AN ABSTRACT FOR THE THESIS OF

Suzanne W. Simard for the degree of Doctor of Philosophy in Forest Science presented on September 13, 1995 Title: Interspecific Carbon Transfer in Ectomycorrhizal Tree Species Mixtures

Abstract approved: Signature redacted for privacy. Perry
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The overall goal of this study was to investigate influences of ectomycorrhizae (EM) and interspecific carbon transfer on seedling performance in species mixtures. The objectives were to: *(i)* determine the potential for EM to link paper birch and Douglas-fir, *(ii)* quantify gross and net interspecific carbon transfer, and *(iii)* evaluate effect of transfer on seedling performance.

A soil bioassay showed that paper birch and Douglas-fir shared seven EM morphotypes in common over 90% of their root tips, indicating potential for hyphal connections. The number and percent colonization of shared morphotypes were greater when species were grown in dual- than monoculture.

Reciprocal labelling of paper birch and Douglas-fir with $^{13}CO_{2(gas)}$ and $^{14}CO_{2(gas)}$ in laboratory rootboxes and the field resulted in bi-directional transfer, with net gain by Douglas-fir. In rootboxes, gross and net transfer represented 29% and 4% of total isotope assimilated by both species. Net transfer was three times greater and one-way gross transfer to Douglas-fir 50% greater where interconnecting hyphae were left intact than where severed, but high p-values ($p>0.05$) leave in question whether hyphal connections facilitated transfer.

In the field, gross and net transfer between paper birch and Douglas-fir represented 4% and 2%, respectively, of total isotope assimilated in 1993, and 7% and 6%, respectively, in 1994. Net transfer to Douglas-fir occurred where Douglas-fir grew full sun in 1993, and in all light intensities in 1994. The change in amount transferred and shading effect between years coincided with greater root development and improved seedling vigor in 1994 than 1993. Net and gross transfer were two times greater in 5% than 50% or 100% sun treatments in 1994, suggesting transfer was affected by changes in photosynthate sink strength of Douglas-fir. Isotope transferred to western redcedar represented <1-18% of gross transfer between paper birch and Douglas-fir, indicating most carbon was transferred between EM species via interconnecting hyphae.

Douglas-fir seedlings were grown in untrenched and trenched treatments to evaluate the ability of overstory paper birch and Douglas-fir to influence seedling EM inoculation patterns and performance. Greater diversity of EM coincided with higher photosynthesis among seedlings in the untrenched than trenched treatment. The effect on seedling performance was attributed to differences in EM colonization, because trenching had no effect on soil water, soil nutrients, or light availability.

Interspecific Carbon Transfer in Ectomycorrhizal Tree Species Mixtures

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Suzanne W. Simard

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Suzanne W. Simard, Author

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Dedication

I dedicate this thesis to the women who have struggled before and beside me, particularly Ellen June Simard, my mother, Robyn Elizabeth Simard, my sister, and Winnifred Beatrice Gardner, my grandmother.

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Interspecific Carbon Transfer in Ectomycorrhizal Tree Species Mixtures

Chapter 1

Introduction

Mixed species forests in the wet climatic region of the interior (wet belt) of British Columbia are slowly being replaced by single species plantations as a result of cutting, site preparation, planting, weeding and stand tending practices that favor the most commercially valuable conifer species, particularly Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco). Early seral hardwoods such as paper birch (*Betula papyrifera* Marsh.) are being aggressively removed from conifer plantations, a practice which is supported by research that indicates birch competes for light, water and nutrients and thereby threatens conifer survival and growth (Gregory 1966, Mielikainen 1985, Simard 1990). Although conifer seedlings grow quickly following such intensive management, the plantations suffer from lowered species and structural diversity (Simard and Vyse 1994), high conifer mortality due to *Armillaria* and *Phellinus* root diseases (Morrison *et al.* 1991), and the potential for decreased long-term productivity over >1 rotation (Sachs 1995).

Although competition is an important process shaping the structure of forest plantations, paper birch also appears to benefit conifer seedling performance through enhanced nutrient cycling (Hendrickson *et al.* 1987), resistance to common root disease pathogens (Morrison *et al.* 1988), and support of a diverse rhizosphere microbial community (see Simard 1995). For example, *Betula* spp. are rich in ectomycorrhizal (EM) fungi (Watling 1984, Deacon and Fleming 1992, Jones 1995), and residual paper birch trees, stump sprouts or seedlings may provide important sources of inoculum for planted Douglas-fir seedlings. Established hardwoods have been shown in other studies to influence formation of mycorrhizal associations of neighboring seedlings (Amaranthus and Perry 1989, Borchers and Perry 1990, Deacon and Fleming 1992, Massicotte *et al.* 1994, Smith *et al.* 1995). Hyphal contact, connection and nutrient transfer between EM plants is thought common (Newman 1988) due to the low host specificity of many EM fungi (Molina *et al.* 1992). Rapid inoculation with EM fungi allows conifers to capture resources early and increase survivorship and growth (Amaranthus *et al.* 1987, Villeneuve *et al.* 1987, Molina and Amaranthus 1990, Hunt 1992) through the critical regeneration period in harsh environments, such as in deep shade (Miller and Allen 1992), drought (Borchers and Perry 1990), or at high elevation (Perry *et al.* 1987).

Mixed species management recently has gained favor in the wet belt forests of British Columbia as forest managers face high economic costs and unpredicted ecosystem changes associated with single species management, as well as social pressure to recognize non-timber values. The success of mixed species management hinges on a clear understanding of linkages

between above- *and* belowground ecosystem processes, as well as recognition of competitive *and* mutualistic interactions. The intent of this study is to determine the significance of interspecific carbon transfer, via mycorrhizal fungi or other belowground pathways, to tree species performance and community dynamics in paper birch and Douglas-fir mixtures. From a practical position, the results will contribute to knowledge of the ecological basis for spatial and temporal patterning (*i.e.*, density, proportion, spatial distribution, succession) of species mixtures in wet belt ecosystems. From a theoretical position, the results will contribute to the debate surrounding the importance of interspecific carbon transfer to plant community dynamics.

The significance of mycorrhiza-mediated carbon transfer to plant community dynamics has remained a controversial issue among ecologists over the past decade (Read *et al.* 1985, Newman 1988, Miller and Allen 1992). Newman stated in 1988 that "evidence on almost every aspect of mycorrhizal links and their possible roles is inadequate". Although hyphal linkage and nutrient transfer between mycorrhizal plants has long been recognized (e.g., Bjorkman 1960, Francis and Read 1984, Newman and Eason 1993), it is unknown whether net transfer occurs (*i.e.*, that gain in material by one plant exceeds that of its connected neighbor) and whether it is of suitable magnitude or timing to affect plant fitness, and hence plant community dynamics (Newman 1988, Miller and Allen 1992). Several specific problems are *(i)* one-way isotope transfer has been very small (^{14}C, Francis and Read 1984, Read *et al.* 1985) or very slow (^{32}P, Hamel and Smith 1992, Newman and Eason 1993) in most studies, *(ii)* the transfer mechanism and role of interconnecting fungi remain questionable, and *(iii)* the long-term effects of transfer on development of complex plant communities is unexplored (Miller and Allen 1992). If net transfer significantly affects plant fitness, then competitive dominance by some plants may be reduced and species diversity enhanced (Newman 1988). For example, Perry *et al.* (1989) showed that competition between Douglas-fir and ponderosa pine (*Pinus ponderosa*) was reduced when they were inoculated with four different species of EM fungi compared to the single greenhouse contaminant, *Thelephora terrestris*. They suggested that inter-seedling nutrient transfer through shared *Laccaria laccata* may have been responsible for enhanced Douglas-fir growth and nutrient status in mixture.

This thesis consists of five studies that address the potential for mycorrhizal fungi to form interspecific hyphal links and facilitate net carbon transfer among paper birch, Douglas-fir and western redcedar (*Thuja plicata*), and to evaluate the importance of the transfer phenomenon to seedling performance in tree species mixtures. The work was conducted in mixed wet belt forests of interior British Columbia. First, the potential for paper birch and Douglas-fir to form hyphal linkages was evaluated and an isotope labeling procedure developed in the laboratory. Second, net transfer between paper birch and Douglas-fir was quantified both in the laboratory and in the field under varying environmental conditions. Finally, the potential significance of hyphal connections to community dynamics was investigated by examining the influence of overstory trees on EM inoculation patterns and performance of understory Douglas-fir seedlings.

Each chapter of this thesis is written as a stand-alone document. Chapter 2 evaluates *(i)* the ability of paper birch and Douglas-fir to share compatible EM fungi and potentially form hyphal linkages, and *(ii)* the influence of neighboring seedlings on EM development. Seedlings were grown in the greenhouse in monoculture and dual culture in soils collected from a mixed species plantation in southern British Columbia. Other studies have shown that primary mycorrhizal hosts have a strong influence on mycorrhizal associations of neighboring secondary hosts (Eissenstat and Newman 1990, Deacon and Fleming 1992, Massicotte *et al.* 1994, Smith *et al.* 1995). For example, Massicotte *et al.* (1994) and Smith *et al.* (1995) found that some *Rhizopogon* and Dark Brown types colonized secondary hosts, such as *Tsuga heterophylla*, *Rhododendron macrophyllum* and *Gaultheria shallon*, when grown in mixture with a well-colonized primary host, ponderosa pine or Douglas-fir. Colonization is thought to be induced when extramatrical hyphae emanate from primary host roots and contact secondary host roots (Read 1987, Borchers and Perry 1990).

Chapter 3 describes a pilot study *(i)* to develop a flexible $^{13}CO_{2(gas)}$ pulse-labeling procedure portable to the field, and *(ii)* to identify the appropriate $^{13}CO_{2(gas)}$ pulse and chase regime for examining intraspecific carbon allocation patterns and interspecific belowground carbon transfer between paper birch and Douglas-fir seedlings. The stable isotope ^{13}C has previously had limited use as a tracer in physiological and ecological research (e.g. Svejcar *et al.* 1990, Miller and Rose 1992), mainly due to its high cost. Instead, $^{14}CO_{2(gas)}$ has been used to label plant shoots and then examine allocation of photosynthate to plant tissues, respiration, microbial associations, soil and interconnected plants (e.g., Paul and Kucey 1981, Francis and Read 1984, Jones *et al.* 1991). Pulse-labeling plants with ^{13}C is more attractive than ^{14}C for ecological tracer studies, however, because of its lower discrimination relative to ^{12}C during photosynthesis, greater safety, and lack of regulatory barriers for use in the field (e.g., Van Norman and Brown 1952, Svejcar 1990). This study modified procedures of Svejcar *et al.* (1990) for pulse-labeling tree seedlings in order to study carbon allocation patterns and interspecific carbon transfer via aboveground and belowground pathways. The labeling procedures were later applied in associated laboratory (Chapter 4) and field (Chapter 5) transfer experiments.

Chapter 4 describes a laboratory study that examines transfer of carbon isotopes between six-month-old EM paper birch and Douglas-fir seedlings growing in rootboxes in separate, root-restricting pouches filled with field soil. Carbon transfer between tree species was examined by reciprocal labeling with ^{13}C and ^{14}C. Amount of carbon transferred directly through EM connections versus indirectly through the soil pool was examined by comparing rootboxes where interconnecting hyphae were left intact versus where they were severed immediately prior to labeling. Earlier studies have demonstrated one-way inter-plant transfer of water, carbon and nutrients via interconnecting mycorrhizal fungi using isotope tracers (e.g., Duddridge *et al.* 1980, Francis and Read 1984, Newman and Eason 1993, Arnebrant *et al.* 1993). However, the extent of

net transfer between plants and whether it is sufficiently large to affect plant performance remain unknown (Newman 1988, Miller and Allen 1992).

