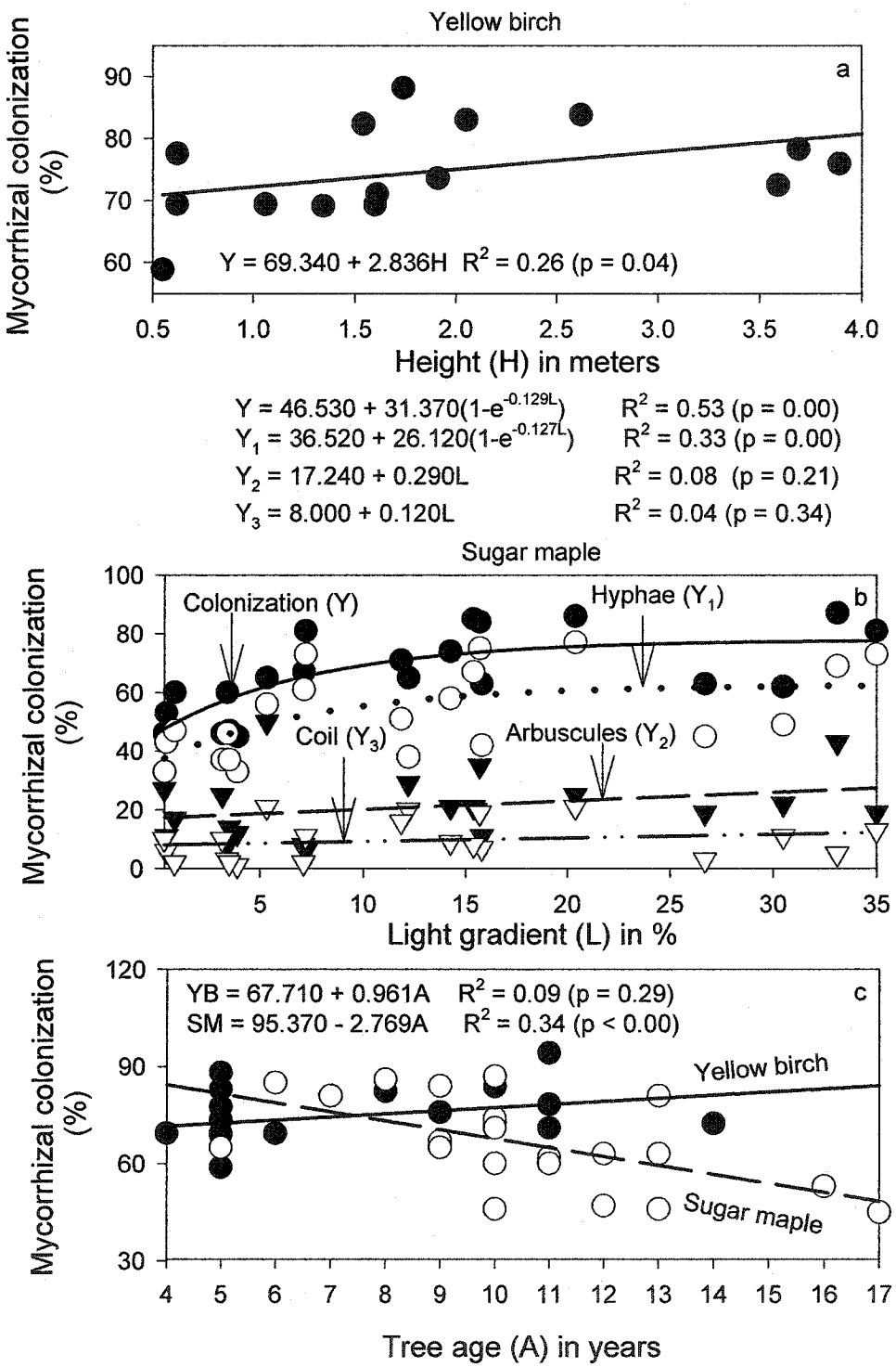
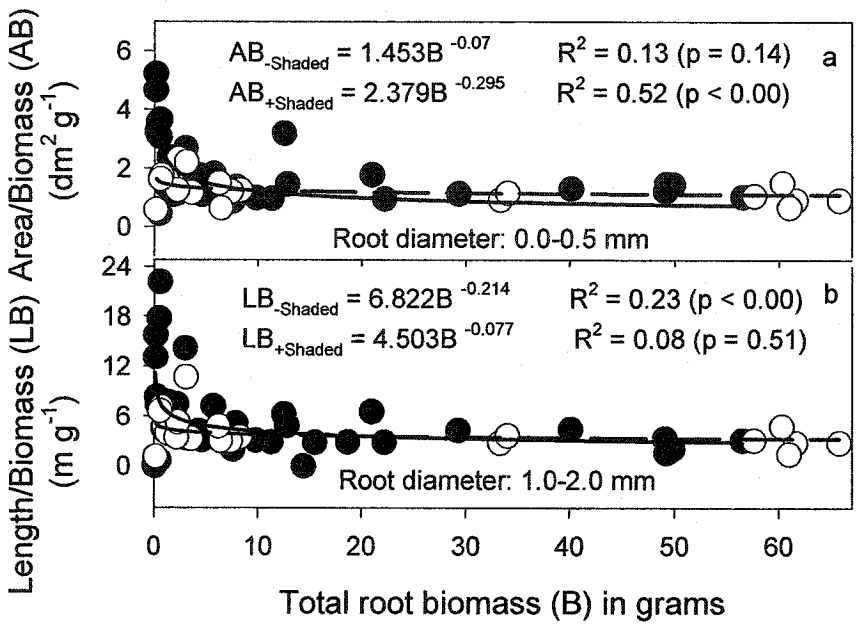

Yellow birch
a
Mycorrhizal colonization (%)
Y = 69.340 + 2.836H R² = 0.26 (p = 0.04)
Height (H) in meters
Y = 46.530 + 31.370(1-e^-0.129L) R² = 0.53 (p = 0.00)
Y1 = 36.520 + 26.120(1-e^-0.127L) R² = 0.33 (p = 0.00)
Y2 = 17.240 + 0.290L R² = 0.08 (p = 0.21)
Y3 = 8.000 + 0.120L R² = 0.04 (p = 0.34)
Sugar maple
b
Colonization (Y)
Hyphae (Y1)
Coil (Y3)
Arbuscules (Y2)
Light gradient (L) in %
c
YB = 67.710 + 0.961A R² = 0.09 (p = 0.29)
SM = 95.370 - 2.769A R² = 0.34 (p < 0.00)
Yellow birch
Sugar maple
Tree age (A) in years

Table 4. Analysis of covariance for the effects of shading cloth, tree species and their interactions on the surface area/biomass ($dm^2\ g^{-1}$), length/biomass ($m\ g^{-1}$), endings/biomass ($no.\ g^{-1}$) and branches/biomass ($no.\ g^{-1}$) ratios in the small fine root classes. All the non- and shaded trees grew in the light regime between 0.3 and 16% of full sunlight. Total root biomass is the covariate. F is the mean square ratio. * = significant at 95%. ** = significant at 99% significant at 99%. The bolded numbers indicate a significant difference. Data for non-significant interaction (shading cloths × species) is not shown.

Source	Root diameter class (mm)	Area/Biomass F	Length/Biomass F	Endings/Biomass F	Branches/Biomass F
Shading (Sh)	0.0-0.5	**11.01****	0.304	1.360	0.315
	>0.5-1.0	**13.30****	**4.351***	0.655	0.329
	>1.0-2.0	**6.053***	**4.432***	**4.509***	0.143
Species (Sp)	0.0-0.5	1.310	1.739	**8.290****	**6.818***
	>0.5-1.0	2.811	0.579	**9.404****	**9.605****
	>1.0-2.0	3.650	0.502	0.031	**28.80****
Total root biomass (B)	0.0-0.5	**30.89****	3.437	0.111	**107.8****
	>0.5-1.0	**38.75****	**15.00****	0.009	**285.3****
	>1.0-2.0	**32.92****	**15.49****	0.343	**420.2****
Sh × B	0.0-0.5	**11.92****	0.984	0.102	0.026
	>0.5-1.0	**13.84****	**4.262***	0.023	0.252
	>1.0-2.0	**5.140***	**4.405***	0.671	0.695
Sp × B	0.0-0.5	**8.421****	0.011	0.674	2.044
	>0.5-1.0	**12.39****	0.497	3.883	0.006
	>1.0-2.0	**13.40****	0.577	3.517	0.082
Sh × Sp × B	0.0-0.5	2.497	0.103	0.186	0.549
	>0.5-1.0	**4.031***	0.016	1.241	0.052
	>1.0-2.0	1.462	0.011	**4.310***	0.007

Fig. 7. Interactive effects of shading cloths (shaded (+) = ●, solid line; non-shaded (-) = ○, dash line) × total root biomass (g) on the root surface area/biomass ($dm^2\ g^{-1}$) ratio in 0.0-0.5 mm sized roots and length/biomass ($m\ g^{-1}$) ratio in >1.0-2.0 mm sized roots of both species grown in a similar range of light. The affected ratios in the other root classes have similar patterns.


Length/Biomass (LB) Area/Biomass (AB)
(m g-1) (dm2 g-1)
AB-Shaded = 1.453B -0.07
AB+Shaded = 2.379B -0.295
R2 = 0.13 (p = 0.14)
R2 = 0.52 (p < 0.00)
a
Root diameter: 0.0-0.5 mm
LB-Shaded = 6.822B -0.214
LB+Shaded = 4.503B -0.077
R2 = 0.23 (p < 0.00)
R2 = 0.08 (p = 0.51)
b
Root diameter: 1.0-2.0 mm
0
2
4
6
0
6
12
18
24
0
10
20
30
40
50
60
Total root biomass (B) in grams

contents of the leaves (Jakobsen 1999). In AM, carbon assimilation can increase by 5-10% and in ECM by 10-20 % (Read 1999). Meanwhile, the concentrations of soluble carbohydrates (Jakobsen 1999) and starch (Hampp and Schaeffer 1999) in the small fine roots decrease as the intensity of mycorrhizal colonization and the length of colonized roots increases (Jakobsen 1999, Hampp and Schaeffer 1999). These decreases create a sink that causes more carbon to be assimilated by photosynthesis and transported to the root system (Hampp and Schaeffer 1999).

Biomass allocation within the root system

As total root biomass increases, the proportion allocated to fine roots progressively decreases whereas the proportion of biomass allocated to the coarse roots progressively increases, thus establishing a strong root skeleton. The rapidly developing coarse roots physically support the aboveground growth of the tree and provide a good storage component for reserves. The absolute amount of small fine root biomass, however, increases continuously, thus supplying nutrients for aboveground growth, but the rate of increase is gradually reduced as total root biomass increases. This is very similar to the behavior of the leaf component aboveground, which continuously increases with tree size, but to a lesser extent than the branches and the trunk (Messier and Nikinmaa 2000, Delagrange et al. 2004). The proportions of biomass allocated to the various size classes of roots become more or less constant after the root systems of the yellow birch reach a total biomass of ~60 g (Fig. 2). The change in investment of root biomass to various root size classes and structures may balance the requirements for aboveground and belowground growth of the tree. In very young understory sugar maples, the biomass allocated to the small fine roots more efficiently expands the root area than does that of yellow birch, based on the higher coefficient of

determination (R^2 in Fig. 3a), as a result of producing many tiny roots to explore for soil nutrients. The biomass in small fine roots is, therefore, more efficiently used to contribute to root architectural traits associated with soil exploration and nutrient absorption. However, the ratios of root area and branches to biomass in the small fine roots decreases with total root biomass (Fig. 3). One possible reason is that, as the tree increases its size, more carbohydrates are used by the fine roots to increase root respiration (Roberts et al. 1993) to support water and nutrient uptake and transport, and to increase the formation of symbiotic associations and exudations that support microbial activities in the soil (Lambers 1990).

Our data emphasize the importance of the small fine roots, as they have the largest numbers of tips and branches (Figs. 4 and 5) and explore the largest volume of soil in relation to the coarse roots. In a coarse root, the epidermis has been modified to become impermeable (McCully 1990). Coarse roots, therefore, lose almost all of their absorptive ability and function mainly as storage organs for starch for future growth and as an anchoring system for physically supporting the aboveground growth of the tree. The living endings (tips) of fine roots, however, are permeable to soil water and mineral nutrients (McCully 1990), with the maximum uptake occurring in the first 4-5 cm of the root (Kolek and Kozinika. 1992). A high number of tips and branches boosts absorption by the roots and enhances their ability to competitively exploit the soil volume for water and mineral nutrients. There are large differences between the architectural traits (Fig. 4) and their relationship to root biomass (Fig. 5) for small vs. coarse fine roots. Our data show that root surface area, length, endings and branches are mainly formed in the small fine roots, where there is maximal respiration, water and nutrient transport (McCully 1990, Pergitzer et al.

1997). As these functions are essential for the survival of saplings, more research attention should be focused on the small fine roots.

Roots of yellow birch grow more rapidly than those of sugar maple. The average age for both species was similar, around 9 years old for yellow birch and 11.5 years old for sugar maple. For total root biomass, Fig. 1b indicates that, for a given age, the total biomass of yellow birch roots is greater than that of sugar maple roots. Architecturally, yellow birch generally tends to have a greater root area, length and more endings and branches per unit of root biomass than sugar maple (Fig. 5), suggesting that the roots of yellow birch develop faster and occupy more soil volume than do those of sugar maple. Compared to sugar maple, yellow birch is a moderately shade-tolerant species and has higher photosynthetic, transpiration and respiration rates (Walters and Reich 2000, Delagrange et al. 2004). Therefore, to satisfy its physiological needs, yellow birch in the understory needs to take up more water and nutrients from the soil by the rapid development of the root system.

Mycorrhizal colonization

Different types of mycorrhizae have different responses to a changing environment, since biotic factors may interactively affect mycorrhizal development. The % of the root system colonized by ectomycorrhizal fungi (yellow birch) or % arbuscular mycorrhizal colonization of sugar maple increases as tree size or light increases (Table 3, Figs. 6a and 6b). However, the ectomycorrhizal colonization of yellow birch appears mainly to respond to size, not to the light gradient (Table 3). High light availability results in rapid height growth in the understory yellow birch. The increased height coincidently promotes the crown development for more production of photosynthates which will promote ectomycorrhizal development. For understory sugar maple, the light gradient affects the

arbuscular mycorrhizal colonization, but the coefficients of determination (Fig. 6b) are low. The different responses of mycorrhizal colonization to changing light in both species suggest that the effect of light on mycorrhizal development is indirect. Increased light generally increases leaf carbohydrate levels, such as starch and sugars. This results in higher translocation to the roots (Corre 1983, Hodge et al. 1997), sequentially enhancing the exudation of sugars, amino acids, amides and phenolic acids to the soil (Grayston et al. 1996). This promotes the development of mycorrhizal and rhizosphere associations. In the arbuscular mycorrhizae of sugar maple, the intramatrical hyphae increased with light whereas the arbuscules and coils do not (Fig. 6b).

The absolute amount of mycorrhizae is positively correlated with increased root biomass in both species. Our data shows that total root biomass has no effect on ectomycorrhizal or arbuscular mycorrhizal colonization rates (Table 3) and no significant relationship between the rates of colonization and the biomass of the small fine roots of either species are found. Increased total root biomass increases the absolute amount of small fine root biomass, leading to increases in the surface area, length, endings and branches of the small fine roots. Although the mycorrhizal colonization rates do not change, the total amount of mycorrhizal fungus in the root systems of both species must increase as total root biomass increases. The total number of mycorrhizae in a root system will, therefore, have a positive relationship with the total root biomass.

Tree age affects the arbuscular mycorrhizal colonization of sugar maple roots only (Fig. 6c). However, mycorrhizal fungi are normally only associated with the very fine roots of trees, which generally have a very high annual turnover rate. Thus, tree age will influence total ectomycorrhizae in yellow birch and total arbuscular mycorrhizae in sugar maple

because older trees with larger root systems will have relatively fewer fine roots available for colonization.

Effects of a sudden reduction in light availability

The presence of shading cloths, which suddenly decreased the light availability by 50%, affects some architectural traits of the roots of both species, but the effect is mainly found in the very small trees. This finding is in contrast to our original hypothesis. It is hard to explain such results since we thought that the larger trees that have adapted their crown and root architecture to a much higher light intensity would have had a difficult time adjusting to such a drastic and rapid light reduction. One possible explanation is that the shading cloth affects some micro-environmental conditions such as wind patterns, evaporation, light quality, temperature etc. which have a greater effect on smaller trees. Shading cloth affects some of the physiological parameters of the leaves and some of the small trees looked less vigorous than the unshaded ones receiving the same amount of light. As an example, some of the small shaded trees had very low rates of photosynthesis (Delagrange et al. 2004). It is possible that the sudden decrease in light imposed on very small trees provoked a greater stress than on larger trees due to the lack of reserves in smaller trees.