Chapter 5 also examined carbon isotope transfer among seedlings, but was conducted on one- and two-year-old seedlings out-planted in the clearcut where soil was collected for the bioassay and rootbox studies. Two dual-isotope labeling experiments were conducted in 1993 and 1994 in order to examine *(i)* whether carbon isotope is translocated among EM paper birch and Douglas-fir and arbuscular mycorrhizal (AM) western redcedar seedlings, and *(ii)* how shading Douglas-fir affects the amount of carbon isotope transferred. Since western redcedar formed only AM and paper birch and Douglas-fir formed only EM, western redcedar was used to signal isotope transferred indirectly between seedlings via the soil pool. Carbon transfer between EM trees has previously been observed in the field by Bjorkman (1960) and Read *et al.* (1985), who labeled spruce or pine trees with ^{14}C and later detected it in neighboring EM but not AM plants. Laboratory studies also have shown that extent of mycorrhiza-mediated isotope transfer is affected by changes in source-sink relationships, such as those established by interspecific variation in net photosynthetic rate or nutrient status (Francis and Read 1984, Hamel and Smith 1992, Arnebrant *et al.* 1993). None of these studies have quantified the extent of net transfer in the field, however, leaving in question the ecological significance of the transfer phenomenon.

Chapter 6 takes a field bioassay approach to examine the ability of overstory trees to influence EM inoculation patterns (composition, richness and diversity) and performance (photosynthesis and growth) of Douglas-fir seedlings growing in the understory. Seedlings were grown in Untrenched and Trenched treatments in three mixed forests of paper birch and Douglas-fir. The ability of mycorrhizae to contact, colonize and form hyphal connections between species in the Betulaceae and Pinaceae families has previously been demonstrated by Read *et al.* (1985) and Arnebrant *et al.* (1993). Hyphal links to larger, established trees may be critical to survival of deeply shaded understory seedlings through rapid EM infection (Perry *et al.* 1987), direct transfer of organic nutrients (e.g., Francis and Read 1984, Arnebrant *et al.* 1993) and increased access to inorganic nutrients or water in a greater volume of soil (Eissenstat and Newman 1990). Conifer seedlings planted near an established tree may also form a greater diversity and different EM types than those planted in isolation (Borchers and Perry 1990, Deacon *et al.* 1983). Access to a diversity of EM inoculum is important to seedling fitness because the EM fungi differ in physiology (e.g., Dosskey *et al.* 1990, Dighton 1991) and allow seedlings to acclimate to changing environmental conditions over time (Perry *et al.* 1987).

Chapter 7 synthesizes results from all chapters and concludes the overall study.

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Chapter 2

Occurrence of Ectomycorrhizae on *Pseudotsuga menziesii* and *Betula papyrifera* Seedlings Grown in Mixture in Soils from Southern British Columbia

Abstract

Seedlings of *Pseudotsuga menziesii* and *Betula papyrifera* were grown in the greenhouse in monoculture and dual culture in soils collected from a young mixed species plantation in the southern interior of British Columbia. The objectives of the study were *(i)* to evaluate the ability of *Pseudotsuga menziesii* and *Betula papyrifera* to share compatible ectomycorrhizal fungi in order to assess their potential for hyphal linkages, and *(ii)* to study the influence of neighboring seedlings on ectomycorrhiza occurrence. Eleven ectomycorrhizal morphotypes were recognized, seven of which *Pseudotsuga menziesii* and *Betula papyrifera* seedlings shared in common over 90% of their root tips. The abundance and/or frequency of *Rhizopogon*, *Wilcoxina* and *Tuber* types on *Pseudotsuga menziesii*, and the frequency of *Lactarius*, *Hebeloma* and *Cenococcum* types on *Betula papyrifera* were affected by the presence of a neighboring seedling. The number of ectomycorrhizal morphotypes shared in common and colonization of root tips by common types were slightly greater when *Pseudotsuga menziesii* and *Betula papyrifera* were grown in dual culture than in monoculture.

Introduction

Douglas-fir (*Pseudostuga menziesii* Mirb. Franco) and paper birch (*Betula papyrifera*) often are cohorts in mixed seral forests in the wet southern climatic region of interior British Columbia. Douglas-fir frequently is planted and paper birch quickly seeds or sprouts following either wildfire or clearcutting with broadcast burning or mechanical site preparation (Simard and Vyse 1994). Paper birch is considered a major competitor with Douglas-fir for light and water in young plantations because of its prolific reproduction habits and rapid growth rates (Haeussler and Coates 1986, Simard 1990). As a result, paper birch has commonly been removed from plantations with the intent of promoting rapid growth of more commercially valuable Douglas-fir. Although competition is an important process shaping the structure of Douglas-fir plantations, paper birch also may benefit conifers by providing mycorrhizal inoculum, as well as improving site productivity (Hendrickson *et al.* 1987, Sachs 1995) and root disease resistance (Morrison *et al.* 1988).

Plant community composition has been shown to influence the formation of mycorrhizal associations of seedlings (Amaranthus and Perry 1989, Borchers and Perry 1990, Eissenstat and Newman 1990, Deacon and Fleming 1992, Massicotte *et al.* 1994, Smith *et al.* 1995).

Extramatrical hyphae emanating from hardwood roots, for example, may contact neighboring conifer roots and induce colonization (Read 1987, Borchers and Perry 1990). The ubiquity of extramatrical hyphae and low host specificity of AM fungi and many EM fungi (Molina *et al.* 1992) has led to the hypothesis that hyphal links between plants are quite common (Newman 1988). The ability of different plant species to form mycorrhizae with the same fungal species has additional ecological consequences, including inter-plant transfer of carbon or nutrients (e.g., Francis and Read 1984, Finlay and Read 1986, Hamel and Smith 1992, Newman and Eason 1993, Arnebrant *et al.* 1993), transfer of nutrients from dying to living roots (Ritz and Newman 1985, Eason and Newman 1990), and alteration of the balance of plant-plant interactions (Grime *et al.* 1987, Newman 1988, Perry *et al.* 1989, Miller and Allen 1992, Perry *et al.* 1992). Groups of host plants that share common belowground mutualists (e.g., mycorrhizal fungi) have been referred to by Perry *et al.* (1989a) as "guilds", or associations which function "for mutual aid and the promotion of common interests".

Both Douglas-fir and paper birch form predominantly ectomycorrhizae (EM) (Harley and Harley 1987), although minor amounts of arbuscular mycorrhizae (AM) have been observed in roots of Douglas-fir (Cazares and Smith 1992, 1994) and paper birch (Malloch and Malloch 1981). Although paper birch and Douglas-fir are each exclusive hosts to some genera of ectomycorrhizal fungi (Molina *et al.* 1992), they also appear to share several fungi in common (Jones 1994, 1995), and hence potentially belong to the same plant guild based on their mycorrhizal associates and possibly other beneficial soil organisms. Ectomycorrhizae common to both Douglas-fir and paper birch growing in British Columbia soils include *Thelephora terrestris*, *Mycelium radicis atrovirens*, E-strain, *Laccaria* sp., *Cenococcum geophilum*, *Lactarius* sp, *Hebeloma* sp, and *Tomentella* sp. (Jones 1995). Douglas-fir also is frequently colonized by *Rhizopogon* section *Villosuli* (Molina and Trappe 1982b, Massicotte *et al.* 1994, Jones 1995, Smith *et al.* 1995). Ectomycorrhizal genera commonly associated with *Betula* include *Russula* (Jones 1995), *Leccinum*, *Boletus* and *Cortinarius* (Fleming 1985, Deacon and Fleming 1992, Molina *et al.* 1992). Many of these ectomycorrhizae form abundant extramatrical hyphae, which function in the mobilization and capture of nutrients and water in the soil (Read 1992), and some form strands or rhizomorphs, which are thought to function in the translocation of water, carbon and nutrients between plants (Brownlee *et al.* 1983, Duddridge *et al.* 1980 and 1988, Cairney 1992).

Mycorrhizae may affect plant-plant interactions in mixed species plantings by exchanging nutrients through interconnecting hyphae as well as improving general plant vigor through improved nutrition, improved water status, protection from pathogens, or detoxification of allelochemicals (Perry *et al.* 1989b). Perry *et al.* (1989b) found that competition between Douglas-fir and *Pinus ponderosa* was reduced when they were inoculated with four different species of ectomycorrhizal fungi compared to the single greenhouse contaminant, *Thelephora terrestris*. Seedling biomass and N and P uptake were greater for both tree species with the inoculated mycorrhizal species than

with the contaminant. Pioneer species, such as many early seral hardwoods which regenerate from surviving propagules, appear to retain a reservoir of soil organisms that benefit conifer establishment. Legacies of soil organisms provided by pioneer plant species following disturbance have been shown to influence mycorrhizal composition and performance of conifers in field studies (Amaranthus and Perry 1989) and in greenhouse soil bioassays (Borchers and Perry 1990, Smith *et al.* 1995, Massicotte *et al.* 1994). For example, Massicotte *et al.* (1994) and Smith *et al.* (1995) found that some *Rhizopogon* types or Dark Brown types colonized secondary hosts, such as western hemlock (*Tsuga heterophylla*), rhododendron (*Rhododendron macrophyllum*) and salal (*Gaultheria shallon*), when grown in mixture with a well-colonized primary host, ponderosa pine (*Pinus ponderosa*) or Douglas-fir. The ability of paper birch to function as a reservoir of ectomycorrhizal inoculum for neighboring Douglas-fir in southern British Columbia has not been evaluated.

To determine the potential for host plant species to be connected by compatible ectomycorrhizal fungi, the occurrence of ectomycorrhizae in single and dual species (*i.e.,* common garden) bioassays can be examined. This study focuses on results from a greenhouse bioassay with Douglas-fir and paper birch seedlings inoculated with soils collected from a planted (to Douglas-fir) clearcut in the southern interior of British Columbia that was originally forested with paper birch, Douglas-fir, and other ectomycorrhizal tree species. Our objective was to describe the type and abundance of ectomycorrhizae formed on Douglas-fir and paper birch seedlings when grown alone and in mixture. The influence of host specificity and host species interactions on patterns of colonization are discussed in relation to inter-plant hyphal connections, nutrient transfer and plant community dynamics.

Methods

Site description

Soil was collected in March, 1993, from the same site as that for the laboratory and field labeling experiments described in Chapters 4 and 5, respectively. The study site is located within the Clearwater Forest District, Kamloops Forest Region, of south-central British Columbia. It occurs within the Thompson variant of the Moist Warm Interior Cedar Hemlock biogeoclimatic subzone (Lloyd *et al.* 1991). The subzone is characterized by warm, moist summers and cold, snowy winters, with mean temperatures of 19°C in July and -6°C in January, and mean annual precipitation of 670 mm, of which 290 mm falls as rain during the growing season (Environment Canada 1980). The submesic-mesic site is a flat terrace (0-5% slope) at 700 m elevation, just above the North Adams River valley bottom. The soil is a Humo-Ferric Podzol (Canadian Soil

Survey Committee 1978), formed over a granitic alluvial blanket. The soil surface layers (to 50 cm) are sandy loam to loamy sand, with coarse fragment content less than 10%.

The original mixed forest of Douglas-fir (*Pseudotsuga menziesii*), paper birch (*Betula papyrifera*), western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), lodgepole pine (*Pinus contorta*), western white pine (*Pinus monticola*), and trembling aspen (*Populus tremuloides*) was clearcut in 1987 and planted to Douglas-fir in 1988. Areas between the planted seedlings were occupied by native hardwoods (primarily paper birch and trembling aspen), shrubs (primarily raspberry and thimbleberry), and forbs (primarily spreading dogbane and fireweed).

Soil collection and preparation

Soil was collected from the area (approximately 0.25 ha) where the field labeling experiments were conducted (Chapter 5). Soil was collected to 15 cm depth, including forest floor and buried organic material, from five sample points randomly located between Douglas-fir seedlings and paper birch sprouts. The five samples were combined to make one sample. The composite sample was placed in plastic bags, set on ice in a cooler, and then transported to the laboratory, where it was immediately sieved to 4 mm, homogenized, and mixed (3:1 by volume) with perlite to minimize compaction. Small woody debris was not removed from the sample. The soil sample was split, and one portion was left untreated and the other autoclaved at 180°C for 3 h for preparation of controls to detect ectomycorrhizal contaminants from the greenhouse. The soil mixtures were then distributed to sixty 50-mL (2-D) sterilized leach tubes: 30 tubes were filled with untreated soil mixture, and 30 with autoclaved mixture. The leach tubes were set up in a greenhouse at Oregon State University, Corvallis, Oregon.

Study design

The greenhouse bioassay consisted of tree species grown in monoculture and dual culture as "bait" for ectomycorrhiza fungal inoculum in the untreated field soil. The three treatments were represented by mycorrhizal host species: Douglas-fir alone (1 seedling per tube), paper birch alone (1 seedling per tube), and Douglas-fir and paper birch in mixture (2 seedlings per tube). The three treatments were replicated 16 times in a completely randomized design. Due to seedling mortality during the growing period, however, the number of replicates actually harvested was 16 of Douglas-fir alone, 12 of paper birch alone, and 9 of Douglas-fir and paper birch in mixture. The autoclaved control tubes also were planted to the three species mixtures to detect ectomycorrhizal contaminants from the greenhouse.

Seedling preparation and growth conditions

Douglas-fir seeds were surface sterilized and stratified by soaking first in 30% H_2O_2 for 2 min. and then in 3% H_2O_2 for 5 h, rinsed with distilled water, and dried at room temperature for 24 h. The same procedure was used for paper birch, except seed was soaked in aerated 10% H_2O_2 for 15 min. Five seeds per species were planted per monoculture leach tube on May 1, 1993. Sterile silica sand was spread over Douglas-fir seeds to 1 cm depth, and over paper birch seeds to a bare covering, to stabilize the surface and minimize mortality due to damping-off fungi. Ten seeds were planted in the dual culture tubes (5 seeds per species) and covered with 0.25 cm silica sand. Most seeds germinated within 2 weeks, and germinants were thinned to one per species per tube after 4 weeks. Every 6 months, each leach tube received 50 mL of Peters solution (20:20:20 N-P-K) to maintain seedling growth. The seedlings were grown for 16 months.

Seedlings were grown in the greenhouse under sodium-vapor lamps with a 16h/8h light/dark cycle complementing natural light and providing a minimum intensity of 280 µmol m^{-2} s^{-1}. Air temperature ranged between 24°C (light) and 18°C (dark). Leach tubes were watered on alternate days, and relocated on the greenhouse bench monthly to reduced environmental differences.

Mycorrhiza assessments

Leach tubes were randomly harvested and root systems examined over a one month period when seedlings were 16 months old. Seedlings were removed from leach tubes, roots of different species carefully separated, and then thoroughly washed with tap water. Ectomycorrhizal types were examined according to guidelines of Agerer (1987) and Ingleby *et al.* (1990). Root squashes and hand sections were examined microscopically to characterize fine details and determine the presence of a Hartig net. Distinguishing features were photographed on Kodak Ektachrome 160T Tungsten film using a Zeiss compound microscope and a Zeiss dissecting microscope.

Abundance of each EM type was determined by placing the entire root system over a clear plastic grid with numbered (2.5 cm^2) squares (Smith *et al.* 1995). Root tips were sampled in randomly selected squares to at least 100 tips per seedling. The proportion of root tips colonized by each of the various EM types was calculated for each seedling root system. For each treatment, *abundance* of a particular ectomycorrhizal type was the average proportion (%) of root tips per seedling per species colonized by that type (based on all replicates per treatment). *Frequency* was the number of seedlings per species on which a particular type was present. After mycorrhizae were identified and enumerated, root tissue was oven dried at 80°C for 48 h and weighed.

Statistical analysis

The ability of host tree species to share compatible ectomycorrhizal fungi was evaluated based on composition of ectomycorrhizal types on seedling root tips. Ectomycorrhizal abundance and seedling root biomass were compared between paper birch and Douglas-fir hosts using *T*-tests. Ectomycorrhizal abundance and seedling root biomass were also compared among host species and host species associations in a 2x2 factorial set of treatments (Douglas-fir alone, Douglas-fir in mixture, paper birch alone, paper birch in mixture) using analysis of variance (ANOVA) for unbalanced data in a completely randomized design. Interspecific associations of ectomycorrhizal types were tested using Chi-square tests of independence. All analyses were performed using SAS procedures (SAS Institute Inc.).

Results

Ectomycorrhizal types

Eleven ectomycorrhizal types were identified on paper birch and Douglas-fir hosts (Table 1). Seven types associated with both Douglas-fir and paper birch to varying degrees, one was unique to Douglas-fir and three unique to paper birch. Morphological characteristics allowed for identification to the genus level for all ectomycorrhizal types and the species level for some. The types are described below in order of abundance.

Ectomycorrhizal types common to Douglas-fir and paper birch

MRA type

MRA, *Mycelium radicis atrovirens* Melin, colonized 96% of Douglas-fir and paper birch seedlings, but was more dominant on the roots of paper birch (mean abundance 67%) than Douglas-fir (mean abundance 45%, Table 1). The ectomycorrhizae were cylindrical, unbranched, smooth, dark brown to black, with a loose mantle. The seedling root tip was usually uncolonized because it grew through the mantle. The mantle surface was an irregular locking synenchyma, or

Table 1. Abundance of ectomycorrhiza types on Douglas-fir and paper birch seedlings grown in potted soil from a mixed Douglas-fir and paper birch stand in the southern interior of British Columbia.

Ectomycorrhiza type[1]	Host	No. of seedlings colonized (controls)[2]	No. of colonized seedlings with <5% colonization (controls)	Mean abundance (%) of EM type on host[4]
MRA type	Douglas-fir Paper birch Host total	23/25 (0%) 21/21 (0%) 44/46 (0%)	5/23 (na)[3] 0/21 (na) 5/44 (na)	45.0 (6.1)[5]b[6] 66.5 (3.8)a p=0.006
Wilcoxina type	Douglas-fir Paper birch Host total	22/25 (77%) 20/21 (35%) 42/46 (56%)	3/22 (0%) 0/20 (0%) 3/42 (0%)	35.8 (6.2)a 15.3 (2.3)b p=0.006
Cenococcum type	Douglas-fir Paper birch Host total	5/25 (0%) 19/21 (23%) 24/46 (12%)	3/5 (na) 18/19 (67%) 21/24 (67%)	0.7 (0.3)a 1.8 (0.5)a p=0.053
Tuber type	Douglas-fir Paper birch Host total	1/25 (0%) 13/21 (0%) 14/46 (0%)	1/1 (na) 6/13 (na) 7/14 (na)	0.1 (0.1)b 4.2 (1.3)a p=0.001
Thelephora type	Douglas-fir Paper birch Host total	3/25 (73%) 5/21 (77%) 8/46 (75%)	1/3 (5%) 2/5 (0%) 3/8 (3%)	1.0 (0.7)a 1.3 (0.7)a p=0.749
Humaria type	Douglas-fir Paper birch Host total	3/25 (0%) 5/21 (0%) 8/46 (0%)	0/3 (na) 2/5 (na) 2/8 (na)	1.1 (0.7)a 5.6 (3.2)a p=0.145
Laccaria type	Douglas-fir Paper birch Host total	2/25 (0%) 2/21 (81%) 4/46 (40%)	2/2 (na) 2/2 (5%) 4/4 (5%)	0.1 (0.0)a 0.2 (0.1)a p=0.416
Rhizopogon type	Douglas-fir Paper birch Host total	23/25 (0%) 0/21 (0%) 23/46 (0%)	2/23 (na) 0/0 (na) 2/23 (na)	15.7 (2.1)a 0.0 (0.0)b p=0.000
Hebeloma type	Douglas-fir Paper birch Host total	0/25 (0%) 12/21 (0%) 12/46 (0%)	0/0 (na) 8/12 (na) 8/12 (na)	0.0 (0.0)b 1.6 (0.6)a p=0.007
Lactarius type	Douglas-fir Paper birch Host total	0/25 (0%) 10/21 (0%) 10/46 (0%)	0/0 (na) 4/10 (na) 4/10 (na)	0.0 (0.0)b 3.1 (1.4)a p=0.020
Tuber-like type	Douglas-fir Paper birch Host total	0/25 (0%) 2/21 (0%) 2/46	0/0 (na) ½ (na) ½ (na)	0.0 (0.0)a 0.4 (0.3)a p=0.164

[1]Ectomycorrhizal types in descending order of abundance.
[2]Mean percent abundance on root tips of control seedlings.
[3]Not applicable because type absent from all control host seedlings.
[4]Mean abundance is the average percent of root tips colonized.
[5]Numbers in parentheses are standard errors.
[6]Values followed by the same letter are not significantly different at the 5% level.

fine jigsaw pattern (Smith 1993), with large (10 µm wide), pillowy hyphal cells. Extramatrical hyphae were 2-4 µm wide, light brown, septate, without clamps. Strands were absent.

Wilcoxina type

Wilcoxina type, or E-strain, colonized 91% of Douglas-fir and paper birch seedlings, but was more dominant on the roots of Douglas-fir (mean abundance 36%) than paper birch (mean abundance 15%, Table 1). Fruiting bodies of *Wilcoxina* were found in several leach tubes with paper birch and Douglas-fir. Features of *Wilcoxina* type corresponded closely with those described by Danielson (1982) for E-strain on *Pinus banksiana*. The ectomycorrhizae were branched, smooth, pale to reddish brown with a white tip. The mantle was a discontinuous, thick (4-12 µm wide), irregular synenchyma lock, with large (2-8 µm wide), thick-walled hyphal cells. Extramatrical hyphae were rare, light brown, 3-10 µm wide, branched, without clamp connections. Loose, reddish brown strands were rare.

Cenococcum type

Cenococcum geophilum type colonized 20% of Douglas-fir and 90% of paper birch seedlings, but usually (88% of those colonized) occupied less than 5% of the root systems (Table 1). The ectomycorrhizae were cylindrical, unbranched, up to 5 mm long, black, with a smooth texture under abundant emanating hyphae. The mantle was compact, black, net synenchyma, with a radiating isocentric pattern (Trappe 1971). Emanating hyphae were bristly, fragile, black, smooth, 3-5 µm wide, septate, without clamp connections. Strands were absent.

Tuber type

Tuber type was present on 62% of paper birch and only 4% of Douglas-fir seedlings, and was more abundant on the root systems of paper birch (mean abundance 4%) than Douglas-fir (mean abundance <1%, Table 1). The ectomycorrhizae were single to infrequently branched, stout, smooth, creamy buff colored, with abundant, needle-like, tapering setae bristling out from the mantle surface. The setae were 50-150 µm long and 3-4 µm wide. The mantle was a smooth, compact, irregular interlocking synenchyma, 10-20 µm wide. Emanating hyphae were rare, hyaline, smooth, rarely branched, 2-5 µm wide, septate, without clamp connections. Strands were absent.

Thelephora type

Thelephora type occurred on 17% of paper birch and Douglas-fir and occupied only 1% of their root systems (Table 1). The ectomycorrhizae were pinnately branched, light to medium brown. The compact mantle was smooth to rough textured, with infrequent emanating hyphae. The emanating hyphae were smooth, hyaline, 4 µm wide, infrequently branched, with clamp connections. Cystidia were hyaline, 4 µm wide and 100 µm long, with basal clamp connections. Light brown strands were occasionally present.

Humaria type

Humaria type was present on 17% of paper birch and Douglas-fir seedlings, and was more abundant on root systems of paper birch (mean abundance 6%) than Douglas-fir (mean abundance 1%, Table 1). The *Humaria* type is referred to as E-strain by Ingleby *et al.* (1990). The ectomycorrhizae were single, rough, dark brown with a pale tip. The mantle was a discontinuous, compact, irregular synenchyma lock, with large (up to 8 µm wide) hyphal cells. Emanating hyphae were few, hyaline, 4-5 µm wide, minimally branched, without clamp connections. Strands were absent.

Laccaria type

Laccaria type occurred on 9% of paper birch and Douglas-fir seedlings, and occupied less than 5% of their root systems (Table 1). *Laccaria laccata* fruited in some of the leach tubes where this type occurred. The ectomycorrhizae were single, 8-10 mm long and 3-4 mm wide, whitish to dark brown, cottony textured. The loose, cottony mantle was a net prosenchyma of variable density. Abundant emanating hyphae were hyaline to light brown, 3-4 µm wide, moderately branched, tortuous, verrucose, with abundant clamp connections 2-4 µm wide. Loose cottony strands were present.

Ectomycorrhizal types unique to Douglas-fir

Rhizopogon type

Rhizopogon section *Villosuli* type occurred on 92% of Douglas-fir seedlings, where it occupied on average 16% of the root systems (Table 1). It was absent from paper birch seedlings. On Douglas-fir, ectomycorrhizae were single to pinnately-branched to pinnately-tuberculate-clumped, dark brown to light brown to white. The mantle was a rough, crusty, felt prosenchyma

with abundant external hyphae. Emanating hyphae were hyaline to light brown, 2-3 µm wide, smooth, with infrequent branching and without clamp connections. Cystidia were up to 10 µm wide and 3 mm long, and frequently bent. Brown strands were abundant, 25 µm wide.