Conclusions

The ability of understory tree saplings to modify their belowground allocation, root traits and mycorrhizae is greatly influenced by tree size and much less by light. These findings agree with recent similar conclusions reported in the literature for aboveground

traits. In this context, many previous studies that associated changes in belowground allocation, root traits and mycorrhizae to light may need to be reevaluated.

Acknowledgments

This study was supported by Concordia University Graduate Fellowship to S. Cheng and by an NSERC strategic grant to the junior author C. Messier (PI). The authors are also grateful to Joel Coburn, Sylvain Delagrange, Marie-Hélène Croisetière, Julie M. Richard, Nathalie Bourdonnais-Spear, Jocelyn Poissant, Mario Bonneau, Alexandre Piboule, Johanne Campbell and Rebecca Tittler for assistance in the field.

Chapter 3. Canopy gaps, liming and plant competition influence the growth and mycorrhizae of yellow birch and sugar maple

Abstract

The effects of canopy gaps, liming and plant competition on aboveground and belowground traits and mycorrhizae of naturally regenerating yellow birch (*Betula alleghaniensis* Britton) and sugar maple (*Acer saccharum* Marsh) saplings were investigated in a temperate deciduous forest in Eastern Canadian four years after selective logging. The experiment consisted of 3 canopy gap sizes (≤50, 101-300 and 701-1200 m^2), 2 liming levels (0 and 653 kg ha^{-1}) and 2 competition levels (partial removal or non-removal of woody plants). Sapling size (height) was used as a covariate to remove its effect from the analyses and isolate the effects produced by the 3 abiotic and biotic factors. The aboveground and belowground responses of both species to combinations of factors were similar with gap size being clearly the most important factor. The gap size had a significant effect on the aboveground traits only after the removal of tree size effects. Liming had no effect on either aboveground or belowground traits except for increased arbuscules in sugar maple roots. The removal of woody plants increased mycorrhizal colonization of both species. The browsed yellow birch quickly sprouted new shoots to compensate for the loss of aboveground biomass and the root architecture remained unchanged.

Key words: Light, Lime, Mycorrhizae, Plant competition, Understory saplings.

Introduction

In the temperate deciduous forest regions of the world, due to regular seasonal variation, small-scale gap creation and closure, caused by the death of a single tree, and large-scale disturbances caused by insect outbreaks, the forest floor is subject to large and unpredictable fluctuations in light and nutrient availability (Zak et al. 1993). This temporal and spatial heterogeneity allows for both competitive and stress-tolerant tree species (Grime 1977) to co-exist in these forests. In the deciduous forests of eastern North America, sugar maple and yellow birch are typical shade-tolerant and moderately shade-tolerant species, respectively (Barker 1949, Canham 1989). The relative contribution of stress-tolerance and competitive ability may be expected to differ somewhat (Grime 1977) to the survival of the two species. Some studies have investigated how the aboveground and belowground systems of both species acclimate to changes in available light in natural or controlled environments (Beaudet and Messier 1998, Delagrange et al. 2004). However, very few studies have investigated in a comprehensive factorial experiment, especially how gap size, liming and understory competition interact to affect both aboveground and belowground growth of these 2 species during the crucial 5 years following establishment.

Understory tree saplings in these forests potentially compete not only aboveground, with the overstory species, for light (Watt et al. 2003, Chertov et al. 2003), but also belowground, with the surrounding overstory and understory species, for soil resources. Furthermore, the study of this competition is complicated in that it involves various functional types of plants, from the herbs to the overstory trees. Various co-existing species have differing competitive effects. Increased competition inevitably affects changes in the morphological traits of the tree. The stronger the competition is, the greater are the changes

in the traits. How woody competitors affect aboveground and belowground traits of understory dominant yellow birch and sugar maple saplings in the complex changing environment is unknown.

Soil nutrients have an important influence on the photosynthetic performance and growth of a plant (Field and Mooney 1983). Soil nutrients are positively correlated with net photosynthesis (Hunt et al. 1985), stomatal conductance (Timmer et al. 1983) and tree growth (Sheriff et al. 1986). High concentrations of Ca (calcium) and Mg (magnesium) in the soil can improve the nutrient-absorption capacity of the roots (Ericssion et al. 1995), whereas high concentrations of Al reduce the uptake (Binns 1985, Bakker and Nys 1999). A low pH value (<5.0) increases the solubility of Al (aluminium) bound to clays and mineral compounds (Havas and Jaworski 1986, Tarabula 2000), depletes Ca and Mg (Binns 1985) and decreases the aboveground (Thornton et al. 1986, Helmisaari et al. 1999) and belowground (Nowotny et al. 1998, Bakker 1999) growth of the plant. The addition of lime ($CaCO_3$) can counteract Al toxicity (Bakker et al. 1999), improve soil nutrient availability (Nohrstedt 2002) and increase the P (phosphorus), S (sulphur), Mg, Ca and K (potassium) contents of the leaves, stems and twigs (Bakker et al. 1999, Moore et al. 2000). It can also increase the longevity (Bakker et al. 1999), biomass, length and nutrient uptake ability (Bakker et al. 2000) of the fine roots and increase shoot (Bakker et al. 1999) and diameter growth (Moore et al. 2000) by increasing the pH value (Hausenbuiller 1985, Majdi and Kangas 1997, Hahn and Marschner 1998, Qian et al. 1998, Bakker 1999).

Finally, mycorrhizae play an important role in increasing soil nutrient uptake by the roots (Linderman 1988) and, thus, benefit plant growth. Soil acidification reduces the ability of ectomycorrhizal fungi to release nutrients by cellulose breakdown (Chang and Alexander

1984), the number of spores present (Wang et al. 1993) and may hamper mycorrhizal colonization (Entry et al. 1987). A moderate dose of lime (1,400 kg ha^{-1}) increases mycorrhizal colonization (Bakker and Nys 1999, Bakker et al. 2000). The effects of low-doses of lime (≤ 1,000 kg ha^{-1}) on mycorrhizae and growth of both the species in a higher vegetation density are unclear.

In this study, yellow birch and sugar maple saplings were grown in a fully factorial experiment under 3 gap sizes (≤50, 101-300 and 701-1200 m^2), 2 liming levels (limed vs. unlimed) and 2 competition levels (partial removal of woody plants vs. non-removal controls). Both aboveground and belowground traits and mycorrhizal colonization were measured in order to test the following hypotheses: 1) the growth and development of understory saplings of yellow birch and sugar maple are limited by the interactive effects of low light, lack of lime and the high level of competing understory woody vegetation; 2) interspecific competition from woody plants has a weak effect on aboveground and belowground traits of the understory dominant species; 3) the mycorrhizal colonization of these 2 species is positively affected by liming and the removal of competing woody plants, due to changes in soil chemistry and available light.

Materials and methods

Study site

The study was conducted at Rivière à Pierre in the Réserve Faunique de Portneuf 47° 04' N and 72° 15'W, Québec, Canada. The site was characterized by an undifferentiated till of approximately >1 m depth. The soil was an ortho-humo-ferric podzol with an average pH of 5.4 and an average $NO_3$of 2.917 mg g^{-1}, NH_4 of 35.513 mg g^{-1}, P of 0.072 mg g^{-1}, Ca of

0.381 mg g^{-1}, K of 1.970 mg g^{-1} and Mg of 0.151 mg g^{-1}, respectively, based on 227 soil samples collected in 1997. The altitude ranged between 320 and 400 m, the mean annual precipitation was 1200 mm and mean daily temperature ranged from -13 °C in January to 28 °C in July (Environment Canada 1982).

Vegetation

Yellow birch and sugar maple made up 75 % of the overstory vegetation cover. Other common trees included American beech (*Fagus grandifolia* Ehrh.), red pine (*Pinus resinosa* Mill.), red maple (*Acer rubrum* L.), and balsam fir (*Abies balsamea* (L.) Mill.). The understory vegetation included mountain maple (*Acer spicatum* Lam.), American yew (*Taxus canadensis* Marsh), striped maple (*Acer pensylvanicum* L.), pincherry (*Prunus pensylvanica* L. f.), mooseberry (*Viburnum alnifolium* Marsh.) and beaked hazelnut (*Corylus cornuta* Marsh.).

Experimental design

Canopy gaps of size ≤50, 101-300 and 701-1200 m^2 were created by single and group selective cutting at a 40 ha forest site in the fall of 1996. In this study, 48 small, 24 medium and 12 large gaps were used. Four 7 m × 7 m plots were established in each of the 12 large gaps, one for each of the four possible combinations of treatments. The competition removal treatment consisted of removing understory woody plants, except for sugar maple and yellow birch, by cutting back to ground level twice (in spring and summer) in 1997, 1998, 1999 and 2000. Limed plots received a mixture of 3.2 kg of lime powder (equaling 653 kg ha^{-1}) containing $CaCO_3$ 92% (Ca: 36%) and $MgCO_3$ 0.76% (Mg: 0.35% including 0.16 kg of KCl), as did a 0.5 m buffer strip surrounding each plot (total area of 64 m^2).

These treatments were divided equally among the 48 small gaps (1 plot per gap) and among the 24 medium gaps (2 plots per gap), thus replicating each combination 12 times.

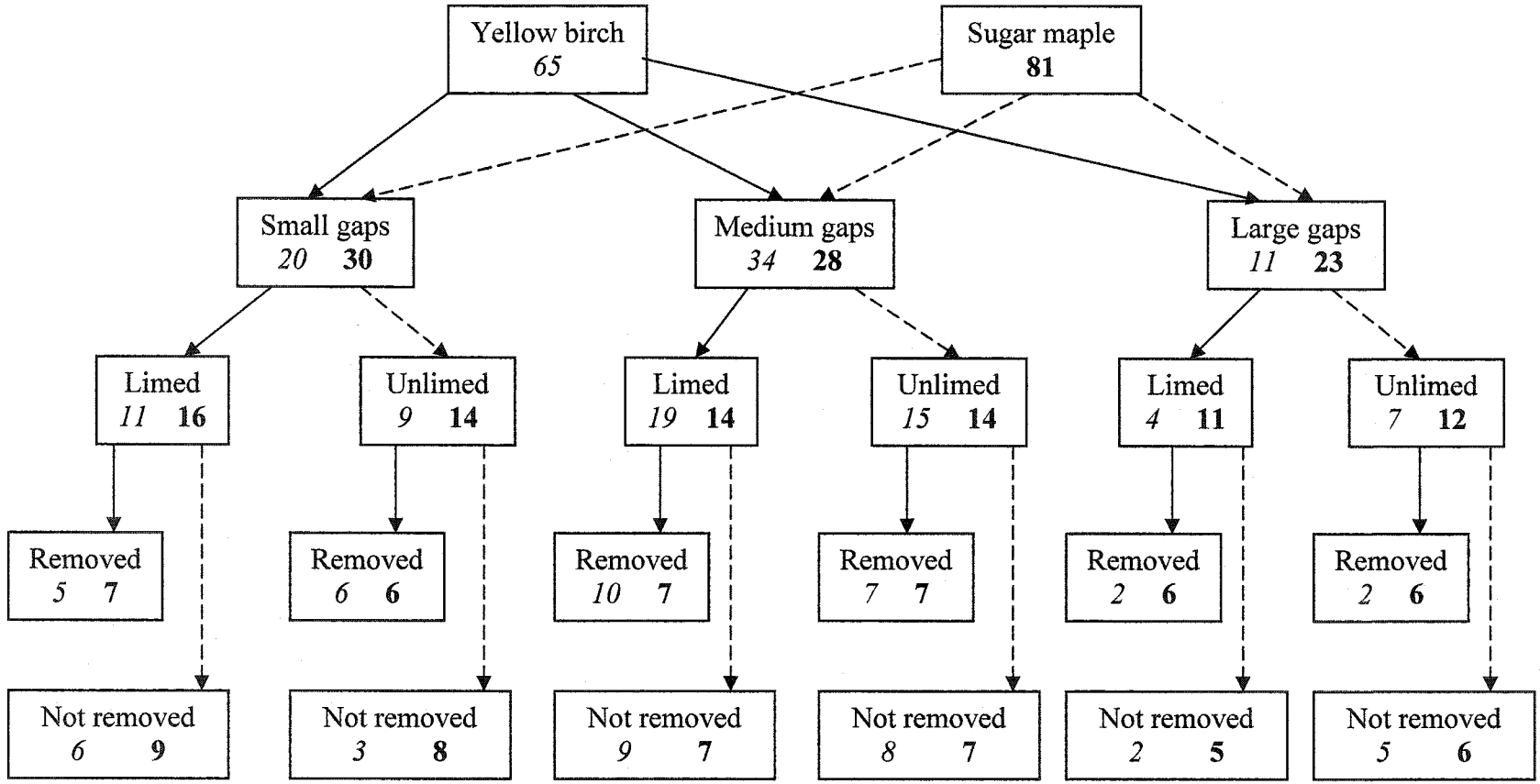
Sampling

A total of 93 yellow birch, including 28 that had been browsed by animals (probably moose) and 81 sugar maples were sampled from each treatment in 2001. All the non-browsed plants were harvested from the different treatments shown in Fig. 1. In each plot, one or two individuals of each species were randomly selected among a group of 10 that had previously been tagged when the experiment was established in 1997. The presence of obvious browsing (which only occurred on the yellow birch) was recorded. All of the selected saplings were carefully pulled out by hand after the aboveground variables had been measured. All the leaves from each seedling were separated from the trees and collected in a paper bag. The root system of each tree was placed in a Ziploc bag, then in a cooler with ice, to minimize water loss, and transported to the field laboratory. In the laboratory, soil particles were washed from the root systems under the tap. From each of the root systems, one to three intact small fine roots (≤ 2 mm in diameter, measured with a digital caliper), approximately 20 cm long, were selected for root measurements and another two or three roots were selected to assess mycorrhizal colonization.

Light measurements

For each of the saplings to be harvested, the light was simultaneously measured in the middle of the crown and in a completely open site nearby on a completely overcast day using a pair of Li-186 point quantum sensors (Li-Cor. Inc., Lincoln, NE). To calculate the percentage of total overstory light available, the values recorded in the middle of the crown were divided by the reference value taken at the same time in the open area. Such

Fig. 1.Sixty three non-browsed yellow birch and eighty one sugar maple saplings were sampled from different sized gaps (small, medium and large gaps), liming (limed and unlimed) and plant removal (woody plant removal and non-removal) treatments are shown as follows. The italic or bold numbers are for the yellow birch or sugar maple saplings from the different treatments.


Yellow birch
65
Sugar maple
81
Small gaps
20 30
Medium gaps
34 28
Large gaps
11 23
Limed
11 16
Unlimed
9 14
Limed
19 14
Unlimed
15 14
Limed
4 11
Unlimed
7 12
Removed
5 7
Removed
6 6
Removed
10 7
Removed
7 7
Removed
2 6
Removed
2 6
Not removed
6 9
Not removed
3 8
Not removed
9 7
Not removed
8 7
Not removed
2 5
Not removed
5 6

instantaneous light measurements taken under overcast conditions have been shown to be highly correlated with the mean seasonal daily light percentage (Gendron et al. 1998). The relative available light for the yellow birch and sugar maple saplings ranged from 1.1 to 88.6 and 0.5 to 83.1 %, respectively.

Traits investigated

Aboveground, the base diameter (mm), height (cm), leaf biomass (g) and stem (including crown branch) biomass (g) were measured. The following parameters were then calculated: 1) height/diameter ratio (cm mm^{-1}), 2) leaf biomass ratio (%) (defined as the ratio of the leaf biomass/aboveground biomass for the tree) and) leaf area (m^2). The leaf areas were calculated by the following equations: for yellow birch, total leaf area = 684.526 + 266.116 × leaf biomass, $R^2 = 0.90$; for sugar maple, total leaf area = 393.000 + 227. 939 × total leaf biomass, $R^2 = 0.92$, (M. Beaudet, personal communication). Belowground, the root surface area (dm^2), root length (m), root tips (no.) and forks (no.) for the samples of small fine roots were measured using a McRhizo system (Regent Instruments Inc., Québec City, Québec, Canada). The small fine root biomass, leaf biomass and stem (including branch biomass) (g) were measured using a digital balance after drying at 70 °C for 48 h. The following traits were then calculated for the roots: 1) surface area/biomass ($dm^2\ g^{-1}$), 2) length/biomass (m g^{-1}) and 3) tips/biomass (no. g^{-1}), and 4) branches/biomass (no. g^{-1}) as calculated by forks/$(biomass)^2$. Tree age (year) was recorded by counting the rings on the basal disk of each tree using a microscope. The total percentage of root tips colonized by ectomycorrhizal fungi in yellow birch and percentage of intramatrical hyphae, coils, vesicles and arbuscules in the small fine roots of sugar maple were recorded. The method of McGonigle et al. (1990) was used to measure arbuscular mycorrhizal colonization of sugar

maple roots and that of Goodman and Trofymow (1998) for the ectomycorrhizal colonization of yellow birch roots. See Chapter 2 for details.

Data analyses

Analysis of covariance (ANCOVA) (Fu 1979, Huitema 1980) was used to investigate the effects of canopy gap size, species, liming, woody plant removal, browsing and their interactions on the aboveground or belowground traits. Due to the significant effects of tree size on many morphological traits, height (or base diameter) was used as a covariate representing the size to remove its direct effects on the aboveground and belowground traits. If the gap size affected one of the traits, the LSD (least significant distance) test was applied to determine which gap size had a significant effect. Stepwise regression analyses were used to examine the effects of available light ao belowground and aboveground traits. Analysis of variance (ANOVA) was used to examine the effects of liming, plant removal, canopy gap size and their interactions on the mycorrhizal colonization of each species.

All the variables were graphically examined for normality using histograms and for homogeneity of variance using scatter plots. When necessary, variables were transformed logarithmically. All the data were tested for and satisfied the assumptions for ANCOVA, ANOVA and regression analyses. SPSS (version 10) statistical software (SPSS Inc. Chicago, USA) was used to perform the analyses.

Results

Effects of abiotic and biotic factors on both species

Species: the aboveground and belowground traits differed between species. Yellow birch saplings had smaller base diameters, leaf biomass ratios and higher height/diameter

ratios, leaf area ratios and specific leaf areas than sugar maple saplings (Table 1). Belowground, sugar maple had lower ratios of root surface area, length and branches to biomass than yellow birch (Table 1).

Lime: the liming had no effect on any of the measured traits (Table 1).

Plant removal: the removal of woody plants resulted in higher leaf biomass ratios and lower specific leaf areas for both species (Table 1).

Canopy gaps: the effects of the size of the canopy gaps could only be seen aboveground (Table 1). As the gap size was increased, the base diameter of both species was increased, but the height/diameter, leaf biomass ratio, leaf area ratio and specific leaf area ware decreased (Table 1).

Tree size: tree height had a significant effect on most of the aboveground and belowground traits for both species (Table 1) and thus was a proper variable to represent tree size as the covariate in the analyses.

Interactions: the interactions of species × plant removal × liming affected the height/diameter ratio. In the competition removal plots, this ratio increased for sugar maple and decreased for yellow birch (Fig. 2A); liming increased the height/diameter ratio in both species when woody competitors were not removed (Fig. 2B). This might be because dense vegetation promoted a rapid development of the height of the tree to compete for light.

Stepwise regression analyses (Table 2) showed that light is more strongly correlated with the aboveground traits than with the root traits of either species. Among the root traits, the length/biomass ratio was the most highly correlated with the aboveground traits.

Table 1. Analyses of covariance for the effects of species, liming, plant removal, canopy gaps and their interactions on the tree base diameter, ratio of height to base diameter (H/D), leaf biomass ratio (LBR), leaf area ratio (LAR), specific leaf area (SLA), root surface area/biomass (A/B), length/biomass (L/B), tips/biomass (T/B) and branches/biomass (B/B) ratios. Tree height is a covariate representing tree size. For the main effects, means of different treated levels are shown. Data for non-significant interactions are not shown. The numbers of yellow birch and sugar maple samples are 65 and 81, respectively. p is the probability. * = significant at 95%, ** = significant at 99%. The bolded numbers indicate a significant difference.

Source		Diameter (mm)	H/D (cm mm^{-1})	LBR (%)	LAR ($m^2 g^{-1}$)	SLA ($m^2 g^{-1}$)	A/B ($dm^2 g^{-1}$)	L/B ($m g^{-1}$)	T/B ($10^2 \times$ no. g^{-1}).	B/B ($10^2 \times$ no. g^{-1}).
Species (S)	Sugar maple	5.830	5.995	41.293	2.228	2.252	1.573	8.224	8.223	2.854
	Yellow birch	**4.088****	**8.683****	**35.584****	**2.966****	**3.196****	**2.226***	**10.957***	14.879	**6.430***
Liming (L)	Unlimed	4.904	7.268	39.089	2.614	2.702	1.872	9.337	13.343	4.229
	Limed	4.855	7.409	37.789	2.580	2.664	1.870	9.641	14.425	4.340
Removal (R)	Removed	4.953	4.348	41.326	2.545	2.535	1.788	8.917	13.105	4.088
	Non-removed	4.811	7.330	**35.552****	2.649	**2.840****	1.958	10.074	14.688	4.491
Gaps (G)	Large	**5.507***	6.451	35.279	2.339	2.646	1.709	8.793	12.293	2.699
	Medium	4.797	7.527	37.627	2.640	2.617	1.943	10.196	15.674	4.302
	Small	4.402	**8.039***	**42.410****	**2.811****	**2.890***	1.972	9.526	13.873	6.780
Height (H)	p	**0.000****	**0.000****	0.608	**0.000****	**0.000****	**0.005****	**0.001****	**0.006****	0.347
S × R	p	0.059	**0.044***	0.380	0.942	0.172	0.842	0.995	0.934	0.124
L × R	p	0.070	**0.043***	0.199	0.194	0.241	0.322	0.363	0.288	0.377

Fig. 2. Interactions between species (■ = yellow birch, □ = sugar maple) and plant removal in graph A and between plant removal and liming (■ = limed; □ = non-limed) on ratio of height to diameter (cm mm^{-1}) in graph B. Error bars present the standard error (SE) of the mean. Means with the same letter do not differ significantly from each other ($p > 0.05$).

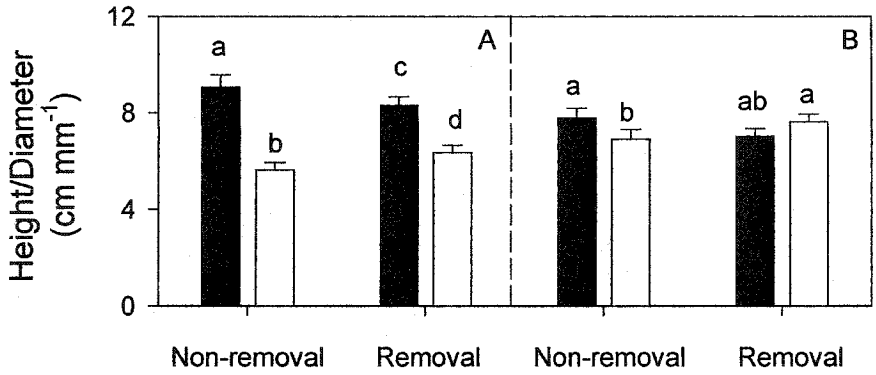

Height/Diameter
(cm mm-1)
12
8
4
0
a
b
c
d
a
b
ab
a
A
B
Non-removal
Removal
Non-removal
Removal

Table 2. Stepwise regression analyses for the effects of light (%), root surface area/biomass (dm^2 g^{-1}), length/ biomass (m g^{-1}), tips/biomass (10^2 × no. g^{-1}) and branches/biomass (10^2 × no. g^{-1}) ratios on the diameter (mm), height/diameter (cm mm^{-1}), leaf biomass ratio (%), leaf area ratio (m^2 g^{-1}) and specific leaf area (m^2 g^{-1}) of yellow birch and sugar maple saplings, respectively. R^2 is the coefficient of determination. * = significant at 95%, ** = significant at 99%. The bolded numbers indicate a significant relationship.

Tree	Dependent variable	Independent variable	R^2
Yellow birch	ln(Diameter)	= 0.640 + 0.360ln(Light)	**0.26****
	(Probability)	(0.000)	(0.00)
	Height/Diameter	= 13.948 – 4.933ln(Length/Biomass) + 2.570ln(Tips/Biomass)	**0.20****
	(Probability)	(0.002) (0.032)	(0.00)
	Leaf biomass ratio	= 46.626 – 3.958ln(Light)	**0.09***
	(Probability)	(0.014)	(0.01)
	Leaf area ratio	= 4.194 – 0.501ln(Light)	**0.61****
	(Probability)	(0.000)	(0.00)
	ln(Leaf specific area)	= 1.204 – 0.230ln(Light) + 0.184ln(Length/Biomass)	**0.32****
	(Probability)	(0.000) (0.015)	(0.00)
Sugar maple	ln(Diameter)	= 1.276 + 0.218ln(Light) – 0.290ln(Area/Biomass)	**0.44****
	(Probability)	(0.000) (0.000)	(0.00)
	Height/Diameter	Non-significant	
	(Probability)		
	Leaf biomass ratio	= 42.581 – 1.172ln(Branch/Biomass)	**0.05***
	(Probability)	(0.049)	(0.05)
	Leaf area ratio	= 2.247 – 0.193ln(Light) + 0.235ln(Length/Biomass)	**0.40****
	(Probability)	(0.000) (0.003)	(0.00)
	ln(Leaf specific area)	= 0.853 – 0.155ln(Light) + 0.188ln(Length/Biomass)	**0.42****
	(Probability)	(0.000) (0.005)	(0.00)

Table 3. Analyses of covariance for comparison of aboveground traits between non-browsed and browsed yellow birch and the effects of browsing on their root ratios. The base diameter is the covariate as a substitute for tree height. For the main effect, means ± standard error of different treated levels are shown. p is the probability. Data for not significant interaction (browsing × base diameter) is not shown. The number of the non- and browsed yellow birch is 65 and 28, respectively. * = significant at 95%, ** = significant at 99%. The bolded numbers indicate a significant difference.

Comparison of the aboveground traits

Source		Aboveground Biomass (g)	Leaf biomass ratio (%)	Leaf area ratio ($m^2\ g^{-1}$).	Specific leaf area ($m^2\ g^{-1}$)
Browsing (B)	Browsed	17.586 ± 7.100	35.636 ± 2.308	2.557 ± 0.082	**2.768*** ± 0.046
	Non-browsed	17.027 ± 4.017	35.042 ± 1.306	2.631 ± 0.046	2.293 ± 0.026
Diameter (D)	p	**0.000****	0.069	**0.000****	**0.000****

Effects of browsing on the roots

Source		Area/Biomass ($dm^2\ g^{-1}$)	Length/Biomass ($m\ g^{-1}$)	Tips/Biomass ($10^2 \times$ no. g^{-1}).	Branches/Biomass ($10^2 \times$ no. g^{-1})
Browsing (B)	Browsed	1.866 ± 0.089	8.706 ± 0.102	13.092 ± 0.124	5.160 ± 0.238
	Non-browsed	1.962 ± 0.059	9.450 ± 0.067	13.236 ± 0.081	5.265 ± 0.156
Diameter (D)	p	0.447	0.386	0.331	0.957

Table 4. Analyses of variance for the effects of liming, plant removal, canopy gaps and their interactions on mycorrhizal colonization in yellow birch and sugar maple roots. For the main effects, means ± standard error of different treated levels are shown. Data for non-significant interactions are not shown. * = significant at 95%. ** = significant at 99%. The bolded numbers indicate a significant difference.

Species		Yellow Birch	Sugar Maple				
Source		Ectomycorrhizael Colonization (%)	Endomycorrhizal Colonization (%)	Arbuscules (%)	Coil (%)	Hyphae (%)	Vesicles (%)
Liming (L)	Unlimed	68.71 ± 2.29	47.73 ± 3.14	12.27 ± 2.24	13.17 ± 1.60	34.32 ± 2.84	2.25 ± 0.23
	Limed	73.77 ± 2.22	54.11 ± 3.13	**19.92*** ± 2.05	15.61 ± 1.50	34.99 ± 2.76	1.50 ± 0.26
Removal (R)	Removed	**75.90**** ± 2.59	**56.53*** ± 3.17	17.44 ± 2.18	**17.80**** ± 1.58	37.47 ± 2.83	1.83 ± 0.26
	Non-removed	66.58 ± 1.78	45.31 ± 3.10	14.74 ± 2.11	10.97 ± 1.53	31.84 ± 2.77	2.00 ± 0.23
Gaps (G)	Large	**79.90**** ± 3.80	**60.19*** ± 4.12	15.72 ± 2.70	13.10 ± 2.03	**41.12*** ± 3.68	2.75 ± 0.31
	Medium	67.04 ± 1.66	46.14 ± 3.73	12.78 ± 2.62	14.45 ± 1.82	31.10 ± 3.31	2.00 ± 0.31
	Small	66.78 ± 2.24	46.42 ± 3.64	19.78 ± 2.56	15.61 ± 1.85	33.75 ± 3.29	1.33 ± 0.29

The effect of browsing on roots of yellow birch

Browsing had no effects on the roots of yellow birch, but did affect the specific leaf area (Table 3).

Mycorrhizal responses

Mycorrhizal colonization in both species was higher in large canopy gaps (Table 4). Liming increased colonization by arbuscules in sugar maple roots. When the competing plants had been removed, the mycorrhizal colonization in both species and the coils in sugar maple roots were increased (Table 4).

Discussion

Effects of gap size, liming and understory woody plants on both species

The major response of both species was to the canopy gaps in the complicated changing environment, but the effects are small after removal of tree size effects. In this study, the average age of yellow birch and sugar maple was approximately 4.5 and 4.6 years, respectively. Yellow birch had smaller base diameters and leaf biomass ratios; greater height/diameter ratios, leaf area ratios, leaf specific area, ratios of root surface area, length and branches to biomass than sugar maple (Table 1). But, these traits responded globally in the same manner to the combination of increasing gap sizes, liming and removal of competing woody plants for both species. The interaction of canopy gap size × liming × plant competition had no effect on the saplings. These data do not support the initial hypothesis of the study. The most important factor affecting these traits is clearly the gap size. In low light, either caused by woody competitors or overstory canopy trees, both species generally invest energy to produce higher leaf biomass ratios, leaf area ratio, specific

leaf area and height growth. This is necessary to capture the limited available light for the production of photosynthate needed for growth and survival, but no direct effect of light on any belowground trait was detected (Table 1). Among the three sized gaps, the effects of the medium (101-300 m^2) and large gaps (701-1200 m^2) on the traits are similar (Table 1). The relatively small effect of light alone on the aboveground traits and the lack of response of the belowground traits may seem surprising, but in this analysis, the effects of sapling size (height in the ANCOVA analysis of Table 1) is controlled for. Similar results have been shown recently in several papers (Messier and Nikinmaa 2000, Claveau et al. 2002, Delagrange et al. 2004 and this thesis Chapter 2). Many previous studies that have reported a strong effect of light on aboveground and belowground traits may have mistakenly interpreted the effect of tree size (or ontogenetic effects) to be an effect of light. Of course, in high light, saplings grow faster and so any change in these traits is indirectly the result of increasing light, but as shown here and elsewhere, the effect is mainly indirect.

Tree growth synergistically involves aboveground and belowground development. Changes in belowground traits are correlated with changes in the aboveground traits. Among these root traits, length/biomass ratio is the most highly correlated with changes in the aboveground traits (Table 2). This suggests that the occupation of soil volume by small fine roots may have an important effect on the aboveground growth of the young trees. When multiple factors are considered, however, the effects of light on the aboveground traits are generally stronger than those on the root traits (Table 2).

Any effect of liming on the aboveground and belowground traits in either species depends on the amount used and the level of plant competition. In this study, the level of lime of 653kg ha^{-1} was used, because the lime was put directly around the sampled saplings.