Ectomycorrhizal types unique to paper birch

Hebeloma type

Hebeloma type occurred on 57% of paper birch seedlings, where it generally occupied <5% of the root systems (Table 1). It was absent from Douglas-fir. On paper birch, ectomycorrhizae were single to pinnately branched, yellow to blackish, crusty textured, with abundant adhering soil. The compact mantle was rough, with yellowish-hyaline hyphae up to 8 µm wide. Emanating hyphae were rare, hyaline, smooth, 4 µm wide, with very infrequent branching and without clamp connections. Loose strands were present.

Lactarius type

Lactarius type occurred on 48% of paper birch seedlings, where it occupied on average 3% of the root system (Table 1). It was absent from Douglas-fir. Ectomycorrhizae on paper birch were single to pinnately branched, smooth, swollen, long, white to yellowish. The compact, smooth mantle was a net synenchyma with lactiferous hyphae up to 10 µm wide. Emanating hyphae were 2 µm wide, hyaline, smooth, unbranched, with rare, flattish clamp connections. Compact, white-yellow strands were present.

Tuber-like type

Tuber-like type occurred on 10% of paper birch seedlings, where it occupied <1% of the root system (Table 1). It was absent from Douglas-fir. The ectomycorrhizae were single to infrequently branched, smooth, brown, with few, very fine, hyaline cystidia emanating from the mantle surface. The cystidia were 50 µm long and 2 µm wide. The dark color of the mantle as well as scarcity and small size of cystidia were the main features distinguishing *Tuber*-like type from *Tuber* type. The mantle was a smooth, compact, irregular interlocking synenchyma, 10-15 µm wide. Emanating hyphae were rare, hyaline, smooth, rarely branched, 2-3 µm wide, without clamp connections. Strands were absent.

Greenhouse contaminants

The greenhouse contaminants on Douglas-fir control seedlings were *Wilcoxina* type and *Thelephora* type (Table 1), which each infected 75% of the seedlings and occupied 65±8% and 58±9% of their root tips, respectively. *Wilcoxina* type also occurred on the majority of Douglas-fir in unsterilized soil, but occupied on average only 36±6% of the root systems. *Thelephora* type was detected on <20% of Douglas-fir in unsterilized soil, and occupied on average only 1% of the root systems. The greenhouse contaminants on paper birch control seedlings were mainly *Thelephora* type, *Laccaria* type and *Wilcoxina* type and, occasionally, *Cenococcum* type (Table 1). *Thelephora* and *Laccaria* types each infected >75% of control paper birch seedlings, and occupied 65±8% and 54±9% of their root tips, respectively. Both types were detected on <25% of paper birch seedlings in unsterilized soil, and occupied <2% of their root systems. *Wilcoxina* and *Cenococcum* types each infected <35% of control paper birch seedlings, and occupied only 4±1% and 1±0.5% of their root tips, respectively. Both types increased in both frequency (>90%) and abundance in unsterilized soils. The dramatic difference in abundance of *Wilcoxina*, *Thelephora*, and *Laccaria* types on Douglas-fir or paper birch between control and unsterilized soils suggests that the importance of their presence as contaminants in the greenhouse was diminished in the company of types local to Adams Lake.

Comparison of ectomycorrhizal colonization between Douglas-fir and paper birch

Douglas-fir and paper birch shared in common MRA type, *Wilcoxina* type, *Cenococcum* type, *Tuber* type, *Thelephora* type, *Humaria* type and *Laccaria* type (Table 1). Ectomycorrhizal types were determined as "common" where their morphological characteristics matched between tree species. *Rhizopogon* type was unique to Douglas-fir and *Hebeloma*, *Lactarius* and *Tuber*-like types were unique to paper birch. The dominant types on paper birch were MRA (mean abundance 67%), *Wilcoxina* (15%), *Humaria* (6%), *Tuber* (4%) and *Lactarius* (3%). The dominant types on Douglas-fir were MRA (mean abundance 45%), *Wilcoxina* (36%) and *Rhizopogon* (16%). The percent of root tips infected by common types averaged 84% for Douglas-fir and 96% for paper birch.

Of the shared types, all were of similar frequency among the two tree species, except *Cenococcum* and *Tuber* types were more frequent on paper birch (>60% of seedlings) than Douglas-fir (<20% of seedlings). Mean abundance of MRA, *Cenococcum* and *Tuber* types was greater on paper birch than Douglas-fir ($p<0.05$), and mean abundance of *Wilcoxina* type was greater on Douglas-fir than paper birch ($p<0.01$, Table 1).

Comparison of ectomycorrhizal colonization between seedlings grown alone and in mixture

Tuber type was absent from Douglas-fir when grown alone, but occurred on 11% (1/9) of the Douglas-fir seedlings grown in mixture with paper birch; it colonized on average 62% of paper birch seedlings in mixture and monoculture. Additional ectomycorrhizal types were not detected on root systems of paper birch when grown in mixture compared with when grown alone; however, frequency (number of seedlings colonized) and abundance (average percent of root tips colonized per seedling) of several types on paper birch and Douglas-fir were affected by the association. On Douglas-fir, *Wilcoxina* and *Tuber* types occurred on 10+% more seedlings in mixture (9/9 and 1/9, respectively) than monoculture (13/16 and 0/9, respectively), while *Rhizopogon* (7/9 in mixture versus 16/16 in monoculture), *Thelephora* (0/9 versus 3/16), *Humaria* (0/9 versus 3/16), and *Laccaria* (0/9 versus 2/16) types occurred on 10+% fewer seedlings (Table 2). On paper birch, *Cenococcum*, *Hebeloma* and *Lactarius* types increased in frequency by 10+% in mixture (9/9, 7/9 and 8/9, respectively) versus monoculture (10/12, 5/12 and 2/12, respectively), whereas *Thelephora* (1/9 in mixture versus 4/12 in monoculture) and *Laccaria* (0/9 versus 2/12) types decreased in frequency by 10+%. The differences in frequency of ectomycorrhizal types on Douglas-fir and paper birch when grown alone compared with in mixture resulted in significant variation in their frequency distributions as detected by Chi-square tests of independence ($p=0.000$, Table 2).

Ectomycorrhizal abundance was significantly affected by the host species association (grown in isolation versus mixture) for 2 of the 11 types: *Wilcoxina* type ($p=0.091$) and *Rhizopogon* type ($p=0.050$, Table 3). *Wilcoxina* type increased in abundance from 25% when Douglas-fir was grown alone, to 54% when grown in mixture with paper birch. Similarly, it increased in abundance from 13% to 19% on paper birch when mixed with Douglas-fir. Abundance of *Rhizopogon* type decreased from 20% when Douglas-fir was grown alone, to 9% when in mixture with paper birch. There were no interactions between host species and host species association in abundance/seedling for any of the ectomycorrhizal types.

Influence of inter-seedling competition on ectomycorrhizal colonization of hosts

We observed that paper birch grew faster and shaded Douglas-fir seedlings in the mixture leach tubes. As a result, root biomass of Douglas-fir in mixture with paper birch was only 15% of that grown in monoculture ($p=0.001$, Table 4). Conversely, paper birch growth appeared to benefit from the association with Douglas-fir; its root biomass was 1.7 times greater in mixture than monoculture ($p=0.079$). Shoot biomass was not measured for either species; as a result, we cannot determine whether biomass differences between mixture and monoculture seedlings

Table 2. Comparisons of frequency of ectomycorrhiza types on root systems of Douglas-fir and paper birch between those grown alone and those grown in mixture.

EM type	Douglas-fir alone	Douglas-fir in mixture	Mix 10%[2]	Paper birch alone	Paper birch in mixture	Mix 10%[3]
MRA type	16/16 (100%)[1]	9/9 (100%)		12/12 (100%)	9/9 (100%)	
Wilcoxina type	13/16 (81%)	9/9 (100%)	+	11/12 (92%)	9/9 (100%)	
Cenococcum type	3/16 (19%)	2/9 (22%)		10/12 (83%)	9/9 (100%)	+
Tuber type	0/16 (0%)	1/9 (11%)	+	7/12 (58%)	6/9 (67%)	
Thelephora type	3/16 (19%)	0/9 (0%)	-	4/12 (33%)	1/9 (11%)	-
Humaria type	3/16 (19%)	0/9 (0%)	-	3/12 (25%)	2/9 (22%)	
Laccaria type	2/16 (13%)	0/9 (0%)	-	2/12 (17%)	0/9 (0%)	-
Rhizopogon type	16/16 (100%)	7/9 (78%)	-	0/12 (0%)	0/9 (0%)	
Hebeloma type	0/16 (0%)	0/9 (0%)		5/12 (42%)	7/9 (78%)	+
Lactarius type	0/16 (0%)	0/9 (0%)		2/12 (17%)	8/9 (89%)	+
Tuber-like type	0/16 (0%)	0/9 (0%)		1/12 (8%)	1/9 (11%)	
Chi-square test[4]	p=0.000			p=0.000		

[1]Numbers in parentheses are percent of seedlings.
[2]Difference of +/- 10% between Douglas-fir in mixture and Douglas-fir alone.
[3]Difference of +/- 10% between paper birch in mixture and paper birch alone.
[4]Levels of significance were determined using Chi-square test of independence.

resulted from uniform changes in whole seedling growth or rather from shifts in allocation between roots and shoots. The lower root biomass of Douglas-fir in mixture corresponded with reduced frequency and abundance of *Rhizopogon* type, as well as reduced frequency of *Thelephora*, *Humaria*, and *Laccaria* types. Note, however, that frequency of *Thelephora* and *Laccaria* types also decreased among paper birch in spite of its increased root biomass in mixture versus isolation. contrast, greater root biomass of paper birch in mixture corresponded with increased frequency of *Cenococcum*, *Hebeloma* and *Lactarius* types.

Table 3. Abundance of ectomycorrhiza types on Douglas-fir and paper birch alone and in mixture.

Ectomycorrhiza type[1]	Douglas-fir alone	Douglas-fir in mixture	Paper birch alone	Paper birch in mixture	p-values[4]
MRA type	50.3[2](8.3)[3]	35.7 (8.1)	70.8 (4.8)	60.8 (5.9)	S, p=0.016** A, p=0.297 SxA, p=0.820
Wilcoxina type	25.4 (7.6)	54.4 (7.6)	12.6 (1.9)	19.0 (4.8)	S, p=0.030** A, p=0.091* SxA, p=0.230
Cenococcum type	0.7 (0.4)	0.7 (0.6)	1.9 (0.8)	1.8 (0.5)	S, p=0.079* A, p=0.639 SxA, p=0.940
Tuber type	0.0 (0.0)	0.3 (0.3)	4.0 (1.6)	4.4 (2.1)	S, p=0.001** A, p=0.360 SxA, p=0.381
Thelephora type	1.5 (1.1)	0.0 (0.0)	2.1 (1.2)	0.1 (0.1)	S, p=0.944 A, p=0.199 SxA, p=0.975
Humaria type	1.8 (1.0)	0.0 (0.0)	3.4 (3.3)	8.6 (6.2)	S, p=0.371 A, p=0.935 SxA, p=0.623
Laccaria type	0.1 (0.1)	0.0 (0.0)	0.3 (0.2)	0.0 (0.0)	S, p=0.471 A, p=0.287 SxA, p=0.634
Rhizopogon type	19.5 (2.6)	8.9 (2.7)	0.0 (0.0)	0.0 (0.0)	S, p=0.000** A, p=0.050** SxA, p=0.204
Hebeloma type	0.0 (0.0)	0.0 (0.0)	2.0 (1.0)	1.1 (0.5)	S, p=0.007** A, p=1.000 SxA, p=1.000
Lactarius type	0.0 (0.0)	0.0 (0.0)	2.5 (2.4)	4.0 (0.8)	S, p=0.061* A, p=0.562 SxA, p=0.581
Tuber-like type	0.0 (0.0)	0.0 (0.0)	0.5 (0.5)	0.2 (0.2)	S, p=0.170 A, p=0.980 SxA, p=0.980

[1]Ectomycorrhiza types in descending order of abundance.
[2]Mean abundance is the average percent of root tips colonized.
[3]Numbers in parentheses are standard errors.
[4]ANOVA of 2x2 factorial in completely randomized design, where S factor=species, A factor=association, and SxA=interaction.
ANOVA detected significant differences at *p<0.10 and **p<0.05.