These young trees deplete soil nutrients less than mature trees. However, this low dose of lime had no effect on any of the traits (Table 1). Anderson et al. (1995) and Sikström (1997) indicated that low-dose liming of 500 and 1000 kg ha^{-1} does not affect the diameter growth and aboveground biomass of mature Scots pine and Norway spruce. This lack of response is because the low dose of lime used is insufficient to increase soil pH and improve soil nutrients. But, this study has showed an interactive effect of the liming and plant competition on both species. The low dose of lime increased the height/diameter ratios of both species grown in the dense vegetation where the woody competitors had not been removed (Fig. 2b). The stronger competition caused by the lack of removal competitors could result in a further limitation of soil nutrients and light for the saplings. In this situation, the growth of the understory saplings seems to be more sensitive to the addition of lime than it was in the competition removal plots, even though the dose is low. Liming may be more important to understory saplings grown in dense vegetation, since the plants with nutrient starvation more strongly acquire the energy to increase net photosynthesis in the leaves for enhancing survival and growth (Ericssion et al 1996, Hodge et al 1997). A moderate dose of lime between >1,000 and 5,000 kg ha^{-1} (Anderson et al. 1995, Sikström 1997, Helmisaari and Hallbacken 1998, Duliere et al. 2000) increases tree growth. Some studies have reported a strong response of Norway spruce (*Picea abies* (L.) Karst.), sessile oak (*Quercus petraea* (M) Liebl.), sugar maple, Scots pine (*Pinus sylvestris* L.), *Quercus petraea and Q. robur* to liming from 1400 to 4000 kg ha^{-1} (Lethto 1994, Hahn and Marschner 1998, Wilmot et al. 1996, Helmisaari et al. 1999, Bakker et al. 2000) in similar acidic soil. These doses of lime have a positive effect on foliar mineral nutrition (Tarabula 2000), growth and survival of fine roots of Scots pine (Helmisaari et al. 1999) and Norway spruce (Hahn and Marschner 1998)

and on fine root length, biomass and tips of sessile oak (Bakker 1999, Bakker et al. 2000). Wilmot et al. (1996) have reported that liming of 3000 kg ha^{-1} changes soil pH from 3.6 to 4.5; significantly improves foliar K, Ca and P concentrations, crown vigor of overstory sugar maple (Long et al. 1997, Wright el at. 1999), diameter growth by 11% (Long et al. 1997) for mature sugar maple and total biomass by 37% (Burke and Raynal 1998) for 2-year sugar maple, but does not affect the fine root biomass (Burke and Raynal 1998).

A high intensity of cutting is necessary to improve the growth of saplings of both understory species. In the forest, yellow birch and sugar maple comprised 75% of the vegetation cover, whereas other woody plants comprised 25%. After the partial removal of understory woody plants, neither height nor diameter growth was statistically improved (Table 1), because the tree density in the removal plots was still high. The competition from other plants is both either interspecific (other woody plants) or intraspecific (other yellow birch and sugar maple). In forest management, removing more than 25% of the vegetation cover, including partial cutting of yellow birch and sugar maple saplings growing abnormally, would be necessary for their rapid regeneration.

Effects of browsing on roots

The ability of browsed saplings to restore their aboveground biomass determines the effects of browsing on the roots. Height has a strong positive correlation with total root biomass, consequently the root biomass directly affects the ratios of area, length, tips and branches to biomass (Chapter 2). Loss of height by browsing causes a change in these root ratios due to a decrease in the growth of root biomass. Surprisingly, browsing does not alter the aboveground biomass, leaf biomass ratio and leaf area ratio (Table 3). However, the traits of fine roots of the browsed yellow birch are unchanged (Table 3), which indicates that

the growth of the root biomass is not affected. Yellow birch has the ability to sprout new shoots when damaged physically. It is stimulated to produce 3-6 sprouts generally and to increase specific leaf area (Table 3), allowing for more carbohydrates to be formed to compensate for the loss of the aboveground biomass. The balance between aboveground and belowground biomass is likely to be maintained at a relatively constant level. This may be a reason why the growth and architectural traits of the roots are not changed.

Effects of gap size, liming and understory woody plants on the mycorrhizal system

Light increases mycorrhizal colonization (Chapter 2). Increased light generally increases the production of photosynthates, such as starch and sugars in the leaves. Sequentially, more carbohydrates are allocated to the roots (Corre 1983, Hodge et al. 1997), in turn, enhancing the exudation of sugars, amino acids, amides and phenolic acids to the soil (Grayston et al. 1996). This benefits the development of the mycorrhizal fungi and the rhizosphere associations thus increasing the growth and survival of the understory saplings by increasing their uptake of soil nutrients. One possible reason is that the extramatrical hyphal length increases as mycorrhizal colonization increases, ranging from 300 (Read and Boyd 1986) to 2000 m m^{-1} root (Jones et al. 1991). This fungal mycelium may more effectively take up nutrients than the roots (Read 1999). For example, absorbing hyphae generally are substantially smaller in diameter (2.5 μ m) than the root hairs (10-20 μ m) (Varma 1999). The thin hyphae are better suited to use P from soil micro-pores (diameter <10 μ m), which are inaccessible to roots or root hairs (Jakobsen 1999).

A number of studies have indicated that the effects of liming on mycorrhizae is also dependant on the dose (Bakker and Nys 1999, Bakker et al. 2000, Duliere et al. 2000). High dose liming (>5,000 kg ha^{-1}) has been shown to have a negative effect on mycorrhizae and

roots (Helmisaari and Hallbacken 1998, Duliere et al. 2000). A moderate dose of 1,400 kg ha^{-1} enhances mycorrhiza formation and improves root absorption function (Bakker and Nys 1999, Bakker et al. 2000). The low dose used in this study increased arbuscule formation in the roots of sugar maple but did not affect ectomycorrhizal colonization of yellow birch (Table 4). The exchange of organic and mineral nutrients between roots and fungi might occur on the arbuscular surfaces in the root tissue. The increased number of arbuscules suggests that this may be related to an increase in mineral nutrients in the plant tissue.

Mycorrhizal colonization in both species shows an inverse relationship with vegetation density. The colonization was higher when some competitors had been removed (Table 4). This effect may have resulted from changes in available light, which benefits the development of mycorrhizal fungi and mycelium of the host plant (Jongen and Jones 1998).

Conclusions

In this study, after removal of the effects of tree size, no interactive effect of canopy gaps × liming × plant competition on the understory saplings was found. Light was the most important factor affecting the aboveground traits and mycorrhizal colonization of both species, however the effect was small. In many previous studies, the effects of tree size have been confounded with the effects of light. Liming and removal of at least 25% of the total vegetation cover more effectively increased the growth of understory saplings in dense vegetation. The arbuscules in the roots of sugar maple were more sensitive to an addition of lime than the other intramatrical structures.

Acknowledgments

This study was supported by a Concordia University Graduate Fellowship and a Power Corporation of Canada Graduate Fellowship to S. Cheng and by an NSERC strategic grant to the junior authors (C. Messier (PI)). We thank Julie Poulin, Pascal Rochon, Judith Bauer, Kate Julig, Virginie Coucou, Julie Messier and Pascal Perron for assistance with the field work.

Chapter 4. CO_2 and soil organisms influence light thresholds in yellow birch and sugar maple seedlings

Abstract

To examine the effects of elevated CO_2 and mycorrhizae on the light thresholds for growth and net photosynthesis, an experiment consisting of a completely randomized design was performed. The experiment was conducted with 3 light (μ mol. m^{-2} s^{-1}) levels (2.9 ± 0.1 %, 7.7 ± 0.3% and 26.1 ± 1.2% of full sunlight) × 2 CO_2 levels (350 and 700 <± 10 ppm) × 2 "mycorrhizal" treatments (sterilized vs non-sterilized natural soil) × 2 species (yellow birch and sugar maple seedlings) with 4 replications in a phytotron. The light thresholds for growth and net photosynthesis of the seedlings varied for the different species and for the different organs of each species. In ambient CO_2, the average light threshold for yellow birch was 26.1% of full sunlight compared to 19.1% for sugar maple. The threshold for leaf biomass growth was the lowest in both species. The leaf organ may play the most important role in the survival of the light-stressed seedlings. In 700 ppm CO_2, the average for the thresholds decreased to 20.9% for yellow birch and to 12.2% for sugar maple. This decrease might cause both species to grow better in the low light resulting from the denser vegetation projected as a result of future global climate change, especially for the moderately light-tolerant yellow birch. Soil organisms altered the biomass allocation by increasing the root biomass, root biomass ratio and decreasing leaf biomass ratio. Soil organisms also generally increased the light thresholds. Elevated CO_2 did not increase arbuscular mycorrhizal colonization of sugar maple roots.

Key words: Global change, Light threshold, Mycorrhizae, Phytotron, Seedlings

Introduction

As global climate change continues, the area of the temperate forest is predicted to expand northward by 193 to 251% (Environment Canada 1988). The temperate forest may, therefore, play a more important ecological role in the future. As a result of global warming, the current average July soil temperature of 15 °C in temperate regions (Stathers and Spittlehouse 1990) is predicted to increase to 19 °C by 2070 (Wegley 1999). This increased soil temperature will be in the optimal range of between 19 and 27 °C for growth of many tree species in North America in ambient CO_2 (Cheng 1999). As a consequence, the community composition of the temperate forest will progressively change and the density of vegetation may increase in the future (Environment Canada 1988). For saplings in the understory, the denser vegetation will create higher aboveground competition for light and belowground competition for soil nutrients. The increase in biomass and photosynthesis resulting from elevated CO_2 (Pospisilova and Catsky 1999) would be associated with increased soil nutrient uptake. Yellow birch and sugar maple are commercially important species in temperate forests of eastern North America (Burns and Honkala 1990) and it is, therefore, important to understand the responses of seedlings of these species in the understory to the environmental changes predicted for the future.

Goldstein et al (1985) and Cheng (1999) have discussed the concept of threshold levels for abiotic factors essential for tree growth. The calculation of a threshold uses the point of inflection, as described in calculus (Leithold 1976), but the inflection point is explained ecologically (Cheng 1999). Leithold (1976) pointed out that if there is a point on the graph of a function at which the sense of concavity changes, the point is called a point of inflection. Cheng (1999) stated that the threshold for soil temperature is the point at which

the rate of change in ecophysiological traits (e.g. net photosynthesis, stomatal conductance, transpiration, respiration etc.) of a tree changes inversely as the temperature decreases from high to low or increases from low to high. Below the threshold, these traits start to be limited (Cheng 1999). Above the threshold, these traits start to increase rapidly. However, there are thresholds for other abiotic factors, such as, light, air temperature etc. Elevated CO_2 and other abiotic or biotic factors may interactively affect the growth of trees by changing these thresholds.

Elevated CO_2 affects mycorrhizal colonization, as mycorrhizae depend on the photosynthetic products of the host (Tester 1986). Arbuscular mycorrhizal (AM) fungi may act as a sink for 10% of the photosynthate in citrus trees (Koch and Johnson 1982). Because of this, Diaz et al (1993) proposed that AM colonization will be increased by elevated CO_2. The effects of elevated atmospheric CO_2 on ectomycorrhizal (ECM) systems have been widely studied (Hodge 1996, Sadowsky and Schortemeyer 1997). For example, ectomycorrhizal colonization in oak and yellow birch is increased by elevated CO_2 (Seegmuller and Rennenberg 1994, Berntson et al. 1997). For arbuscular mycorrhizae, the percentage root length colonized (% RLC) is increased under elevated CO_2 for many plants (Monz 1994, Sanders 1996, Lovelock et al. 1996, Godbold et al. 1997). Staddon et al (1999) showed that elevated CO_2 increases mycorrhizal hyphal density by up to 65% in *Plantago lanceolata* and over 200% in *Trifolium repens*. However, there is very little information concerning the responses of the various intraradical structures of arbuscular mycorrhizae (Rillig 1998, Staddon and Fitter 1998) which may differentially respond to elevated CO_2 (Rillig 1998).

Many of the possible effects of the multiple changing variables on trees predicted as a result of global climate change are not well understood (Pearcy and Smith 1994). The effects of elevated CO_2 and soil organisms (including mycorrhizal fungi) on the light thresholds for growth and net photosynthesis in yellow birch and sugar maple, or any other species, have, never been investigated. In this study, the interactive effects of available light, soil organisms and elevated CO_2 on those traits of both species were examined. These data were used to test the hypotheses that: 1) there are thresholds for light, above which the rate of change in net photosynthesis and growth of the trees changes positively and below which the rate of change is negative, and that the thresholds vary for different species; 2) elevated CO_2 results in reduced light thresholds; 3) the presence of soil organisms, particularly mycorrhizal fungi, increases plant biomass so as to decrease the thresholds; 4) for sugar maples, the frequency of vesicles, which represent the major carbon sink, increases with increasing CO_2.

Materials and methods

Experimental design

The experiment was established as a completely randomized design (Brown 1995) with 2 CO_2 concentrations × 3 light levels × 2 mycorrhizal treatments × 2 tree species in the McGill University Phytotron. Two chambers were maintained at 350 (SE < ± 10) and two at 700 (SE < ± 10) ppm CO_2 concentrations (Romer 2001). In each growth chamber, 3 light levels (46.62 ± 0.93 μ mol m^{-2} s^{-1} = 2.7 ± 0.1% of full sunlight; 122.92 ± 2.46 μ mol m^{-2} s^{-1} = 7.7 ± 0.3% of the light; 417.66 ± 9.49 μ mol m^{-2} s^{-1} = 26.1 ± 1.2% of the light) were established to mimic closed, small and large canopy gaps in natural forests (<5%, ≥5% - <10% and >20% of full sunlight). The low and medium light levels were created by 78 cm

wide × 130 cm long frames covered by two or four layers of shade and black colored nylon cloths. For the high light treatment, plants were placed on a platform (78 cm wide × 130 cm long × 25 cm high) without shading in each chamber. The 4 growth chambers were used twice, resulting in 4 replications of each treatment.

The growth medium consisted of either sterilized (non-mycorrhizal) or unsterilized (mycorrhizal) forest topsoil for both yellow birch and sugar maple. Under each light level, about 4 seedlings of each species grown in each soil type were randomly placed. Due to the different height growth of each species, the light availability for each seedling changed relatively during the experiment (therefore, light was not treated as a categorical variable, but, as a continuous variable in the ANCOVA analyses). The general linear model (GLM) for the experiment was as follows:

$$GLM = \mu + C_i + L_j + CL_{ij} + M_k + CM_{ik} + LM_{jk} + CLM_{ijk} + S_n + CS_{in} + LS_{jn} + CLS_{ijn} + MS_{kn} + CMS_{ikn} + LMS_{jkn} + CLMS_{ijkn} + \varepsilon_{(ijkn)o}$$

Where μ = the overall mean; C = CO_2 concentration, i = 1, 2; L = Light, j = 1, 2, 3; M = Soil organisms, k = 1, 2; S = Species, n = 1, 2; ε = the random effect, o = 1, 2, 3, 4; the items of more than one letter (treatment factor) combined were the two-, three- and four-way interactions.

Plant materials and germination

Yellow birch and sugar maple seeds were obtained from the Québec Ministry of Natural Resources in 2001. Yellow birch and sugar maple were germinated twice, once in January and once in April of 2002. Before the first batch of germination, the seeds of both species were soaked in tap water for 20 days. Yellow birch seeds were placed in a beaker and covered with water at room temperature, whereas sugar maple seeds were laid among

layers of cotton batting that were soaked in water and placed in a tray. The tray was placed in an incubator at 4 (SE ± 2) °C in the dark. The water in the tray was changed every day. After the soaking period, yellow birch and sugar maple seeds were sown separately in propagation plug trays (28 cm wide × 55 cm long × 6 cm high, Plant Products Co. Ltd, Brampton, Canada). Each tray contained 72 plugs (6 rows × 12 columns) filled with sterilized or non-sterilized soil. The trays of yellow birch seeds were placed in the McGill university greenhouse with a 16 h photoperiod and 25/15 (SE ± 3/2) °C daytime/night time temperature for the germination. The trays of sugar maple seeds were covered with aluminum foil and stratified by placing them in an incubator at 4 (SE ± 1.5) °C for 32 days. The seeds were checked and watered after two weeks of the stratification. At the end of the period, most of the seeds had sprouted cotyledons and all the trays were moved into the greenhouse with the yellow birch seedlings. In the greenhouse, yellow birch and sugar maple seedlings were watered once per week. Soil water was maintained at saturation. Yellow birch and sugar maple were grown in the greenhouse for 64 days and 32 days, respectively. Before being placed in the growth chambers, seedlings were transplanted to12 cm diameter × 12 cm high pots, containing sterilized or non-sterilized soil and kept in the greenhouse for a further 3 days to minimize the disturbance and mortality due to environmental change after the transplantation.

Soil collection and treatment

One cubic meter of natural soil was collected from the A_1H horizon of the Duchesnay Forest in Québec, Canada. After mixing to homogeneity, the soil was stored in a cold room at 4 °C before use. Half of the soil was distributed among 7 autoclave bags. Three or four bags at a time were sterilized by autoclaving three times for 20 min at 15 psi.

Growth chamber environment

In all of the growth chambers, the air temperature was maintained at 25/15 (SE ± 0.05/0.04) °C daytime/nighttime, relative humidity at 60/65% (SE ± 0.86/0.58) daytime/ nighttime and photoperiod at 16 h (6:00 a.m. to 10:00 p.m.). During the light period, the light was gradually increased to the maximum and decreased to zero in order to mimic sunrise and sunset and minimize the potential disturbance of plant processes by changing the light suddenly. All the plant pots under the high light were covered by aluminum foil with a 3 cm diameter hole at the center, to reduce the possible increase in soil temperature. The soil temperatures under the high and low level lights were monitored by thermometers and the difference during the experiment was between 0.5-1 °C. Soil water under the different light treatments in each chamber was monitored by soil water moisture meters. When the meters indicated dry soil, all the plants were watered.

Measurement of photosynthesis

Light intensity at the 5th leaf of each yellow birch and 3rd leaf of each sugar maple from the top of its crown was measured by a LI-1000 datalogger (LI-COR, Lincoln, Nebraska, USA). Relative light was calculated as the light received by a seedling divided by the averaged full sunlight (1,600 μ mol m^{-2} s^{-1}) in a temperate forest measured at Rivière à Pierre in the Réserve Faunique de Portneuf 47° 04' N and 72° 15'W, Québec, Canada. Net photosynthesis was measured by a LI-Cor 6400 instrument (LI-COR, Lincoln, Nebraska, USA) with the individual light intensity on the 5th or 3rd leaf of each seedling. The conditions of the measurements were:- temperature 25 (SE ± 0.1) °C, humidity 60 (SE ± 1) %, CO_2 concentration 350 or 700 (SE ± 5 or 7) ppm.

Harvesting of plants

After measurement of net photosynthesis, 159 yellow birch (83 from non-sterile soil; 76 from sterile soil) and 154 sugar maple (82 from non-sterile soil; 72 from sterile soil) from the different light and CO_2 treatments were harvested. From the 350 ppm CO_2 chambers, 24, 28 and 28 yellow birch and 27, 25 and 24 sugar maple were taken from the 2.7%, 7.7% and 26.1% of light treatments, respectively. From the 700 ppm CO_2 chambers, 24, 31 and 24 yellow birch and 30, 30 and 18 sugar maple were sampled from the same three light levels, respectively. The leaves, stem (including branches) and root system of each seedling were separated. The roots were washed in tap water.

Growth traits investigated

Initial and final height, initial and final base diameter, stem biomass (including branch biomass for yellow birch), leaf biomass, root biomass and mycorrhizal colonization were measured. All of the organs were weighed on a digital balance after drying at 70 °C for 48 hours. Total plant biomass, root/shoot ratio, stem biomass ratio (defined as stem biomass/total plant biomass), leaf biomass ratio (defined as leaf biomass/total plant biomass) and root biomass ratio (defined as total root biomass/total plant biomass) were calculated.

Mycorrhizae

Ectomycorrhizal colonization of yellow birch roots and arbuscular mycorrhizal colonization of sugar maple were measured and calculated as described by Goodman and Trofymow (1998) and McGonigle et al. (1990), respectively.

Data analyses

Analysis of variance (ANOVA) was used to test the effects of the four replications on the seedling growth and to examine the effects of elevated CO_2 on mycorrhizal colonization

for each species. Analysis of covariance (ANCOVA) (Fu 1979, Huitema 1980) was used to investigate the effects of species, soil organisms (including mycorrhizal fungi), elevated CO_2 and their interactions on the net photosynthesis. Tree height and light, which significantly affected all of the organs of a tree, were used as covariates to remove their effects from the analyses. A cubic regression model was used to simulate the relationships between the dependent variables (net photosynthesis, biomass of all individual organs) and the independent variable (light), as this model is better for fitting experimental data in studies of plant physiology and ecology (Botkin 1993, Sullivan et al. 2002, Uddin ey al. 2003). The light threshold was calculated as follows: 1) the first derivative function derived from the cubic model described the rate of change, 2) the second derivative function of the cubic model represents zero for the threshold point as described by Leithold (1976) and Cheng (1999).

The cubic model: $A = B_0 + B_1L + B_2L^2 + B_3L^3$ (1)

Rate of change: $d(A)/d(L) = C_0 + C_1L + C_2L^2$ (2)

Light threshold: $d\left(d(A)/d(L)\right) = 0$ (3)

Where: B_0, B_1, B_2 B_3, C_0, $C_1$and C_2 were coefficients of the equations, A was a dependent variable, L was the independent variable, light.

All the variables were graphically examined for normality using histograms and for homogeneity of variance using scatter plots. If necessary, variables were transformed logarithmically. All of the data were tested for, and satisfied, the assumptions for ANCOVA, ANOVA and regression analyses. SPSS (version 10) statistical software (SPSS Inc. Chicago, USA) was used to perform the analyses.

Results

Seedling growth

The data from all four replications of the experiment were used in the analyses, except for 2 replications of the data for photosynthetic rates, since a technical problem with the LI-Cor instrument occurred in the first run of the experiment. There were no statistical differences between replicates of the seedlings grown in sterile and non-sterile soils in the chambers (Table 1). Overall, the net photosynthetic rate, total biomass, leaf biomass, stem biomass, root biomass, the leaf, stem and root biomass ratio of yellow birch were greater than for sugar maple (Tables 2, 3). In both species, elevated CO_2 increased the rate of photosynthesis and total plant biomass as well as that of leaves and roots (Tables 2, 3), but did not change the biomass allocation patterns (Table 2). Net photosynthesis in yellow birch increased much more than that in sugar maple in elevated CO_2 (Fig. 1). Height and light interactively affected the leaf, root and total plant biomass positively (Table 2). In this study, yellow birch grew well. The plants grown in non-sterilized soil had increased root biomass and root biomass ratios and decreased leaf biomass ratios (Table 2), but their net photosynthetic rates were unchanged (Table 3). Many of the sugar maple seedlings, however, performed poorly and had many leaves either partially or fully desiccated by the airflow from the bottom of the growth chambers.

Elevated CO_2 and light thresholds

The regression models (Table 4) indicated that the coefficients of determination between light and the biomass variables were weaker in sugar maple than in yellow birch grown in non-sterilized soil. This was probably because the leaves of the sugar maples

Table 1. Analyses of variance for the effects of the four replications on biomass of stem (g, including branches), leaf, root, total plant (g), root/shoot ratio (%, R/S), stem biomass ratio (%,SBR), leaf biomass ratio (%, LBR) and root biomass ratio (%, RBR) of yellow birch and sugar maple. 83 and 76 for yellow birch, 81 and 73 for sugar maple seedlings are from the non- and sterilized soils, respectively. p is the probability.

4 Replications	Soil	Stem (p)	Leaf (p)	Root (p)	Total (p)	R/S (p)	SBR (p)	LBR (p)	RBR (p)
Yellow Birch	Non-sterilized	0.569	0.811	0.401	0.745	0.159	0.167	0.184	0.163
	Sterilized	0.686	0.401	0.358	0.422	0.457	0.690	0.719	0.643
Sugar maple	Non-sterilized	0.901	0.851	0.586	0.764	0.801	0.968	0.481	0.487
	Sterilized	0.886	0.450	0.244	0.373	0.348	0.334	0.814	0.452

Table 2. Analyses of covariance for the effects of elevated CO_2, species, soil treatment and their interactions on biomass of stem (%, including branches), leaf, root, total plant (g), root/shoot ratio (%, R/S), stem biomass ratio (%, SBR), leaf biomass ratio (%, LBR) and root biomass ratio (%, RBR). Tree height and light gradient are the covariates. Non- and sterilized levels of the soil treatment present mycorrhizal and non-mycorrhizal seedlings. For the main effects, the exact values of different treated levels are shown. For the other items, their values of probability (p) are shown. The numbers of yellow birch and sugar maple seedlings are 159 and 154, respectively. * = significant at 95%, ** = significant at 99%. The bolded numbers indicate a significant difference. Data for not significant interactions are not shown.

Source		Stem	Leaf	Root	Total	R/S	SBR	LBR	RBR
CO_2 (C)	350 ppm	0.389	1.038	1.005	2.432	2.581	15.120	40.326	39.017
	700 ppm	0.431	**1.154***	**1.189****	**2.774***	2.754	14.717	39.291	40.528
Species (S)	Sugar maple	0.154	0.397	0.506	1.057	3.290	14.154	36.525	46.572
	Yellow birch	**1.099****	**3.022****	**2.361****	**6.482****	2.160	**15.705****	**43.423****	**33.954****
Soil (O)	Non-sterilized	0.430	1.092	1.224	2.746	2.849	14.909	37.863	42.478
	Sterilized	0.391	1.097	**0.976****	2.464	2.497	14.908	**41.846****	**37.225****
Height (H)	p	**0.000****	0.641	**0.003****	**0.033***	0.207	0.760	**0.000****	**0.006****
Light (L)	p	**0.000****	**0.000****	**0.000****	**0.000****	**0.000****	**0.000****	**0.000****	0.372
C × H	p	0.837	0.708	0.387	0.480	0.386	**0.018***	0.558	0.513
S × H	p	**0.000****	**0.000****	**0.000****	**0.000****	0.660	0.982	0.145	0.447
S × L	p	**0.000****	**0.005****	**0.012***	**0.004****	0.517	0.671	0.963	0.549
H × L	p	0.28	**0.021***	**0.040***	**0.020***	0.509	0.656	0.937	0.582

Table 3. Analysis of covariance for the effects of elevated CO_2, species, soil treatment and their interactions on net photosynthesis (μ mol m^{-2} s^{-1}). Light (%) is the covariate. For the main effects, the exact values of different treated levels are shown. For the other items, their values of probability (p) are shown. * = significant at 95%; ** = significant at 99%. The bolded numbers indicate a significant difference. Data for not significant interactions are not shown.

Source		Net photosynthesis
CO_2 (C)	350 ppm	2.081
	700 ppm	**3.298****
Species (S)	Sugar maple	1.277
	Yellow birch	**4.102****
Soil (O)	Non-sterilized	2.727
	Sterilized	2.652
Light (L)	p	**0.000****
C × S	p	**0.000****
S × L	p	**0.000****

were dehydrated, resulting in the drying out of some of the leaves and a decline or cessation of growth before harvesting. The high airflow might also impact net photosynthesis due to stomatal closure by water loss in the leaves. This problem might affect the precision of the results for this species. For the seedlings grown in non-sterilized soil at both 350 and 700 ppm CO_2, the rate of change in total plant, leaf, stem and root biomass, and in net photosynthesis in response to light (the equations shown in Table 5) formed a saddle-shaped curve for yellow birch (except for stem biomass in 700 ppm) (Figs. 2, 3). For sugar maple, these changes generally formed a hump-shaped curve, except for net photosynthesis (Figs. 2, 3). In yellow birch at 350 ppm CO_2, the light thresholds for total plant, leaf, stem, root biomass and net photosynthesis were 18.9%, 26.0%, 30.8% 35.7% and 20.0 % of full sunlight, respectively; at 700 ppm CO_2, the thresholds were 17.0%, 21.2%, 27.3% and 18.2% for the total plant, leaf, root biomass and net photosynthesis (Table 6). For the sugar maple at 350 ppm CO_2, the light thresholds for leaf, stem, root biomass and net photosynthesis were 1.3 %, 26.7%, 36.0% and 12.3%, respectively; at 700 ppm CO_2, the thresholds were 7.8%, 7.6%, 16.7%, 16.6% and 12.1% for total plant, leaf, stem, root biomass and photosynthesis, respectively (Table 6). In 350 and 700 ppm CO_2, the average threshold values were 26.3% and 20.9% respectively for yellow birch and 19.1% and 12.2% respectively for sugar maple (Table 6). For the seedlings in sterilizad soil, the light thresholds generally were lower than for plants grown in the non-sterilized soil (Table 7). Elevated CO_2 decreased these thresholds for the yellow birch in sterilized soil, but, had a weaker effect on the thresholds for the sugar maple seedlings (Table 4).

CO_2 and mycorrhizal colonization in the sterilized soil and non-sterilized soil

Even in the sterilized soil, low levels of mycorrhizal colonization were detected. In

Fig. 1. Interactive effects of species (yellow birch (YB) = ●, solid line; sugar maple (SM) = ○, dashed line) × light and species × CO_2 (350 ppm = □; 700 ppm = ■) on net photosynthesis (μ mol m^{-2} s^{-1}). The number of YB and SM seedlings are 80 and 77. The error bars represent the standard error (SE) of the mean. Different letters present statistical differences.

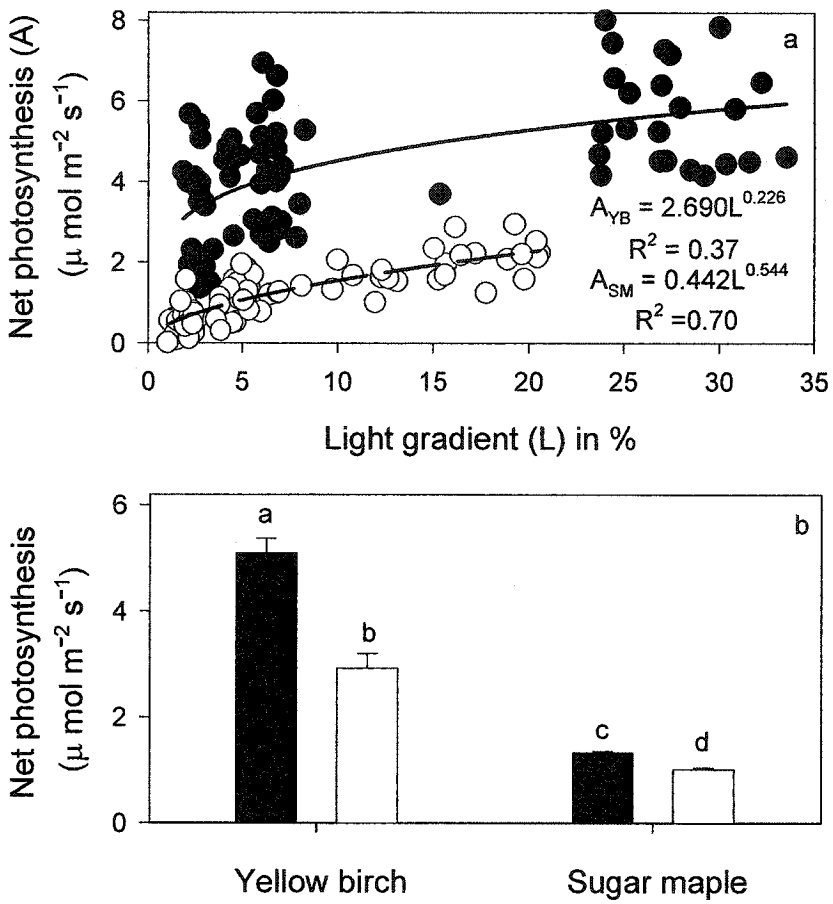

Net photosynthesis (A)
(μ mol m-2 s-1)
8
6
4
2
0
0
5
10
15
20
25
30
35
Light gradient (L) in %
a
AYB = 2.690L0.226
R2 = 0.37
ASM = 0.442L0.544
R2 =0.70
Net photosynthesis
(μ mol m-2 s-1)
6
4
2
0
a
b
c
d
b
Yellow birch
Sugar maple

Fig. 2. Cubic model for the relationships between total plant biomass (g), leaf biomass (g), stem biomass (g), root biomass (g) and light (%) in yellow birch (YB) and sugar maple (SM) seedlings grown in the non-sterilized soil. ● and solid line = YB in 350 ppm CO_2, ○ and dash line = YB in 700 ppm CO_2, ▼ and solid line = SM in 350 ppm CO_2, ▽ and dash line = SM in 700 ppm CO_2. Derivative curves represent the rates of change in their individuals as light changes. All the regression, derivative equations and the light threshold points are shown in Tables 4, 5 and 6.