Table 4. Root dry weight (g) of Douglas-fir and paper birch seedlings grown alone and in mixture in treatment soil.

Species	Alone	Mixture	p-value[3]
paper birch	1.06 (0.17)[1] b[2]	1.80 (0.35) a	0.079*
Douglas-fir	0.54 (0.10) a	0.08 (0.02) b	0.001**
p-values[4] S, p=0.000*** A, p=0.001*** SxA, p=0.000***			

[1]Numbers in parentheses are standard errors.
[2]Values followed by the same letter are not significantly different at the 10% level.
[3]T-test comparison between associations (alone versus mixture) for each species.
[4]ANOVA of 2x2 factorial in completely randomized design, where S factor=species, A factor=association, and SxA=interaction. Variable is natural log of root dry weight.
*T-test detected significant difference at p<0.10.
**T-test detected significant difference at p<0.05.
***ANOVA detected significant differences at p<0.01.

Discussion

Patterns of host specificity

The soil bioassay resulted in formation of ectomycorrhizae on Douglas-fir and paper birch in monoculture and mixture with eleven fungal species: eight EM types occurred on Douglas-fir (in order of abundance: MRA, *Wilcoxina*, *Rhizopogon*, *Humaria*, *Thelephora*, *Cenococcum*, *Tuber*, and *Laccaria* types) and ten on paper birch (in order of abundance: MRA, *Wilcoxina*, *Humaria*, *Tuber*, *Lactarius*, *Cenococcum*, *Hebeloma*, *Thelephora*, *Tuber*-like, and *Laccaria* types). Patterns of host specificity were similar to those observed by Jones (1995), who examined roots of two-year-old out-planted Douglas-fir and paper birch seedlings on the same site where soils were collected for the present study. All of the types identified in our bioassay appeared to correspond with types found on out-planted seedlings by Jones (1995). An additional 19 types occurred on the out-planted seedlings, which may have been a function both of a wider range of environmental niches available for a greater diversity of ectomycorrhizae as well as greater seedling age in field soils than greenhouse bioassay soil. Specifically, Jones (1995) reported that Douglas-fir root systems were dominated by *Rhizopogon*, *Humaria*, E-strain, MRA, *Thelephora*, and *Cenococcum*, with lower abundance of *Tuber*, and *Laccaria*, as well as 14 other minor types. Paper birch root systems were dominated by *Thelephora*, MRA, *Humaria*, E-strain and *Cenococcum*, with lower

abundance of *Lactarius*, *Hebeloma*, and *Laccaria* types, as well as 14 other minor types. Features of the E-strain type described by Jones (1995) matched those of *Wilcoxina* type identified in our study, which previously has been referred to as E-strain (Danielson 1982, Egger *et al.* 1991).

Common ectomycorrhizal fungi on young seedlings in greenhouses and disturbed sites include *Thelephora terrestris* (Molina and Trappe 1984), MRA (Danielson *et al.* 1985, Ingleby *et al.* 1990), E-strain (Danielson *et al.* 1985, Smith *et al.* 1995) and *Laccaria* (Molina and Trappe 1984) types. Occurrence (frequency, abundance) of these types on paper birch and Douglas-fir seedlings in our soil bioassay was similar to out-planted seedlings in Jones' (1995) field study, suggesting that greenhouse contaminants had a minor effect on mycorrhizal composition of our seedlings. All of the types common to both paper birch and Douglas-fir in this study (MRA, *Wilcoxina*, *Cenococcum*, *Tuber*, *Thelephora*, *Humaria* and *Laccaria* types) generally are common on a broad range of coniferous and deciduous hosts (Molina *et al.* 1992 Smith *et al.* 1995, Ingleby *et al.* 1990).

Unique to paper birch were *Lactarius* and *Hebeloma* types. *Lactarius* and *Hebeloma* have been identified as common ectomycorrhizal genera of *Betula* species in Great Britain (e.g., Ford *et al.* 1980, Fox 1983, Watling 1984, Mason *et al.* 1984, Last *et al.* 1984, Deacon and Fleming 1992). Several species of *Lactarius* and *Hebeloma* are listed as broad host ranging and several as narrow host ranging by Molina *et al.* (1992). *Lactarius* and *Hebeloma* types in our study were not identified to species, but were found only on paper birch. In contrast with our results, Jones (1995) found two types each of *Lactarius* and *Hebeloma* on out-planted seedlings of both paper birch and Douglas-fir, indicating that Adams Lake soil has inoculum potential for broad host ranging species of *Lactarius* and *Hebeloma*. The results of Jones (1995) indicate that our soil bioassay did not fully reflect the overlap in ectomycorrhizal types between the two tree species growing in Adams Lake soil.

Most ectomycorrhizal associates identified in our study have "early stage" characteristics (MRA, *Wilcoxina*, *Cenococcum*, *Thelephora*, *Humaria*, *Hebeloma* and *Laccaria* types) and some "late stage" characteristics (*Tuber*, *Lactarius*, and *Rhizopogon* types) described by Deacon and Fleming (1992). Early stage fungi are characterized by low carbon demands, ready establishment from spores or mycelium, and a ruderal behavior strategy. Conversely, late stage fungi are characterized by high carbon demands, establishment on older root systems or in older soil, poor establishment from spores, and usually advanced development of rhizomorphs or strands (Deacon *et al.* 1983, Last *et al.* 1984, Fleming 1983 and 1985, Deacon and Fleming 1992). The predominance of early stage versus late stage fungi in our study may have resulted from the young age of host seedlings and the growth restrictions imposed by the bioassay environment (e.g., small soil volume in leach tubes). Both classes of fungi potentially can form hyphal connections and facilitate nutrient or water transfer between plants. The probability that fungi form hyphal connections between paper birch and Douglas-fir is greatest among the "early stage" types

because most are broad host ranging (Newman 1988, Molina *et al.* 1992). However, the amount of material transferred between paper birch and Douglas-fir potentially is greatest among "late stage" fungi because strands or rhizomorphs are thought to play a greater role in translocation of water, nutrients or carbon than simple extramatrical hyphae (e.g., Cairney *et al.* 1992).

Rhizopogon sp. section *Villosuli* mycorrhizae were abundant on Douglas-fir but absent from all paper birch seedlings in our greenhouse bioassay, which agrees with field study results of Jones (1995). *Rhizopogon* species are numerous in western coniferous forests of North America, and are considered host genus-specific with Pinaceae (Molina and Trappe 1994). There are a few experimentally confirmed exceptions, however; *Rhizopogon* has been shown to form ectomycorrhizae with ericaceous host genera *Arbutus menziesii* Pursh and *Arctostaphylos uva-ursi* (Molina and Trappe 1982a), and *Gaultheria shallon* and *Rhododendron macrophyllum* in mixed plantings with well-colonized Douglas-fir (Smith *et al.* 1995). Read and Finlay (1985) also found that *Rhizopogon roseolus* spread from *Pinus* to *Betula* roots in seedling microcosms. Some have suggested the possibility of *Rhizopogon* links between conifers and companion plants, and subsequent facilitation of inter-plant nutrient transfer (e.g., Read and Finlay 1985, Smith *et al.* 1995). *Rhizopogon* sp. section *Villosuli* specifically, however, is specialized towards mycorrhizal association with Douglas-fir (Molina and Trappe 1994). In our study, *Rhizopogon* sp. section *Villosuli* did not form ectomycorrhizae with paper birch when in dual culture with well-colonized Douglas-fir seedlings. Members of the section *Villosuli* have had limited mycorrhizal development on western hemlock in pure culture syntheses (Molina and Trappe 1994), but not in dual culture soil bioassays where western hemlock was grown in mixture with well-colonized Douglas-fir hosts (Massicotte *et al.* 1994). In contrast, Smith *et al.* (1995) found in another polyculture soil bioassay that ectomycorrhizae of *Rhizopogon* section *Villosuli* repeatedly occurred on western hemlock, and they suggested that the length of time in the presence of a well-colonized primary host (Douglas-fir) may have influenced hemlock's colonization with the host specific fungus. In the present study, Douglas-fir and paper birch were grown in mixture for 16 months before their mycorrhizae were assessed, which is longer than other dual culture experiments where *Rhizopogon* inoculation of companion plants occurred (e.g., Read and Finlay 1985, Smith *et al.* 1995).

Effect of mixture

The change in ectomycorrhiza colonization of Douglas-fir and paper birch when grown together in mixture compared with monoculture included both appearance of an additional type on Douglas-fir as well as variation in frequency and abundance of several types on Douglas-fir and paper birch. *Tuber* type associated only with paper birch in single species tubes, but colonized both paper birch and Douglas-fir in one of nine dual culture tubes. The *Tuber* type was more abundant on paper birch (20% of root tips colonized) in that particular dual culture tube than in the

others where it was present (on average 4±1% of root tips colonized), suggesting that it may have spread from well colonized paper birch to Douglas-fir roots. More extensive colonization of paper birch with *Tuber* type increases the probability that its hyphae contact and initiate mycorrhizae on the secondary host, Douglas-fir. The result also appears to support the view that variation in colonization potential depends on whether a mycorrhizal fungus is already linked to a compatible host (Massicotte *et al.* 1994). The carbon requirements of some fungi to form ectomycorrhizae may not be satisfied by some "less compatible" hosts until it is supplemented by photosynthate from a linked "more compatible" host. Massicotte *et al.* (1994) drew a comparison with mycorrhizal succession on *Betula pendula* Roth. (e.g., Fleming 1983, Fleming *et al.* 1984), where robust groups of "late stage" fungi, such as *Lactarius* and *Tuber*, formed ectomycorrhizae with birch seedlings grown either in "mycorrhizal soil" (*i.e.*, soil from immediately beneath a birch tree) in the greenhouse or with access to roots of mature birch trees in the field. Conversely, "early stage" fungi, such as *Thelephora* and *Hebeloma*, formed ectomycorrhizae with birch seedlings grown either in "nonmycorrhizal soil"(*i.e.*, soil taken far away from a birch tree) or in isolation from mature birch trees in the field.

Rhizopogon abundance on Douglas-fir was significantly lower when it was grown in mixture with paper birch (9±3%) than when grown alone (20±3%). The reduction in *Rhizopogon* abundance corresponded with a dramatic reduction in Douglas-fir root biomass (0.08±0.02 g in mixture versus 0.54±0.10 g in isolation), presumably due to resource competition with paper birch. *Rhizopogon* ectomycorrhizae produced prolific rhizomorphs on Douglas-fir seedlings in this study, which is typical of many *Rhizopogon* spp. (Molina and Trappe 1994). Extensive rhizomorph (strand or chord) networks are thought largely responsible for enhanced nutrient uptake (Read 1992), water uptake (Duddridge *et al.* 1980, Brownlee *et al.* 1983), drought stress tolerance (Dosskey *et al.* 1990), and carbon, nutrient and water transfer between linked plants (e.g., Read *et al.* 1985, Duddridge *et al.* 1988). They also form the main linkages between a plant's root system and diffuse mycelial front (Read 1992), which has been shown as a major sink for photosynthate (Finlay and Read 1986). Based on fungal biomass (Deacon and Fleming 1992) and mycorrhizal stimulation of photosynthesis (Dosskey *et al.* 1990), the carbon drain on plants imposed by ectomycorrhizae with extensive rhizomorph networks (e.g., *Rhizopogon* in this study) would be much greater than those with weak sheath and extramatrical hyphal development (e.g., MRA or *Wilcoxina* types in this study). Considerable quantities of current photosynthate are allocated to extramatrical hyphae (e.g., Miller *et al.* 1989), which suggests that smaller Douglas-fir in mixture with paper birch would have lower capacity to support abundant *Rhizopogon* ectomycorrhizae than would more robust, healthier Douglas-fir growing in isolation. Interestingly, larger root biomass of paper birch growing in mixture with Douglas-fir (1.80±0.34 g) than in isolation (1.06±0.17 g) corresponded with a >70% increase in frequency of *Lactarius* type and >35% increase in frequency of *Hebeloma* type, both which were characterized in this study by presence of rhizomorphs.