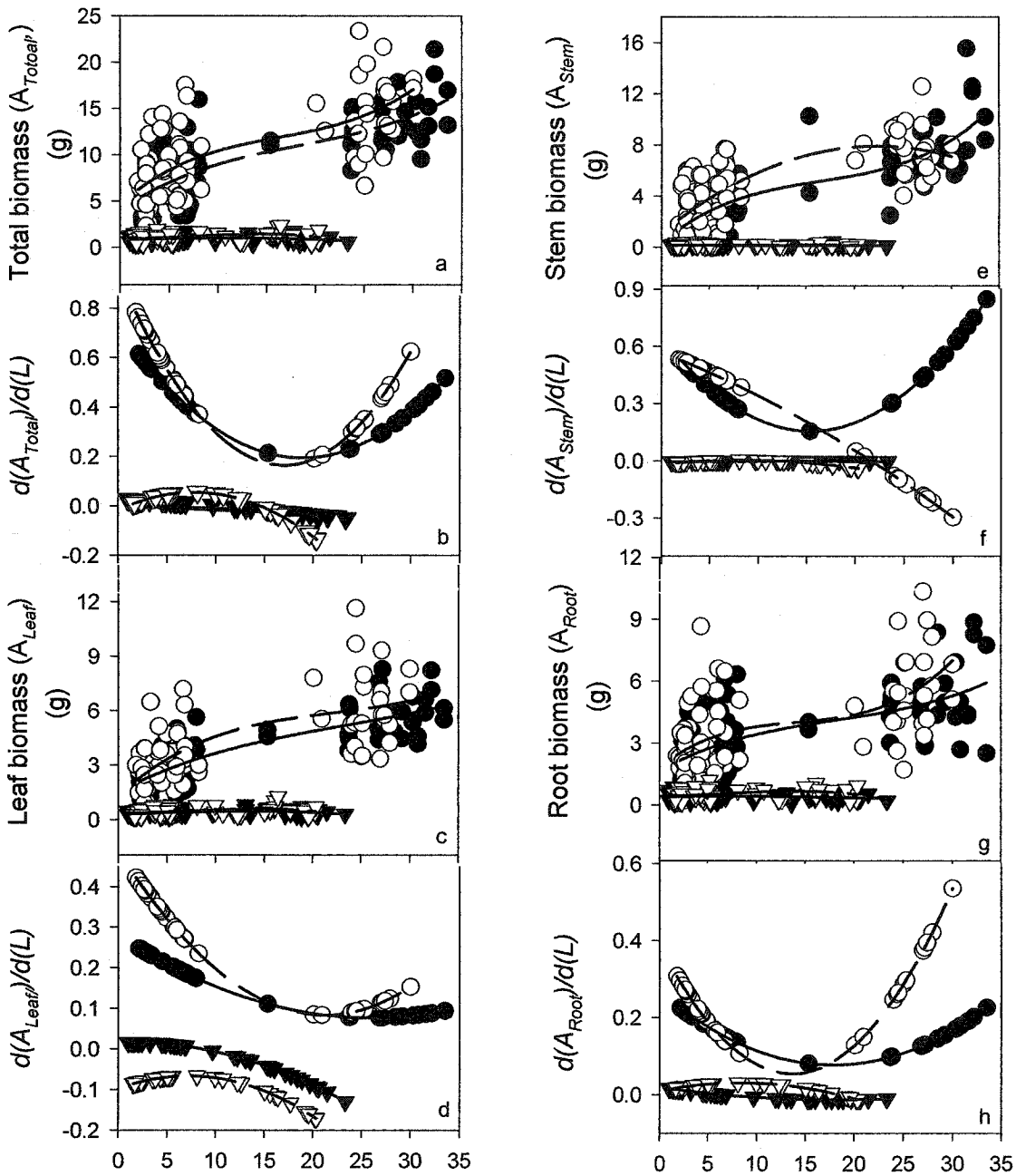

Total biomass (A_{Totoal}) (g)
$d(A_{Total})/d(L)$
Leaf biomass (A_{Leaf}) (g)
$d(A_{Leaf})/d(L)$
Stem biomass (A_{Stem}) (g)
$d(A_{Stem})/d(L)$
Root biomass (A_{Root}) (g)
$d(A_{Root})/d(L)$
a
b
c
d
e
f
g
h
Light gradient (L) in %

Fig. 3. Cubic models for the relationships between net photosynthesis (μ mol m^{-2} s^{-1}) and light (%) in yellow birch (YB) and sugar maple (SM) seedlings grown in the non-sterilized soil. ● and solid line = YB in 350 ppm CO_2, ○ and dash line = YB in 700 ppm CO_2, ▼ and solid line = SM in 350 ppm CO_2, ▽ and dash line = SM in 700 ppm CO_2. Derivative curves represent the rates of change in net photosynthesis as light changes. All the regression, derivative equations and the light threshold points are showed in Tables 4, 5 and 6.

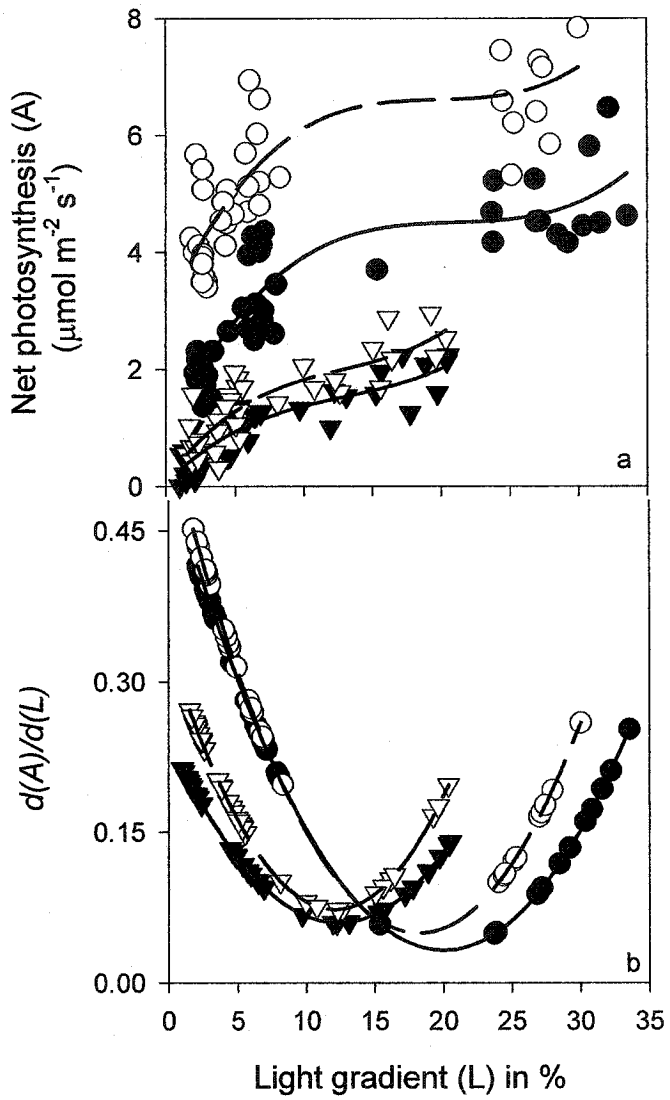

Net photosynthesis (A)
(µmol m-2 s-1)
8
6
4
2
0
a
d(A)/d(L)
0.45
0.30
0.15
0.00
b
0
5
10
15
20
25
30
35
Light gradient (L) in %

Table 4. Regression coefficients and coefficients of determination for the relationships between light (%, L) and total plant biomass (g, A_{Total}), leaf biomass (g, A_{Leaf}), stem biomass (g, A_{Stem}), root biomass (g, A_{Root}) and net photosynthesis (μ mol m^{-2} s^{-1}, A) of yellow birch and sugar maple grown in the non-sterile soil in 350 and 700 ppm CO_2, respectively. The cubic model is: $A = b_0 + b_1L + b_2L^2 + b_3L^3$. R^2 is the coefficient of determination. p is the probability. b_0, b_1, b_2 and b_3 are the coefficients of the models. * = significant at 95%, ** = significant at 99%. The bolded numbers indicate a significant relationship.

Species: Traits	CO_2	R^2	p	b_0	b_1	B_2	b_3
Yellow birch: A_{Total}	350 ppm	**0.61****	<0.00	0.4000	0.7282	-0.0283	0.0005
	700 ppm	**0.47****	<0.00	4.6490	0.9411	-0.0458	0.0009
Sugar maple: A_{Total}	350 ppm	0.02	=0.17	0.8356	0.028	-0.0010	-0.0001
	700 ppm	**0.10***	=0.03	0.9708	-0.0176	0.0093	-0.0004
Yellow birch: A_{Leaf}	350 ppm	**0.66****	<0.00	1.5220	0.2795	-0.0078	0.0001
	700 ppm	**0.49****	<0.00	1.3050	0.4886	-0.0191	0.0003
Sugar maple A_{Leaf}	350 ppm	**0.11***	=0.02	0.2946	0.0152	0.0004	-0.0001
	700 ppm	**0.09***	=0.04	0.3798	-0.0976	0.0043	-0.0002
Yellow birch: A_{Stem}	350 ppm	**0.65****	<0.00	0.2738	0.6521	-0.0323	0.0007
	700 ppm	**0.57****	<0.00	1.1870	0.5683	-0.0099	0.00009
Sugar maple A_{Stem}	350 ppm	0.01	=0.25	0.1618	-0.0064	0.0004	-0.00001
	700 ppm	**0.09***	=0.04	0.1781	-0.0177	0.0025	-0.00009
Yellow birch: A_{Root}	350 ppm	**0.41****	<0.00	1.6240	0.2681	-0.0107	0.0002
	700 ppm	**0.34****	<0.00	1.7780	0.3904	-0.0246	0.0006
Sugar maple A_{Root}	350 ppm	0.02	=0.18	0.3791	0.0194	-0.0018	0.00004
	700 ppm	**0.09***	=0.04	0.4129	0.0098	0.0025	-0.0001
Yellow birch: A	350 ppm	**0.80****	<0.00	0.8152	0.5121	-0.0240	0.0004
	700 ppm	**0.67****	<0.00	2.9222	0.5460	-0.0273	0.0005
Sugar maple: A	350 ppm	**0.81****	<0.00	0.0470	0.2420	-0.0148	0.0004
	700 ppm	**0.74****	<0.00	0.0818	0.3343	-0.0217	0.0006

350 ppm CO_2, mycorrhizal colonization of the non-sterile and sterile soil seedlings was 71.11 ± 1.76% and 8.32 ± 2.34% respectively for yellow birch and 42. 21 ± 3.77% and 6.70 ± 3.76% respectively for sugar maple (Table 8). Elevated CO_2 increased ectomycorrhizal colonization of yellow birch grown in the non-sterilized soil, but did not for plants grown in the sterile soil and for sugar maple grown in either soil (Table 8). In elevated CO_2, the ectomycorrhizal colonization increased to 77.17 ± 1.79% (Table 8). For intramatrical structures of arbuscular mycorrhizae in sugar maple roots, there was on effect of CO_2 condition.

Discussion

Light threshold, elevated CO_2 and soil organisms

The light thresholds varied between the 2 tree species. The growth patterns observed for both species in the artificial environment in 350 ppm CO_2 were similar to those found in a natural environment (Beaudet and Messier 1998). This study demonstrates that, for the shade-tolerant species, sugar maple, the light thresholds for growth at both 350 and 700 ppm CO_2 are generally lower than for the less shade-tolerant yellow birch (Table 6). The higher thresholds indicate that yellow birch is more sensitive to changes in light. This could be a major reason why yellow birch survives with difficulty, but sugar maple can survive well in very low light conditions in the forest floor.

The light thresholds among the organs of a tree vary. Leaves, the organs with the lowest threshold, play the most important role in the survival of the seedling under low light stress. For yellow birch leaves, the threshold is 26.0% compared to 30.8% for the stem and 35.7% for the roots at ambient CO_2 (Table 6). The pigments for light absorption,

Table 5. Coefficients of the first derivative equations derived from the models in Table 4. The first derivative equation is: $d(A)/d(L) = c_0 + c_1 L + c_2 L^2$.

Species:	Rate of change	C_0	C_1	C_2	CO_2
Yellow birch:	$d(A_{Total})/d(L)$	0.7282	-0.0566	0.0015	350 ppm
		0.9711	-0.0916	0.0027	700 ppm
Sugar maple:	$d(A_{Total})/d(L)$	0.0282	-0.0020	-0.0002	350 ppm
		-0.0176	0.0186	-0.0012	700 ppm
Yellow birch:	$d(A_{Leaf})/d(L)$	0.2795	-0.0156	0.0003	350 ppm
		0.4886	-0.0382	0.0009	700 ppm
Sugar maple:	$d(A_{Leaf})/d(L)$	0.0152	0.0008	-0.0003	350 ppm
		-0.0976	0.0086	-0.0006	700 ppm
Yellow birch:	$d(A_{Stem})/d(L)$	0.6521	-0.0646	0.0021	350 ppm
		0.5683	-0.0198	0.0003	700 ppm
Sugar maple:	$d(A_{Stem})/d(L)$	-0.0064	0.0008	-0.00003	350 ppm
		-0.0177	0.0050	0.0003	700 ppm
Yellow birch:	$d(A_{Root})/d(L)$	0.2681	-0.0214	0.0006	350 ppm
		0.3904	-0.0492	0.0018	700 ppm
Sugar maple:	$d(A_{Root})/d(L)$	0.0194	-0.0036	0.0001	350 ppm
		0.0098	0.0050	-0.0003	700 ppm
Yellow birch:	$d(A)/d(L)$	0.5121	-0.0480	0.0012	350 ppm
		0.5460	-0.0546	0.0015	700 ppm
Sugar maple:	$d(A)/d(L)$	0.2420	-0.0296	0.0012	350 ppm
		0.3343	-0.0434	0.0018	700 ppm

Table 6. Light thresholds ($L_{Threshold}$) of A_{Total}, A_{Lea}, A_{Stem}, A_{Root} and A in yellow birch and sugar maple seedlings grown in the non-sterilized soil.

Traits		Yellow birch		Sugar maple	
		$L_{Threshold}$		$L_{Threshold}$	
	CO_2	350 ppm	700 ppm	350 ppm	700 ppm
Total biomass: $d\left(\frac{d(A_{Total})}{d(L)}\right)=0$		18.9%	17.0%	N/A	7.8%
Leaf biomass: $d\left(\frac{d(A_{Leaf})}{d(L)}\right)=0$		26.0%	21.2%	1.3%	7.6%
Stem biomass: $d\left(\frac{d(A_{Stem})}{d(L)}\right)=0$		30.8%	N/A	26.7%	16.7%
Root biomass: $d\left(\frac{d(A_{Root})}{d(L)}\right)=0$		35.7%	27.3%	36.0%	16.6%
Photosynthesis: $d\left(\frac{d(A)}{d(L)}\right)=0$		20.0%	18.2%	12.3%	12.1%
Average		26.3%	20.9%	19.1%	12.2%

Table 7. Light thresholds ($L_{Threshold}$) of A_{Total}, A_{Lea}, A_{Stem}, A_{Root} and A in yellow birch and sugar maple grown in the sterilized soil. Those calculations are as the same as the calculation approach for both species grown in the non-sterilized soil. Those cubic and the first derivative equations are not shown.

Traits		Yellow birch		Sugar maple	
		$L_{Threshold}$		$L_{Threshold}$	
	CO_2	350 ppm	700 ppm	350 ppm	700 ppm
Total biomass: $d\left(d(A_{Total})/d(L)\right)=0$		22.7%	10.7%	14.3%	10.0%
Leaf biomass: $d\left(d(A_{Leaf})/d(L)\right)=0$		26.7%	10.8%	5.8%	10.0%
Stem biomass: $d\left(d(A_{Stem})/d(L)\right)=0$		17.8%	19.4%	16.7%	10.4%
Root biomass: $d\left(d(A_{Root})/d(L)\right)=0$		22.0%	18.1%	11.3%	10.3%
Photosynthesis: $d\left(d(A)/d(L)\right)=0$		19.2%	17.2%	11.3%	11.1%
Average		21.6%	15.2%	11.8%	10.4%

Table 8. Analyses of variance for the effects of elevated CO_2 on mycorrhizal colonization in yellow birch and sugar maple seedlings grown in the non- and sterile soil. The means ± standard error in different treated levels are shown. The data are from the four replications. * = significant at 95%. The bolded indicates the significant difference.

Species		Yellow birch	Sugar maple				
Source		Ectomycorrhizael	Endomycorrhizal	Arbuscules	Coil	Hyphae	Vesicles
CO_2	Soil	Colonization (%)	Colonization (%)	(%)	(%)	(%)	(%)
350 ppm CO_2	Non-sterilized soil	71.11 ± 1.76	42.21 ± 3.77	35.06 ± 5.78	11.33 ± 2.55	22.67 ± 3.07	2.00 ± 0.12
700 ppm CO_2	Non-sterilized soil	**77.17*** ± 1.79	34.11 ± 4.62	33.53 ± 7.08	6.00 ± 3.13	25.00 ± 3.76	2.00 ± 0.11
350 ppm CO_2	Sterilized soil	8.32 ± 2.34	6.70 ± 3.76	5.06 ± 1.23	1.74 ± 0.27	3.07 ± 1.35	0.00
700 ppm CO_2	Sterilized soil	9.46 ± 3.22	6.93 ± 4.23	5.75 ± 2.88	1.87 ± 0.65	3.56 ± 1.01	0.00

contained in the leaf tissues, increase in concentration as light levels decrease (Tang 1997) to more efficiently capture the limited photons. Close to the threshold for the leaves, root and stem growth are greatly restrained, but the leaf biomass increases in order to produce the energy needed for the survival of the tree. Therefore, in a given range of low light, the growth of the leaves determines the survival of the seedling. Because leaf biomass was partially lost, due to the stress of dehydration, the measured threshold for leaf biomass of sugar maple in ambient CO_2 is too low and cannot be taken as a reliable measure (Table 6).

Elevated CO_2 decreases the thresholds (Table 6) and may, therefore, ameliorate the aboveground and belowground competition between understory saplings grown in dense vegetation. The decreased thresholds imply that the seedlings of both species may grow better under low light in a future with elevated CO_2 levels than they do now. Elevated CO_2 is likely to exaggerate increases in root biomass of yellow birch as the rate of change increases sharply above the threshold (Fig. 2h). The increased root biomass would improve the ability of the root system to compete for belowground resources in the denser vegetation of the future. These responses of yellow birch to possible future elevated CO_2 levels are stronger than those seen in sugar maple. Shade-tolerant plants usually have higher stomatal resistance than shade-intolerant species (Environment Canada 1988). Therefore, the shade-intolerant species will have stronger responses to a changing environment than the shade-tolerant species (Environment Canada 1989). For instance, the increase in net photosynthesis of yellow birch grown at 700 ppm CO_2 is much greater than that of the sugar maples (Fig. 1b).

The light thresholds between the artificial and natural environments should differ. In this study, the seedlings were grown in an artificial environment, with optimum air and soil temperatures, photoperiod and available water for growth. This environment is very different

from the natural environment, where, for example, the average soil temperature in July is 15 °C in Ontario, Canada (Stathers and Spittlehouse 1990). The daytime soil temperature in the chambers ranged from 19 to 24 °C, which is optimal for the growth of many tree species in North America (Cheng 1999). Each of the seedlings is grown in a pot without belowground competition from other plants and the light intensity is stable during the daytime. Any of the above factors would result in a better growth response in the seedlings compared to their growth in the natural environment. Thus, the light thresholds in the phytotron are likely to have been higher when compared to those found in nature.

The light thresholds may be also be different as a result of the precision of the cubic modeling. The experiment focuses on low light levels where the light thresholds occur, so most of these seedlings grew below 8% available light. The other seedlings grew in high light between 25% and 35%, especially yellow birch (Fig. 2). There was a gap in the data points 8% and 25% available light. The lack of points in this intermediate light range reduced the precision of the modeling. However, this gap does not change the results in that elevated CO_2 reduces the light thresholds, as changes in the growth of seedlings at both CO_2 levels caused by the increasing intermediate light range are almost similar. Therefore, there is still is a difference in the light thresholds.

Soil organisms, including mycorrhizal fungi, may affect light thresholds for the leaves and roots. Soil organisms alter biomass allocation to the organs of the seedlings. Our data show that the total root biomass of both tree species grown in the non-sterile soil, in which they have acquired normal levels of mycorrhizal colonization, is higher (Table 2). This supports the conclusions of Harris and Paul (1987) and Hampp and Schaeffer (1999), that mycorrhizae form a strong carbon sink and driving force by the accumulation of lipid in

the hyphae, spores and vesicles; exponential production of fungal biomass and 30-40% consumption of total photosynthate for the root respiration (Harris and Paul 1987). The increased root biomass and its ratio increase the light threshold for the mycorrhizal roots. In contrast, soil organisms decrease leaf biomass (although not significantly) and leaf biomass ratio (Table 2), possibly by improving the efficiency of light use in the leaves as a result of increasing mineral nutrient content in the tissue. The higher light thresholds for seedling grown in non-sterile soil suggest that the seedlings need more carbohydrates assimilated by photosynthesis for the development of their mycorrhizal systems and other root-associated microorganisms

Mycorrhizal colonization

Mycorrhizal colonization in both species was similar in the phytotron to that of the natural environment. In ambient CO_2, the colonization of the phytotron yellow birch and sugar maple grown in light from 1.82% to 33.5% and 0.7% to 23.27% was 71.1 ± 1.8 % and 42.2 ± 3.8%, respectively. Under the same light levels, the colonization of 4.5 yr old (average)yellow birch and 4.6 yr old (average) sugar maple, which grew without disturbance in a temperate forest at Rivière à Pierre in the Réserve Faunique, Québec, Canada, were 63.0 ± 3.1% and 38.5 ± 4.2% (data from Chapter 3).

Elevated CO_2 increased ectomycorrhizal colonization in yellow birch roots grown in the non-sterile soil only (Table 8). The increased ectomycorrhizal colonization of the yellow birch roots exposed to elevated CO_2 is similar to the results reported by Berntson et al. (1997) for this species. The mycorrhizal community may also be changed by elevated CO_2 due to different requirements of fungal species in different environments. However, the arbuscular mycorrhizal colonization of the sugar maple roots in non-sterile soil did not

respond to elevated CO_2, thus, the fourth hypothesis for this study and the hypothesis of Diaz et al (1993) are not supported. The high airflow in the chambers that stressed the maple seedlings may have affected these results, which makes it impossible to properly test this hypothesis of the study.

Conclusions

This study first proposes and then demonstrates the existence of light thresholds. The threshold, as an index, reveals the shade-tolerant ability of the plants. The lower the threshold is, the stronger is the shade-tolerance. Elevated CO_2 decreases the light threshold, thus increasing the shade-tolerant ability of the seedlings. Leaf growth is the most important factor that affects the survival of trees under light stress. The presence of soil organisms, including mycorrhizal fungi, increased the light thresholds and altered biomass allocation.

Acknowledgements

The authors thank Mark Romer, Claire Cooney, Christian Mark, Sylvain Delagrange, Martin Lechowicz, Selvadurai Dayanandan, Yevs Claveau, Qing Guo, Alain Leduc for assistance and suggestion in the experiment and statistical analysis. This study was supported by Concordia University Graduate Fellowship and Power Corporation for Canada Graduate Fellowships to S. Cheng and by an NSERC strategic grant to the junior author (C. Messier (PI)).

Chapter 5. General conclusions

The three experiments reported in this thesis have led to several new insights into understanding the growth and mycorrhizal development of yellow birch and sugar maple in a changing environment in the present and future.

Root ecology and belowground development

Contrary to our previous understanding, my data indicated that light had no direct effect on root architecture, but that root biomass had a major effect on the architecture of the root. Light had an indirect effect by increasing tree size (height), which was strongly and positively associated with total root biomass. Thus, tree size had an important influence, not only on the aboveground system, but also on root development and architecture and their acclimation to conditions in the understory.

Root architecture differed between the two species and this different architecture and development belowground coincided with their aboveground growth. Yellow birch roots grew rapidly, producing more tips and branches and exploited a larger soil volume for supporting the rapid aboveground growth. In contrast, the aboveground and belowground systems of sugar maple grew slowly, which was consistent with the conservation of resources associated with a stress-tolerant strategy.

The architecture of small and coarse fine roots differed dramatically, showing that the combining of all fine root classes together that has been done in most previous studies is not justified. As the root biomass in the various root diameter categories increased, the root surface area, length, tips and branches generally were increased in the small fine roots, but the reverse occurred in coarse and coarse fine roots. Thus, the main focus for research on the

relationship between mineral nutrients made available by roots and tree growth should be on small fine roots.

This thesis clearly showed that the dynamics of root biomass investment to various sized roots within a whole root system. The investment of increased root biomass in coarse roots was the priority and, as a result, the relative importance of fine roots was decreased as the tree grew. The proportions of the investment to the various root size classes gradually became constant, presumably to balance the physical and nutritional requirements of tree growth. The point at which the investment to the various root classes became constant may indicate the transition from the establishment phase to the rapid growth phase in these saplings. However, this needs further study.

The development of the aboveground system was strongly related to that of the belowground systems. Of all of the belowground traits, the root length in small fine roots was the most highly correlated with the aboveground traits, but the relationship was weaker than that between light and the aboveground traits. The presence of soil organisms, including mycorrhizal fungi, altered biomass allocation in the Phytotron study by increasing root biomass ratios and decreasing the leaf biomass ratios, suggesting a possible trade-off between leaf biomass and mycorrhizal formation.

Forest ecology

The hypothesis that light is the most important determinant of growth for both species in a complex changing environment was confirmed. Canopy gaps, liming and competing plants, both individually and together, caused complex changes in the microenvironment for saplings of yellow birch and sugar maple. Among these abiotic and biotic experimental factors, light (as affected by canopy gap size) had the most important effect on their growth.

However the effect of light was small when the effects of tree size had been factored out. The saplings of both species responded similarly to the medium (101-300 m^2) and large gaps (701-1200 m^2). Therefore, the effects of light on understory tree growth may have been confounded with tree size in previous studies. Light was also positively correlated with the development of the mycorrhizal system. The dense understory vegetation at Rivière à Pierre reduced mycorrhizal colonization, probably as a result of light limitation.

The phytotron study is the first demonstration of the light thresholds for growth of any tree species. Light thresholds correlated with the shade-tolerance of a species, the higher the threshold was, the less shade-tolerant was the species. Sugar maple had a low threshold that allowed it to survive at very low light, but yellow birch, as expected, had a higher threshold.

Leaves were the most important organ in determining the survival of trees in the stress caused by low light. Among all of the organs of the tree that were studied, the leaves had the lowest light threshold and could be formed at very low light levels at which the growth of other organs (e.g. stems and roots) was critically restricted. This would increase carbohydrate availability for the survival of the tree.

Forest management

The growth (e.g. height and collar diameter) of both species did not increase either in gaps where 25% of the cover had been removed or when the woody competitors had been removed. In the system of selective cutting, the intensity of removal generally ranges from 20% to 40%. The requirement for light of saplings in the understory changes with their size and age. For instance, 3 year old Norway spruce saplings may grow normally in 7% of full sunlight, but, at 18 years they need 16-20 % of light for normal growth (Tang 1997).

Therefore, the determination of the optimal intensity of removal involves multiple abiotic and biotic factors, that need further study. The addition of a low dose of lime only improved growth of the saplings in the plots where competing vegetation had not been removed. Thus, in dense vegetation, liming may have a benefit.

The effects of elevated CO_2

Elevated CO_2 increased the shade-tolerance of both species by decreasing the light thresholds. However, yellow birch would possibly be better able to compete at elevated CO_2 than sugar maple as elevated CO_2 caused a higher increase in photosynthesis and root biomass for yellow birch, which would possibly make it a better competitor in elevated CO_2. These responses, however, need to be investigated in a natural environment. Elevated CO_2 increased the ectomycorrhizal colonization, but probably not arbuscular mycorrhizal colonization.

Literatures Cited

Abbott, L. K. and A. D. Robson. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agriculture, Ecosystems and Environment 35:121-150

Anderson S, Söderström B (1995) Effect of lime ($CaCO_3$) on ectomycorrhizal colonization of *Picea abies* (L) Karst. seedlings planted in a spruce forest. Scandinavian Journal of Forest Research 10: 149-154.

Anderson F, Bergholm J, Hallbäken L, Möller G, Pettersson F, Popovic B (1995) Farabolförsöket – försurning, kalkning och kvävegödsling av en sydöstsvensk granskog (The Farabol experiment – acidification, liming and nitrogen fertilization in a Norway spruce stand in southeast Sweden). Sweden University of Agricultural Sciences, Department of Ecology and Environmental Research, Report No. 70., Uppsala 133p (in Swedish with English abstract).

Barker FS (1949) A revised tolerance table. J. For. 47: 179-181

Bakker MR (1999) The effect of lime and gypsum application on sessile oak (*Quercus petraea* (M) Liebl.) stand at La Croix_Scaille (French Ardennes) II. Fine root dynamics

Bakker MR, Nys C. (1999) Effect of liming on fine root cation exchange site of oak. J. of Plant Nutrition 22: 1567-1575

Bakker MR, Kerisit R, Verbist K, Nys C (1999) Effects of liming on rhizoshere chemistry and growth of fine roots and of shoots of sessile oak (*Quercus petraea*). Plant and Soil 217: 243-257

Bakker M, Garbaye J, Nys C (2000). Effect of liming on the ectomycorrhizal status of oak. For. Ecol. Manage. 126: 121-131

Beaudet M, Messier C (1998) Growth, and morphological responses of yellow birch, sugar maple, and beech seedlings growing under a natural light gradient. Can. J. For. Res. 28: 1007-1015

Berntson GM, Wayne PM, Bazzaz FA (1997) Belowground architectural and mycorrhizal responses to elevated CO_2 in *Betula alleghaniensis*. Funct. Ecol. 11: 684-695

Bethlenfalvay GJ, Pacovsky RS (1983) Light effects on mycorrhizal soybeans. Plant Physiol. 73: 969-972

Binkley, D. and D. Richter. 1986. Nutrient cycles and H^+ budgets of forest ecosystem. Adv. Ecol. Res. 16: 1-15

Binns WO (1985) Effects of acid depositions on forests and soils. The Environmentalist 5: 279-288

Botkin, D.B. (1993) Forest Dynamics : An Ecological Model. Oxford University Press, Oxford, pp 309.

Bowes G (1993) Facing the inevitable: Plants and increasing atmospheric CO_2. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44:309-332

Brown KM (1995) Design and analysis of experiments. Lakehead University, Canada. 188 p

Burke M.K, Raynal DJ (1998) Liming influences growth and nutrient balances in sugar maple (Acer saccharum) seedlings on an acidic forest soil. Environmental and Experimental Botany 39: 105-117

Burns RM, Honkala BH (1990) Silvics of North America. USDA, Forest Service. 637 p

Canham CD (1988) Growth and canopy architecture of shade-tolerance trees: Response to canopy gaps. Ecology 69: 786-795

Canham CD (1989) Different responses to gaps among shade-tolerant tree species. Ecology 70: 548-550

Chang F, Alexander M (1984) Effects of simulated acid precipitation on decomposition and leaching of organic carbon in forest soils. Soil Science 138: 226-234

Cheng. S. 1999. Influence of soil temperature on ecophysiological traits of four boreal tree species. M. Sc. F. Thesis. Lakehead University. 73p

Cheng S, Widden P, Messier C (2004) Light, artificial shading and tree size influence belowground development in yellow birch and sugar maple. Submitted to Plant and Soil.

Chertov O, Komarov A, Kolstrom M, Pitkanen S, Strandman H, Zudin S, Kellomaki S (2003) Modlling the long-term dynamics of populations and communities of trees in boreal forests based on competition for light and nitrogen. For Ecol. Manage. 176: 355-371

Claveau Y, Messier C, Comeau PG, Coates KD (2002) Growth and crown morphological responses of boreal conifer seedlings and saplings with contrasting shade tolerance to a gradient of light and height. Can. J. For. Res. 32: 458-468

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

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

DeBellis TD (2000) The effects of age and size of canopy gaps on the mycorrhizae of yellow birch (*Betula alleghaniensis*) and sugar maple (*Acer sacchrum*) from deciduous forests of the Québec city region. MS. Thesis, Concordia University, Montreal, Canada.

Delagrange S, Messier C, Lechowicz MJ, Dizengremel P (2004) Physiological, morphological and allocational plasticity in understory deciduous trees: importance of individual size and light availability. Tree Physio. Accepted for publication Nov. 2003.

Diaz S, Grime JP, Harris J, McPherson E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364: 616-617

Duliere JF, DeBruyn R, Malaisse F (2000) Changes in the moss after liming in a Norway spruce (Picea abies (L.) Karst.) stand of Eastern Belgium. For. Ecol. Manage. 136: 97-105

Entry JA, Cromack KJr, Stafford SG, Castellano MA (1987) The effect of pH and aluminum concentration on ectomycorrhizal formation in Anbies balsamea. Can. J. For Res. 17: 865-871

Environment Canada (1982) Canadian climate normals. Temperature and precipitation 1951-1980 Quebec. Atmospherical Environmental service, Environment Canada, Ottawa, Ontario

Environment Canada (1988) Climate Change Digest: The implications of climate change for natural resources in Quebec. CCD 88-08. pp 9

Environment Canada (1989) Climate Change Digest: Exploring the implications of climate change for the boreal forest and forestry economics of western Canada. CCD 89-02. pp18

Ericssion T (1995) Growth and shoot: root ratio of seedlings in relation to nutrient availability Plant and Soil. 168-169: 206-214

Ericssion T, Goransson A, Van Oene H, Gobran G (1995) Interactions between aluminum, calcium, and magnesium-Impacts on nutrition and growth of forest trees. Ecol. bull. 44: 191-196

Ericsson T, Rytter L, Vapaavuor E (1996) Physiology of Carbon Allocation in Trees. Biomass and Bioenergy, Elsevier Science Ltd. 11: 115-127

Field C, Mooney HA (1983) Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a California shrub. Oecologia 56: 348-355

Fitter AH, Graves JD, Self GK, Brown TK, Bogie DS, Taylor K (1998) Root production, turnover and respiration under two grassland types along a latitudinal gradient: influence of temperature and solar radiation. Oecologia 114: 20-30

Franken P, Gnädinger F (1994) Analysis of parsley arbuscular endomycorrhizas: infection development and mRNA levels of defense-related genes. Molecular Plant-Microbe Interactions 7: 612-620

Fu WR (1979) Statistic and Experimental Design. Beijing Forestry University Press, Beijing, pp 305-311.