Wilcoxina type abundance on Douglas-fir was significantly greater when it was grown in mixture with paper birch (54±8%) than when grown alone (25±8%). It was also more abundant on paper birch in mixture than isolation; however, the difference between associations was likely too small to be of ecological significance (19±5% in mixture versus 13±2% in isolation). E-strain mycorrhizae are prolific colonizers on burned sites and in nurseries (e.g., Danielson 1982, Wilcox *et al.* 1983), and are characterized by thin mantles and infrequent extramatrical hyphae (Ingleby *et al.* 1990). That they are host "generalists", have low vegetative capacity (Wilcox *et al.* 1983), and are readily replaced by other more aggressive fungi, is suggestive of the "early stage" descriptions by Deacon and Fleming (1992). On healthy, robust Douglas-fir seedlings, abundance of *Wilcoxina* type would normally decrease and abundance of ectomycorrhizal types such as *Rhizopogon* would increase with seedling age (J.E. Smith, personal communication). The photosynthate foodbase provided by Douglas-fir in mixture with paper birch may have been too small, however, for *Rhizopogon* and other robust fungi to colonize extensively, thereby allowing *Wilcoxina* type to persist.

The effect of species mixtures on mycorrhizal occurrence of host plants expands earlier observations in dual culture and polyculture experiments (e.g., Read and Finlay 1986, Massicotte *et al.* 1984, Smith *et al.* 1995). These polyculture studies showed that secondary host species (e.g., hemlock, birch) became mycorrhizal with atypical fungal associates (e.g., *Rhizopogon*) when grown in the presence of well-colonized primary host species (e.g., Douglas-fir, pine). Our study showed *(i)* that Douglas-fir formed mycorrhiza with an additional fungal type (but only 1/9 seedlings in mixture) and *(ii)* that the frequency and abundance of several other types changed in mixture relative to monoculture. More specifically, Douglas-fir became mycorrhizal with *Tuber* type, was under-represented by *Rhizopogon*-type, and over-represented by *Wilcoxina* type in mixture with paper birch relative to monoculture. At the same time, paper birch was more frequented by *Lactarius, Hebeloma* and *Cenococcum* types in mixture versus monoculture.

That the ectomycorrhizal composition of paper birch was affected by presence of Douglas-fir in spite of the latter's poor condition is suggestive of a "legacy" effect (Perry 1994) of Douglas-fir. In an ecological context, a "legacy" is anything handed down from a previous ecosystem condition (in this case, healthy Douglas-fir mixed with healthy paper birch), and usually influences the successional trajectory of the system. In this study *(i)* early influence of healthy Douglas-fir on advanced ectomycorrhizal composition of paper birch, and conversely *(ii)* influence of paper birch on ectomycorrhizal composition of Douglas-fir, persisted even after Douglas-fir had substantially declined. Alternatively, Douglas-fir seedlings may have spawned the "mixture effect" simply through its low vigor. The potential significance of the tree species "mixture effect" on ectomycorrhizal composition to inter-plant hyphal linkages and nutrient transfer are discussed below.

Potential for interspecific hyphal linkages

The low host specificity of AM and many EM fungi can result in physical hyphal connections between host plants of different species (Molina *et al.* 1992), which has been demonstrated using ^{14}C-labelling and autoradiography (Brownlee *et al.* 1983, Francis and Read 1984, Finlay and Read 1986). Hyphal connections between plants have been demonstrated to facilitate interspecific transfer of carbon, nutrients and water (e.g., Chiariello *et al.* 1982, Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986, Bethlenfalvay *et al.* 1991, Hamel and Smith 1992, Frey and Schuepp 1992, Arnebrant *et al.* 1993). Our study showed extensive overlap between Douglas-fir and paper birch in ectomycorrhizal associates, suggesting a potential for interspecific hyphal connections. Douglas-fir and paper birch growing in monoculture shared in common 6 ectomorphotypes over 80% and 95% of their root tips, respectively. When grown in mixture, the number of common types increased to 7 and proportion of root tips colonized by those types increased to 91% and 98% for Douglas-fir and paper birch, respectively. These results suggest that neighbors can influence transfer and feedback pathways within plant communities. However, it is possible that some of the shared morphotypes in this study were genetically incompatible, reducing the potential for functional links between species. This hypothesis could be tested by developing DNA profiles of these EM types using molecular genetic tools. Additionally, linkages and carbon transfer could more conclusively be demonstrated using isotope labeling techniques in the field, so that their ecological significance can be better evaluated.

Carbon and nutrients are thought to translocate through hyphal connections along electrochemical potential gradients from source to sink plants which differ in some way such as nutrient status or photosynthetic rate (Read *et al.* 1985, Newman 1988, Bethlenfalvay *et al.* 1991, Arnebrant *et al.* 1993). Rhizomorphs are viewed as important conduits for transfer (Duddridge *et al.* 1980, Brownlee and Jennings 1982, Brownlee *et al.* 1983, Duddridge *et al.* 1988), and their spatially separated inner vessel hyphae and outer living cortex hyphae are thought to function in bi-directional transfer of carbon compounds and nutrient ions, respectively (Cairney 1992). Of the common morphotypes identified in the present study, strands were frequently observed on *Thelephora terrestris*, occasionally on *Laccaria* type, and rarely on *Wilcoxina* type (note that the terms rhizomorph and strand are used interchangeably by Read (1992)). Occasionally a few extramatrical hyphae on MRA mycorrhizae appeared loosely aggregated (Ingleby *et al.* 1990). The strongest strand-formers, *Thelephora terrestris* and *Laccaria* type, usually colonized <5% of the root tips on both Douglas-fir and paper birch. These results suggest that carbon or nutrient transfer between Douglas-fir and paper birch via interconnecting ectomycorrhizal strands is possible, but the low frequency of the strongest strand-forming common fungi indicate that the magnitude of such exchange pathways may be limited.

Other strand-forming ectomycorrhizal fungi which were specific to only one host species were *Rhizopogon* type associated with Douglas-fir, and *Lactarius* and *Hebeloma* types associated with paper birch. Because of the restricted host range of *Rhizopogon* section *Villosuli*, it is unlikely to form interspecific linkages between Douglas-fir and paper birch. Intraspecific *Rhizopogon* section *Villosuli* linkages may occur among Douglas-fir individuals, however, as demonstrated with host family specific *Suillus bovinus* (Molina *et al.* 1992) linkages among *Pinus sylvestris* individuals (Read *et al.* 1985). The advanced and frequent strand formation among *Rhizopogon* spp. section *Villosuli* implies potential for substantial direct exchange pathways between Douglas-fir hosts. *Lactarius* and *Hebeloma* types only associated with paper birch in our study, but two common morphotypes of both genera were identified on out-planted Douglas-fir and paper birch seedlings by Jones (1995). Results of Jones (1995) provide further evidence for potential linkages between Douglas-fir and paper birch by strand-forming ectomycorrhizae in the field.

Extramatrical hyphae of EM should not be discounted as unimportant to interspecific linkages and nutrient transfer, however. Read *et al.* (1985) used autoradiography after feeding *Pinus sylvestris* donor seedlings $^{14}CO_2$ to show that carbon isotope had translocated through the entire fan-shaped mycelial network (extramatrical hyphae in addition to strands) of *Suillus bovinus* interconnecting donor and receiver seedlings. In addition, carbon and nutrient transfer has repeatedly been demonstrated to occur between plants interconnected by arbuscular mycorrhizae (e.g, Hirrel and Gerdemann 1979, Francis and Read 1984, Newman and Ritz 1986, Ritz and Newman 1986, Eissenstat 1990, Bethlenfalvay *et al.* 1991, Frey and Schuepp 1992, Hamel and Smith 1992, Newman and Eason 1993), which are more delicate in structure and do not form hyphal aggregates as seen in ectomycorrhizal systems (Read 1992). Given demonstration of inter-plant carbon and nutrient transfer through extramatrical hyphae in previous studies, the large number and abundance of types shared in common by paper birch and Douglas-fir, as well as occurrence of some shared strand-forming ectomycorrhizae, we expect that interspecific hyphal linkages are likely between Douglas-fir and paper birch. Hyphal linkages between species in the Pinaceae and Betulaceae families have already been documented by others. Arnebrant *et al.* (1993), for example, found that 5-15% of $^{15}N_{2(gas)}$ fixed by the association between *Alnus glutinosa* (Betulaceae) and *Frankia* was transferred to *Pinus contorta* (Pinaceae) via *Paxillus involutus* connections, and that approximately 20% of the nitrogen found in pine was derived from N_2-fixation by alder. Read and Finlay (1985) also found that *Rhizopogon roseolus* spread from ectomycorrhizae formed with *Pinus* spp. (Pinaceae) to roots of *Betula* sp. (Betulaceae) in seedling microcosms.

Summary

The greenhouse bioassay showed that mixtures of Douglas-fir and paper birch grown in field soil from south British Columbia shared in common 7 ectomycorrhizal types over 90% of their root tips. These results suggest there is potential for hyphal linkages to occur between the two species, and facilitate direct exchange of carbon, nutrients or water. However, the low levels of colonization by shared strand-forming ectomycorrhizae on Douglas-fir and paper birch as well as limitations of the greenhouse environment must be considered. Our results are in general agreement with EM colonization of out-planted Douglas-fir and paper birch (Jones 1995), suggesting that types and amounts found in our experiment reflect natural clearcut conditions in British Columbia. It is not known, however, whether the levels of colonization observed in our bioassay and by Jones (1995) would facilitate carbon or nutrient transfer between Douglas-fir and paper birch. This question can only be answered through careful isotope labeling experiments in a field setting.

Mycorrhizal-mediated carbon or nutrient transfer may be considered an ecologically significant process if it affects plant fitness. Possible consequences of ecologically significant transfer in plant communities are decreases in interspecific competition and enhancement of coexistence and species diversity (Newman 1988). Following disturbance such as wildfire or clearcut logging, surviving birch stumps may serve as necessary reservoirs of mycorrhizal inoculum to Douglas-fir germinants or planted seedlings. In mixed stands, Douglas-fir may directly benefit from association with nutrient-rich paper birch (see Chapter 5) through improved nutrition in nutrient-poor or patchy environments, or through supplemental carbon gain during conditions of low photosynthetic potential such as shade or drought. Conversely, paper birch may benefit from the association with Douglas-fir if the direction of transfer is reversed during early spring or fall when paper birch foliage is absent. If ectomycorrhizal connections and transfer are ecologically significant processes, then inter-pant interactions have more dimensions than resource competition alone. Further research is needed to evaluate the ecological consequences of these processes to plant community dynamics.

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Chapter 3

Intraspecific Carbon Allocation and Interspecific Carbon Transfer between *Betula papyrifera* and *Pseudotsuga menziesii* Seedlings using a ^{13}C Pulse-Labeling Method

Abstract

One year-old paper birch and Douglas-fir seedlings were pulse-labeled with $^{13}CO_{2(gas)}$ in the laboratory using a procedure that is portable to the field. A range of pulse (100-mL and 200-mL 99 atom% $^{13}CO_{2(gas)}$) and chase (0, 3 and 6 d) treatments were applied to identify the appropriate pulse-chase regime for examining intraspecific carbon allocation patterns and interspecific belowground carbon transfer. The amount of $^{13}CO_2$ fixed immediately after 1.5 h exposure was greatest for both paper birch (40.8 mg excess ^{13}C) and Douglas-fir (22.9 mg excess ^{13}C) with the 200-mL pulse, but higher ^{13}C loss and high sample variability resulted in little difference in excess ^{13}C content between pulse treatments after 3 d for either species. The average excess ^{13}C root/shoot ratio of paper birch and Douglas-fir changed from 0.00 immediately following the pulse to 0.61 and 0.87 three and six days later, which reflected translocation of 75% of fixed isotope out of foliage following the pulse and continued enrichment in fine roots over 6 d. Based on these results, the 100-mL $CO_{2(gas)}$ and 6-d chase were considered appropriate for carbon allocation and belowground transfer studies.

Greater pulse labeling efficiency of paper birch compared to Douglas-fir was associated with its two-fold and 13-fold greater leaf and whole seedling net photosynthetic rates, respectively, 53% greater biomass, and 35% greater root/shoot ratio. Species differences in isotope allocation patterns paralleled differences in tissue biomass distribution. After 6 d, paper birch had allocated 49% (average 9.5 mg) and Douglas-fir 41% (average 5.8 mg) of fixed isotope to roots, of which over 55% occurred in fine roots in both species.

The $^{13}CO_2$ pulse-labeling procedure was used to study belowground transfer of carbon from paper birch to Douglas-fir in laboratory rootboxes. A pulse of 100 mL $^{13}CO_{2(gas)}$ was applied to paper birch and transfer to neighboring Douglas-fir measured after 6 d. Of the excess ^{13}C fixed by paper birch, 4.7% was transferred to neighboring Douglas-fir, which distributed the isotope evenly between roots and shoots. Of the isotope received by Douglas-fir, it was estimated that 11% was taken up by foliage as $^{13}CO_{2(gas)}$ respired by paper birch shoots, none was taken by roots as $^{13}CO_{2(gas)}$ respired by paper birch roots, and the remaining 89% was taken up either directly via connecting mycorrhizal fungi or indirectly via the soil carbon pool.

Introduction

Paper birch (*Betula papyrifera* Marsh.) and Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) and are common cohorts in wet belt forests of the southern interior of British Columbia (Simard and Vyse 1994). Paper birch grows more rapidly than neighboring Douglas-fir in young plantations, indicating that its physiology and carbon allocation patterns are better suited to early community domination. Although paper birch competes with Douglas-fir for light and soil water (Simard 1990), it also has beneficial effects on long-term site productivity (Sachs 1995, in press) through high tissue nutrient concentrations (Hendrickson *et al.* 1987, Hendrickson 1987, Wang *et al.*, in press) and resistance to the common wet belt root diseases, *Armillaria ostoyae* and *Phellinus weirii* (Morrison *et al.* 1988). This competitive balance may also be affected by belowground carbon and nutrient transfer (Newman 1988), because paper birch and Douglas-fir share in common several ectomycorrhizal morphotypes (Chapter 2). Given the benefits of maintaining species mixtures (e.g., Perry *et al.* 1992), paper birch density is being carefully manipulated in Douglas-fir plantations to minimize adverse competitive effects. Defining mixtures optimal for sustaining site productivity requires an understanding of intraspecific carbon allocation and interspecific carbon transfer patterns.

Allocation of photosynthate to plant tissues, respiration, microbial associations, and soil has commonly been measured following exposure of plant shoots to $^{14}CO_{2(gas)}$ (e.g., Paul and Kucey 1981, Miller *et al.* 1989, Jones *et al.* 1991). The radioactive isotope has also been used to examine transfer of carbon between plants through inter-linking mycorrhizal fungi (Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986, Söderström *et al.* 1988, Duddridge *et al.* 1988, Arnebrant *et al.* 1993) or between ramets of clonal plants through connecting rhizomes or stolons (Jonsdottir and Callaghan 1989, Alpert *et al.* 1991). In contrast to ^{14}C, the stable isotope ^{13}C has had limited use as a tracer in ecological research. Although some studies have used ^{13}C tracers to study metabolism, carbon allocation or decomposition in grasses and herbs (Kouchi and Yoneyama 1984, Kim and Suzuki 1989, Svejcar *et al.* 1990, Berg *et al.* 1991, Miller and Rose 1992), none have implemented the technique in forest ecosystem studies. Most ecological research with ^{13}C instead has used the natural variation in relative abundances of ^{12}C and ^{13}C, and differential discrimination by C_3 and C_4 plants against $^{13}CO_2$ during photosynthesis and other metabolic pathways (O'Leary *et al.* 1992) to examine, for example, patterns in soil organic matter composition and decomposition (Nadelhoffer and Fry 1988, Balesdent *et al.* 1987), animal diets (Svejcar *et al.* 1993), water use efficiency (Farquar and Richards 1984), or spatial patterns in soil water availability (van Kessel *et al.* 1994).

Pulse-labeling plants with ^{13}C is more attractive than ^{14}C for ecological tracer studies because of its lower discrimination relative to ^{12}C during photosynthesis, greater safety, and lack of regulatory barriers for use in the field (Van Norman and Brown 1952, Buchanan *et al.* 1953, Craig

1954, Svejcar 1990). In particular, Van Norman and Brown (1952) showed that relative to ^{12}C the isotopes ^{13}C and ^{14}C are used during photosynthesis at rates of 0.96 and 0.85, respectively. Assuming that differences in discrimination between ^{13}C and ^{14}C also occur in other metabolic pathways along the translocation route from stomates to mycorrhizae, tracer studies utilizing ^{14}C would underestimate amounts of ^{12}C translocated within and between plants relative to ^{13}C.

Early ^{13}C tracer studies, where plant tissues were labeled with $^{13}CO_{2(gas)}$ during photosynthesis, involved long-term, continuous exposure to low concentrations of $^{13}CO_2$ inside elaborate, regulated labeling chambers (Kouchi and Yoneyama 1984, Kim and Suzuki 1989, Berg *et al.* 1991). A simpler pulse-labeling procedure, which has greater potential for application to field ecological studies, was developed by Svejcar *et al.* (1990) for use in a laboratory carbon allocation study. They used gas-tight plexiglass exposure chambers to label potted cheatgrass (*Bromus tectorum* L.) seedlings with 150 mL 99 atom % $^{13}CO_2$. The labeling procedure resulted in ambient total CO_2 concentration of 640 ppm inside the chamber, and increased $\delta^{13}C$ in shoots by nearly 450‰ 1 h postdose relative to baseline values. The same technique was applied in a field agricultural study by Miller and Rose (1992), who examined carbon allocation in *Agropyron desertorum.* One hour following pulse-labeling with 40 mL 99 atom % $^{13}CO_2$, $\delta^{13}C$ in shoots reached approximately 250‰ and roots -3‰, representing a substantial enrichment over natural abundance (-25‰).

The procedures of Svejcar *et al.* (1990) for labeling grasses in the laboratory need to be modified for pulse-labeling tree seedlings or other woody vegetation in the field. These modified procedures can be used to trace the fate of assimilates in plants and their natural environment, estimate the flux of assimilates through plant, fungal or microbial tissues, or identify the various C compounds which are stored or mobilized. This study in particular used ^{13}C labeling to study carbon allocation patterns within paper birch and Douglas-fir, and to quantify interspecific carbon transfer via aboveground and belowground pathways. The labeling procedures were later applied in associated field experiments (Chapter 5).

The objectives of this study were threefold: (1) Modify the procedure of Svejcar *et al.* (1990) for pulse-labeling tree seedlings in the laboratory or field. (2) Use the modified procedure to examine carbon allocation patterns in paper birch and Douglas-fir seedlings in the laboratory, and thereby identify the appropriate pulse-chase regime for labeling out-planted seedlings for interspecific carbon translocation experiments. (3) Use the modified procedure to study belowground transfer of carbon from paper birch to Douglas-fir growing in intimate mixture in rootboxes.

Methods

Seedling establishment

For the intraspecific carbon allocation experiment, six-month-old paper birch and Douglas-fir seedlings were transplanted from styroblocks to plastic pots in January, 1993, and then grown in a greenhouse for 6 months. The seedlings were of the same age, size, seed source and nursery as those planted for the field carbon translocation experiments (Chapter 5). The sandy loam mineral soil used to fill the pots was collected from the same field experimental site where the carbon translocation experiments were conducted, which was in a clearcut located in the Interior Cedar Hemlock (ICH) biogeoclimatic zone (Lloyd *et al.* 1991) of south-central British Columbia. Mineral soil was collected to 15 cm depth from 10 randomly located sample points, and composited into a single sample. The composite sample was transported in a cooler to the greenhouse, where it was sieved (2 mm), homogenized, mixed (3:1 by volume) with perlite, and distributed to 50 1-L plastic pots. Seedlings were planted in the soil mixture at a density of one per pot.

The soil mixture was also used to grow pairs of seedlings in rootboxes for the interspecific carbon translocation experiment, and individual seedlings in leach tubes for anapleurotic uptake measurements. For the carbon translocation experiment, six rootboxes were constructed using a modification of the root-mycocosm design described by Rygiewicz *et al.* (1988). Each acrylic plastic rootbox was 22 cm high, 20 cm wide and 3 cm thick, with a single root chamber. The root chamber was filled with the soil mixture, and Douglas-fir seed planted on one and paper birch on the other side of each rootbox. In preparation for planting, Douglas-fir seeds were surface sterilized and stratified by soaking first in 30% H_2O_2 for 2 min. and then in 3% H_2O_2 for 5 h, rinsed with distilled water, and dried at room temperature for 24 h. The same procedure was used for paper birch, except seed was soaked in aerated 10% H_2O_2 for 15 min. Five seeds of each species were planted per rootbox, and covered with sterile silica sand (½ cm depth) to stabilize the surface and minimize mortality due to damping-off fungi. The rootboxes were wrapped in aluminum foil to ensure darkness and reduce evaporation. After 4 weeks, seedlings were thinned to one per species. For anapleurotic uptake measurements, individual seedlings were grown in leach tubes using these same planting procedures.

All seedlings for both experiments were grown in the greenhouse under high intensity light (280 $\mu mol\ m^{-2}\ s^{-1}$), 16h/8h light/dark cycle, temperature range of 18°C (dark) to 24°C (light), and a daily watering regime. They were fertilized with 50 mL of Peters solution (20:20:20 N-P-K) monthly for 3 months to maintain seedling growth. Rootboxes and pots were relocated monthly to reduce environmental differences. Research was conducted at Oregon State University, Corvallis, Oregon.

Net photosynthetic rate

Net photosynthetic rate of potted paper birch and Douglas-fir seedlings was measured using a portable closed CO_2 gas analyzer (Li-Cor 6200, Lincoln, Nebraska). Net photosynthetic rate was used to estimate the approximate length of time required to reach the seedlings' CO_2 compensation point inside the labeling chamber (*i.e.*, appropriate pulse period). Light saturation curves for each species also were developed to determine the appropriate PAR (photosynthetically active radiation) intensity for maximum CO_2 uptake during the pulse period.

Measurements were made one week before the pulse-labeling experiment was conducted. Three randomly selected seedlings of each species were moved from the greenhouse into the laboratory 2 days prior to measurement, so that they could equilibrate with the measurement environment. Net photosynthetic rate was measured across a broad range of light intensities (0, 100, 500, 1000 and 2100 $\mu E\ m^{-2}\ s^{-1}$) on a new leaf from each seedling (the third leaf of paper birch, a lateral branch from top whorl of Douglas-fir). Each sample leaf or branch per seedling was measured four times. Air temperature (21±1°C), relative humidity (16±1%), and initial CO_2 concentration (385 ppm) were monitored at the same time to ensure consistency among samples. Sample leaves were immediately harvested and leaf area (one side) measured using a leaf area meter (Li-Cor 3100, Lincoln, Nebraska). Biomass was measured after leaves were oven-dried at 80°C for 48 h. Specific leaf area ($cm^2\ g^{-1}$) was calculated as the ratio of leaf area to corresponding leaf weight. Specific leaf area, whole seedling foliar biomass and leaf net photosynthetic rate were used to estimate whole seedling net photosynthetic rate.

Pulse-labeling procedure

Labeling chambers were designed to house a single seedling and permit injection and assimilation of $^{13}CO_{2(gas)}$. Aboveground air space of one-year-old seedlings was first isolated from the soil by sealing the top of the pots and root collar with plastic wrap and duct tape. A tomato frame was then placed over the seedling and fastened to the pot. The frame was used to support the plastic labeling bag in an upright, expanded position. The labeling bag was a flexible, air-tight, 5 mil thick x 60 cm wide x 90 cm tall, fluoropolymer gas sampling bag (Teflon Brand, Chemware), which was sealed around the entire framed seedling and pot using duct tape. Each sampling bag was fit with a silicone septum in a polypropylene housing for $^{13}CO_{2(gas)}$ injections with a gas-tight syringe. At the time of the first gas injection, the bags were completely sealed by screwing on the septum housing.

Seedlings were pulsed under high intensity lights and a circulating water bath, which maintained the seedling environment at room temperature. A 1.2 m x 1.2 m steel frame light stand was constructed to support the 90 cm x 30 cm x 8 cm open-topped, circulating acrylic plastic water

bath at approximately 1.0 m height. Six high intensity ESD lamps (General Electric) were wired 5 cm above the bath, and light diffusion plates (variegated plastic) taped to the bottom of the bath to diffuse the direct beam. The light intensity under the water bath was approximately 1000 μE m^{-2} s^{-1}. The steel frame was large enough to pulse 4 seedlings at once, which is equivalent to one replicate of pulse treatments (100- and 200-mL $^{13}CO_{2(gas)}$ applied to paper birch and Douglas-fir) for a single chase period.