Gendron F, Messier C, Comeau PG (1998) Comparison of various methods for estimating the mean growing season percent photosynthetic photon flux density in forests. Agricultural and Forest Meteorology 92: 57-73

Godbold DL, Wayne PM, Bazzaz FA (1997) Growth and mycorrhizal colonization of three North American tree species under elevated atmospheric CO_2. New Phytol. 137: 433-440

Goldstein GH, Brubaker LB, Hinckley TM (1985) Water relations of white spruce at tree line in north central Alaska. Can. J. For. Res. 15: 1080-1087

Goodman DM, Trofynow JA (1998) Comparisons of communities of ectomycorrhizal fungi in old-growth and mature stands of Douglas-fir at two sites on southern Vancouver Island. Can. J. For. Res. 28: 574-581

Grayston SJ, Vaughan D, Jones D (1996) Rhizosphere carbon flow in trees in comparison with annual plants: The importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology 5: 29-56

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. The American Naturalist 111: 1169-1194

Hahn G, Marschner H (1998) Effects of acid irrigation and liming on root growth of Norway spruce. Plant and Soil 199: 11-22

Hampp R, Schaeffer C (1999) Mycorrhiza-Carbohydrate and Energy Metabolism. In: Varma A, Hock B (eds) Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. Springer-Verlag, New York, pp 273-303

Harris D, Paul EA (1987) Carbon requirements of vesicular-arbiscular mycorrhizae. Ecophysiology of VA Mycorrhizal Plants edited by Safir, G. R. CRC Press, Inc. pp: 93-105

Hausenbuiller RL (1985) Soil Science-Principles & Practices. 3th Edition. Wm. C. Brown Publishers. Dubuque. lowa. pp. 610

Havas M, Jaworski JF (1986) Aluminum in the Canadian environment. pp 331

Hayman DS (1974) Plant growth responses to vesicular-arbuscular mycorrhiza. New Phytologist 73: 71-80

Helmisaari HS, Hallbacken L (1998) Fine-root biomass and biomass production in limed and fertilized Norway spruce (Picea abies (L.) Karst) stands. For Ecol. Manage. 112: 99-111

Helmisaari HS, Makkonen K, Olsson M, Viksna A, Malkonen E (1999) Fine-root growth, mortality and heavy metal concentrations in limed and fertilized *Pinus silvestris* (L.) stands in vicinity of a Cu-Ni smelter in SW Finland. Plant and Soil 209: 193-200

Hodge A (1996) Impact of elevated CO_2 on mycorrhizal associations and implication for plant growth. Biol. Fertil. Soils. 23: 388-398.

Hodge A, Paterson E, Thornton B, Millard P, Killham, K (1997) Effects of photon flux density on carbon partitioning and rhizosphere carbon flow of *Lolium perenne*. J. of Experimental Botany 48: 1797-1805

Huitema BE (1980) The Analysis of Covariance and Alternatives John Wiley & Sons, Inc. pp 445

Hunt ERJr, Weber JA, Gates DM (1985) Effects of nitrate application on *Amaranthus powellii* Wats: III. Optimal allocation of leaf nitrogen for photosynthesis and stomatal conductance. Plant Physiol. 79: 619-624

Jakobsen I (1999) Transport of Phosphorus and Carbon in Arbuscular Mycorrhizae. In: Varma A, Hock B (eds) Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. Springer-Verlag, New York, pp 305-332

Jones MD, Durall DM, Tinker PB (1991) Fluxes of carbon and phosphorus between symbikonts in willow ectomycorrhizae and their changes with time. New Phytol. 119: 99-106

Jongen M, Jones MB (1998) Effects of elevated carbon dioxide on plant biomass production and competition in a simulated neutral grassland community. Annals of Botany 82: 111-123

Karen O, Nylun JE (1996) Effects of N-free fertilization on ectomycorrhiza community structure in Norway spruce stands in southern Sweden. Plant and Soil. 181: 295-305

Keyes MR, Grier CC (1981) Above- and below-ground net production in 40-year-old Douglas-fir stands on low and high productivity sites. Can. J. For. Res. 11: 599-605

Koch KE, Johnson CR (1982) Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and non-mycorrhizal root systems Plant Physiol. 75: 26-30

Kolek J, Kozinka V (1992) Physiology of The Plant Root System. Kluwer Academic Publishers, pp 361

Kozlowski TT, Kramer PJ, Pallardy SG (1991) The physiological ecology of woody plants. Academic Press, Inc., CA. 657 pp

Lambers H (1990) Growth, Respiration, Exudation and Symbiotic Associations: The fate of carbon translocated to the roots. In: Gregory PJ, Lake JV, Rose DA (eds) Root Development and Function. Cambridge University Press, pp 125-145

Lee HSJ, Jarvis PG (1996) The effects of tree maturity on some responses to elevated CO_2 in Sitka spruce (*Picea sitchensis* Bong.) Carr). In carbon dioxide and terrestrial ecosystems. Eds. G. W. Koch and H. A. Mooney. Academic Press, London, pp. 53-70

Leithold L (1976) The Calculus With Analytic Geometry. Third Edition. Harper & Row, New York, pp 744

Letho T (1994). Effects of liming and boron fertilization on mycorrhizas of *Picea abies.* Plant and Soil 163: 65-68

Leverenz JW (1996) Shade-shoot structure, photosynthetic performance in the field, and photosynthetic capacity of evergreen conifer. Tree Physiol. 16: 109-114

Lieffers VJ, Stadt KJ, Navratil S (1996) Age structure and growth of understory white spruce under aspen. Can. J. For. Res. 26: 1002-1007

Linderman RG (1988) Mycorrhizal interaction with the rhizosphere microflora: the mycorrhizosphere effect. Phytopath. 78: 366-371

Lorimer CG (1989) Relative effects of small and large disturbances on temperate hardwood forest structure. Ecology 70: 555-567

Long RP, Horsley SB, Liljia R (1997) Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. Can. J. of For. Res. 27: 1560-1570

Lovelock CE, Kyllo D, Winter K (1996) Growth responses to vesicular-arbuscular mycorrhizae and elevated CO_2 in seedlings of a tropic tree, *Beilschmiedia pendula.* Funct. Ecol. 10: 662-667

Majcen Z, Richard Y (1993) Resultats apres 5 ans d un essai de coupe de jardinage dans une erabiere. Can. J. For. Res. 22: 1623-16329

Majdi H, Kangas P (1997) Demography of fine roots in response to nutrient applications in a Norway spruce stand in southwestern Sweden. Ecoscience 4: 199-205

Marschner H (1995) Mineral nutrition of higher plants (2nd ed.). Academic Press, Ltd., CA. 889 pp.

McCully ME (1990) Selected Aspects of The Structure and Development of Field-Crown Roots With Special Reference to Maize. In: Gregory PJ, Lake JV, Rose DA (eds) Root Development and Function, Cambridge University Press, pp 53-70

McGonigle TP, Miller MH, Evans D, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115: 495-501

Messier C, Puttonen P (1995) Spatial and temporal variation in the light environment of developing Scots pine stands: The basis for a quick and efficient method of characterizing light. Can. J. of For. Res. 25: 313 –319

Messier C, Doucet R, Ruel JC, Claveau Y, Kelly C, Lechowicz MJ (1999) Functional ecology of advance regeneration in relation to light in boreal forests. Can. J. For. Res. 29: 812-823

Messier C, Nikinmaa E (2000) Effects of light availability and sapling size on the growth, biomass allocation, and crown morphology of understory sugar maple, yellow birch, and beech. Ecoscience 7: 345-356

Monz CA (1994) The response of mycorrhizal colonization to elevated CO_2 and climate change in *Pascopyron smithii* and *Bouteloua gracilis* Plant soil. 165: 75-80

Moore JD, Camire C, Ouimet R (2000) Effects of liming on the nutrition, vigor, and growth of sugar maple at the Lake Clair Watershed, Quebec, Canada. Can. J. of For. Res. 30: 725-733

Nakazato T, Kadota A, Wada M (1999) Photoinduction of spore germination in *Marchantia polymorpha L.* mediated by photosynthesis. Plant Cell Environ. 40: 1014- 1020

Nohrstedt HO (2002) Effects of liming and fertilization (N, PK) on chemistry and nitrogen turnover in acidic forest soil in SW Sweden. Water, Air and Soil Pollution 139: 343-355

Noland TL, Mohammed GH, Scott M (1997) The dependence of root growth potential on light level, photosynthetic rate, and root starch content in jack pine. New Forests 13: 105-119

Nowotny I, Daehne J, Klingelhoefer D, Rothe GM (1998) Effect of artificial soil acidification and liming on growth and nutrient status of mycorrhizal roots of Norway spruce (Picea abies (L.) Karst.). Plant and Soil 199: 29-40

Ozier-Lafontaine H, Lecompte F, Sillon JF (1999) Fractal analysis of the root architecture of *Gliricidia sepium* for the spatial prediction of root branching, size and mass: model development and evaluation in agroforestry. Plant and Soil 209: 167-180

Parke JL, Linderman RG, Trappe JM (1984) Inoculum potential of ectomycorrhizal fungi in forest soil from southwest Oregon and northern California. For. Sci. 30:300-304

Parsons WFJ, Miller SL, Knight DH (1994) Root-gap dynamics in a lodgepole pine forest: ectomycorrhizal and non-mycorrhizal fine root activity after experimental gap formations. Can. J. of For. Res. 24: 1531-1538

Pearcy RW, Smith DA (1994) Ecophysiological processes above- and belowground. In Exploitation of environmental heterogeneity by plants: Academic Press. New York. pp. 145-174

Pergitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR (1997) Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiol. 18: 665-670

Peterken GF (1996) Natural Woodland: Ecology and Conservation in Northern Temperate Regions. Cambridge University Press. Cambridge, UK. pp 522

Pospisilova J, Catsky J (1999) In handbook of plant and crop stress: effects of increased atmospheric CO_2 concentration on water use efficiency of plants. Marcel Dekker. New York. pp 1163-1184

Qian XM, Kottke I, Oberwinkler F (1998) Influence of liming and acidification on the activity of the mycorrhizal communities in a *Picea abies* (L.) Karst. stand. Plant and Soil 199: 99-109

Read DJ, Boyd R (1986) Water relations of mycorrhizal fungi and their host plants. *In* Water, fungi and plants edited by Ayres, P and L. Boddy. Cambridge University Press, Cambridge, pp 287-303

Read DJ (1999) Mycorrhiza- The State of The Art. In: Varma A, Hock B (eds) Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. Springer-Verlag, New York, pp 3-27

Rillig MC (1998) Arbuscular mycorrhizal percent root infection and infection intensity of *Bromus hordeaceous* grown in elevated atmospheric CO_2. Mycologia 90: 199-205

Roberts SD, Long JN, Smith FM (1993) Canopy stratification and leaf area efficiency: a conceptualization. For. Ecol. Manage. 60: 143-156

Romer M (2001) Carbon dioxide within controlled environments: the commonly neglected variable. In: Proceeding of the International Conference: Controlled Environments In The New Millennium. Norwich, United Kingdom

Runkle JR, Yetter TC (1987) Treefalls revisited: gaps dynamics in the Southern Appalachians. Ecology 68: 417-424

Sadowsky MJ, Schortemeyer M (997) Soil microbial response to increased concentrations of atmospheric CO_2. Glob. Change Biol. 3: 217-224

Sanders IR (996) Plant-fungal interactions in a CO_2 -rich world. Carbon Dioxide, Populations and Communities. pp. 265-272

Saralabai VC, Vivekandan M, Babu RS (1997) Plant responses to high CO_2 concentration in the atmosphere. Photosynthetica. 33: 7-37

Seegmuller S, Rennenberg H (994) Interactive effects of mycorrhizae and elevated carbon dioxide on growth of young pedunculate oak (*Quercus robus* L.) tree. Plant soil. 167: 325-329

Sheriff DW, Nambiar EKS, Fife DN (1986) Relationships between nutrient status, carbon assimilation and water use efficiency in *Pinus radiata* (D. Don) needles. Tree Physiol. 2: 73-88

Sikström U (1997) Effects of low-dose liming and nitrogen fertilization on stemwood growth and needle peoperties of *Picea abies and Pinus sylvestris*. For Ecol. Manage. 95: 261-274

Staddon PL, Fitter AH (1998) Does elevated atmospheric carbon dioxide affect arbuscular mycorrhizae? Tree. 13: 455-458

Staddon PL, Fitter AH, Graves JD (1999) Effect of elevated atmospheric CO_2 on mycorrhizal colonization, external mycorrhizal hyphal production and phosphorus inflow in *Plantage lanceolata* in association with the arbuscular mycorrhizal fungus *Glomus mosseae*. Glob. Change Biol. 5: 936-947

Stathers RJ, Spittlehouse DL (1990) Forest soil temperature manual. Forestry Canada, Victoria, B. C. 47 pp

Stennes, B. E. Krcmar-Nozic and G. C. van Kooten. 1998. Climate Change and Forestry: What policy for Canada? Working Paper. No. 2. pp 13

Sullivan MJ, Damicone JP, Payton ME. (2002) The effects of temperature and wetness period on the development of spinach white rust. Plant Disease 86: 753-759

Tang YH (1997). Light. In: Parsad MNV (eds) Plant Ecophysiology. John Whiley and Sons, Inc. New York, NY. USA. pp 41-56

Tarabula T (2000) The influence of experimental soil acidification and liming on concentration of some basic and trace metals in Scots pine (*Pinus sylvestris* L.) needles. Polish J. of Ecol. 48: 63-75

Tester M (1986) Effects of photon irradiance on the growth of shoots and roots, on the rate of initiation of mycorrhizal colonization units in *Trifolium subterraneum* L.New Phytol. 103: 375-390

Thompson S (1994) Harvesting methods in Canada's Forests. A discussion paper from the national round table on environment and the economy. Working paper 23 document. National Round Table and the Economy (NRTEE).

Thornton FC, Schaedle M, Raynal DJ (1986) Effect of aluminum on the growth of sugar maple in solution culture. Can. J. For. Res. 16: 892-896

Timmer VR, Savinsky HM, Marek GT (1983) Impact of intensive harvesting on nutrient budgets of boreal forest stands. pp. 131-147. *In* Wein, R.W., R.R. Reiwe and I.R. Methven (eds.). 1982. Proceedings of resources and dynamics of the boreal zone, Thunder Bay, ON. A.C.U.N.S. pp 544

Uddin W, Serlemitsos K, Viji G. (2003) A temperature and leaf wetness suring-based model for prediction of gray leaf spot of perennial ryegrass turf. Phytopathology 93: 336-344

Varma A (1999) Function and Application of Arbuscular Mycorrhizal fungi. In. Varma A, Hock B (eds) Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology. Springer-Verlag, New York. pp 521-569

Walters MB, Kruger EL, Reich PB (1993) Relative growth rate in relation to physiological and morphological traits for northern hardwood tree seedlings: species, light environment and ontogenetic considerations. Oecologia 96: 219-231

Walters M.B, Reich PB (1996) Are shade tolerance, survival, and growth liked? Low light and nitrogen effects on hardwood seedlings. Ecology 77: 841-853

Walters MB, Reich PB (2000) Trade-off in low-light CO_2 exchange: a component of variation in shade tolerance among cold temperature tree seedlings. Func. Ecology 14: 155-165

Wang GM, Stribley DP, Tinker PB, Walter C (1993) Effects of pH on arbuscular mycorrhiza. I. Field observations on the long-term liming experiments at Rothamsted and Woburn. New Phytol. 124: 465-472

Watt MS, Whitehead D, Mason EG, Richardson B, Kimberley MO (2003) The influence of weed competition for light and water on growth and dry matter partitioning of young Pinus radiata at a dryland site. For Ecol. Manage. 183: 363-377

Webb DP (1976) Root growth in *Acer saccharum* Marsh. seedlings: effects of light intensity and photoperiod on root elongation rates. Bot. Gaz. 137: 211-217

Wegley TML (1999) The science of climate change, globe and U.S. perspectives. National center for atmospheric research, USA. Report. 51 p

Wilczynski CJ, Pickett STA (1993) Fine root biomass within experimental canopy gaps: evidence for a below-ground gap. J. Veg. Sci. 4: 571-474

Wilmot TR, Ellsworth, DS, Tyree M (1996) Base cation fertilization and liming effects on nutrition and growth of Vermont sugar maple stands. For Ecol. Manage. 84: 123-134

Wright AN, Niemiera AX, Harris JR, Wright RD (1999) Soil Management, Fertilization, and Irrigation - Preplant Lime and Micronutrient Amendments to Pine Bark Affect Growth of Seedlings of Nine Container-grown Tree Species. Hortscience 34: 669-674

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant soil 151: 105-117