Each seedling was pulse-labeled by injecting a fixed quantity of $^{13}CO_{2(gas)}$ into labeling chambers in four equal portions every 20 min. (0, 20, 40, and 60 min.) over a 90 min. period, allowing 30 min. for assimilation following the last injection At the time of each injection, the seedlings were rotated to ensure even light distribution. After the 90 min. pulse period was complete, the labeling chambers were removed, unused $^{13}CO_{2(gas)}$ allowed to escape, and the seedlings returned to the greenhouse for the 3 or 6 d chase period. Seedlings subject to the 0 d chase were placed under grow lights (100 μE m^{-2} s^{-1}) for 30 min. post-pulse and then harvested in a walk-in cooler.

Paper birch growing in rootboxes were labeled using the same technique applied to potted seedlings, except the labeling bags were smaller (15 cm wide x 15 cm tall) and support frames not necessary. In addition, because the rootbox seedlings were smaller than the potted seedlings, 100 mL $^{13}CO_{2(gas)}$ was applied only twice (50 mL at 0 and 15 min.) over a 45 min. pulse period.

The described pulse-labeling procedure represented modifications of the procedure described by Svejcar *et al.* (1990) as follows. *(i)* Instead of shallow plexiglass boxes, labeling chambers consisted of tall flexible bags supported by wire frames, allowing the chamber to be fit to the size of the seedling. *(ii)* Air inside the chambers was kept cool with an overhead circulating waterbath instead of with fans. *(iii)* Instead of a single injection, the pulse consisted of 4 sub-injections to approximate continuous labeling. *(iv)* CO_2 levels were not monitored in the labeling chambers, allowing several plants to be pulsed at once.

Intraspecific carbon allocation study design

The pulse-label experiment consisted of a range of $^{13}CO_{2(gas)}$ pulses and chase periods applied to individually potted paper birch and Douglas-fir seedlings in a 2x2x3 factorial set of treatments, where the three factors were species, pulse, and chase period. The two pulses were 100-mL (4.5 mmol, 58.5 mg ^{13}C) and 200-mL (9.0 mmol, 117 mg ^{13}C) 99 atom% $^{13}CO_{2(gas)}$ (Cambridge Isotope Laboratories), which were injected in four equivalent volumes (25 mL or 50 mL) every 20 min. Based on the chamber volume and atmospheric CO_2 concentration of 0.030% (Perry 1994), we estimate that $^{12+13}CO_2$ concentration in the chambers was 0.035% and 0.040% for the 100-mL and 200-mL pulse, respectively, representing a 15% difference in $^{12+13}CO_2$ concentration between pulses. The three chase periods were 0 (½ h for initial $CO_{2(gas)}$ fixation), 3

and 6 d. The treatments were replicated three times in a completely randomized design, requiring 36 seedlings. An additional three seedlings per species were harvested for ^{13}C natural abundance determinations.

Interspecific carbon transfer study design

Belowground carbon transfer from paper birch to Douglas-fir was examined by pulse-labeling paper birch growing in rootboxes with $^{13}CO_{2(gas)}$, and then measuring excess^{13}C in the neighboring Douglas-fir. Two treatments were applied to 6 rootboxes. The first treatment consisted of pulse-labeling paper birch with 100 mL $^{13}CO_{2(gas)}$ and allowing transfer to occur over 6 d. The second treatment consisted of unlabeled rootboxes planted with paper birch and Douglas-fir (controls) placed between labeled boxes during the chase, at the same interplant distance as paper birch and Douglas-fir within a single rootbox. This treatment was used to estimated the amount of $^{13}CO_{2(gas)}$ which was respired by paper birch and then re-assimilated by foliage of neighboring Douglas-fir in the same rootbox during the chase. It allowed separation of the amount of excess^{13}C in neighboring Douglas-fir which was received by aboveground mechanisms (fixation of $^{13}CO_{2(gas)}$ respired from paper birch shoots) from all belowground mechanisms combined (transfer directly through root grafts or mycorrhizal hyphae, and indirectly through the soil pool of root exudates, sloughed root and fungal cells, and respired $^{13}CO_{2(gas)}$).

Anapleurotic uptake

Three individual seedlings of paper birch and Douglas-fir each were randomly selected from those planted in leach tubes for measurement of anapleurotic uptake of $^{13}CO_{2(gas)}$ by roots. Immediately prior to labeling, seedlings were removed from leach tubes and their roots sealed up to the root collar inside 15 cm x 15 cm labeling bags. The roots were pulse labeled for 1 h with 10 mL $^{13}CO_{2(gas)}$, and then the bags were removed to release residual $^{13}CO_{2(gas)}$. The intact plants were stored in a dark cooler for 6 days, then separated into shoot and root fractions.

Seedling analysis

Prior to harvest, each potted seedling was measured for height and root collar diameter. Leaf area of 3 seedlings per species, representing a wide range in foliar biomass, was measured using a leaf area meter (Li-Cor 3100, Lincoln Nebraska). Seedlings were then harvested and separated into four tissue fractions: leaves, stems, coarse roots (>1 mm diameter), and fine roots (<1 mm diameter). Rootbox and anapleurotic seedlings were separated into shoots and roots only. Soil was gently washed off roots. The tissues were ovendried at 80°C for 48 h, weighed, and then

ground to 20 mesh in a Wiley mill. Ground tissue samples (1 mg) were combusted (Dumas Complete Combustion) for %C and $CO_{2(gas)}$ analyzed for ^{13}C abundance using a Europa Scientific ANCA-MS mass spectrometer.

Isotope calculations

Sample $\delta^{13}C$ (‰) was converted to milligrams C isotope using procedures described by Boutton (1991). The tissue $\delta^{13}C$ values first were converted to the absolute isotope ratio ($^{13}C/^{12}C$) of the sample (R):

$$R_{sample}=^{13}C/^{12}C=[(\delta^{13}C/1000)+1] \times R_{standard}$$

where $R_{standard}=0.0112372$, the international PDB standard. The fractional abundance (A) of ^{13}C relative to $^{13}C+^{12}C$ was then related to R by the equation:

$$A=^{13}C/(^{13}C+^{12}C)=R/(R+1).$$

Fractional abundance and total carbon content (mg) of the sample were used to calculate mg ^{13}C of the sample:

$$mg^{13}C_{sample} = A \times [^{13}C+^{12}C] \text{ (mg)}.$$

The enrichment level of the sample ($mg^{13}C_{sample}$) in excess of natural abundance ($mg^{13}C_{na}$) was calculated as:

$$\text{excess } mg^{13}C_{sample}=mg^{13}C_{sample}-mg^{13}C_{na}.$$

Excess $mg^{13}C$ of the tissue (excess $mg^{13}C_{tissue}$) was calculated as the product of excess $mg^{13}C_{sample}$ (per mg of sample) and tissue biomass (mg). Excess $mg^{13}C$ of the whole plant (excess $mg^{13}C_{plant}$) was determined by summing the excess $mg^{13}C_{tissue}$ of the all tissue types. Pulse labeling efficiency was defined as the ratio of excess $mg^{13}C_{plant}$ in plants harvested on day=0 to $mg^{13}C$ injected into the labeling chamber.

Statistical analysis

Light saturation curves were compared between paper birch and Douglas-fir using chi-square tests. The effects of pulse and chase treatments on excess ^{13}C (mg and ‰) content of

paper birch and Douglas-fir tissues were tested using analysis of variance (ANOVA) in a completely randomized design. In the carbon transfer study, the effects of pulse-labeling paper birch on excess ^{13}C content of neighboring Douglas-fir growing in the same rootbox, and on paper birch and Douglas-fir in control rootboxes, were tested using ANOVA. Means were separated using Waller-Duncan-Baye's multiple range test. All analyses were performed using SAS procedures (SAS Institute Inc. 1985).

Results

Intraspecific carbon allocation study

Seedling size and net photosynthesis

Paper birch were larger than Douglas-fir seedlings in height ($p=0.0245$), root collar diameter ($p=0.0001$) and total biomass ($p=0.0001$, Table 5). In particular, foliage and total root biomass were 59% and 82% greater among paper birch than Douglas-fir seedlings. The higher foliage biomass ($p=0.0012$) and specific leaf area ($p=0.0001$) amounted to higher whole seedling leaf area ($p=0.0001$) of paper birch than Douglas-fir. Paper birch distributed proportionately more biomass to coarse roots (25% versus 18%) and less to stems (25% versus 32%) than Douglas-fir, which was reflected in its 35% higher root/shoot ratio ($p=0.0001$).

The net photosynthetic response of individual leaves to increasing PAR differed considerably between species, with paper birch out-performing Douglas-fir at all light intensities except complete darkness ($p_{\chi 2}=0.0001$, Figure 1). At PAR of 1000 $\mu E/m^2/s$, leaf net photosynthetic rate was over 3 times higher for paper birch than Douglas-fir ($p=0.0001$, Table 5). The light intensity, 1000 $\mu E/m^2/s$, was considered saturating because average net photosynthetic rate exceeded 85% of maximum (measured at 2100 $\mu E/m^2/s$) for both species. Consequently, labeling for both the pulse-label and carbon translocation experiments was performed at PAR of approximately 1000 $\mu E/m^2/s$ to ensure high labeling efficiency.

Whole plant net photosynthetic rate, calculated from leaf net photosynthetic rate (PAR=1000 $\mu E/m^2/s$) and whole seedling leaf area, was estimated to be 13 times higher for paper birch than Douglas-fir in the labeling environment ($p=0.0001$). Based on average whole seedling net photosynthetic rates of 10.16 and 0.78 mmol s^{-1} for paper birch and Douglas-fir, respectively (Table 5), and assuming an ambient ^{12}C concentration of 0.03% (Perry 1994), the total $^{13+12}CO_{2(gas)}$ in the chamber was estimated to be consumed by both species in minutes. The staggered $^{13}CO_{2(gas)}$ pulses over 1.5 h was sufficient for full $^{13+12}CO_{2(gas)}$ fixation potential in all chambers.

Table 5. Size, biomass, specific leaf area and net photosynthesis of potted 6 month-old paper birch and Douglas-fir seedlings. Values are means and standard errors (parentheses). T-test detected differences between paper birch and Douglas-fir at ** $p<0.01$ and *$p<0.05$, d.f.=21 (d.f.=2 for specific leaf area).

Characteristic	Paper birch	Douglas-fir	p-value
Height (cm)	57.88 (3.38)	48.91 (1.67)	0.0245*
Root collar diameter (cm)	1.09 (0.06)	0.78 (0.02)	0.0001**
Total biomass (g)	30.33 (2.78)	19.76 (1.01)	0.0009**
foliage (g)	8.33 (0.79)	5.24 (0.41)	0.0012**
stem (g)	7.62 (0.81)	6.38 (0.32)	0.1638
coarse roots (g)	8.14 (0.86)	3.52 (0.23)	0.0001**
fine roots (g)	6.62 (0.49)	4.57 (0.30)	0.0009**
Root/shoot ratio	0.96 (0.04)	0.71 (0.03)	0.0001**
Specific leaf area ($cm^2\ g^{-1}$)	188.1 (33.6)	51.1 (0.2)	0.0001**
Whole seedling leaf area (cm^2)	1568 (148)	268 (21)	0.0001**
Leaf net photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$) at PAR 1000 $\mu E\ m^{-2}\ s^{-1}$	6.48 (0.68)	2.92 (0.32)	0.0001**
Whole seedling net photosynthetic rate ($mmol\ s^{-1}$)	10.16 (0.96)	0.78 (0.06)	0.0001**

Seedling isotopic composition

Pulse-labeling resulted in whole seedling excess ^{13}C contents of 25.11 mg and 14.55 mg for paper birch and Douglas-fir, respectively, averaged over all pulse and chase treatments ($p=0.0013$, Figure 2). The lack of species*pulse*chase interaction ($p>0.10$) indicated that paper birch isotope content was consistently higher than that of Douglas-fir across all treatments. Pulse labeling efficiency for paper birch and Douglas-fir was 57% and 25% for the 100-mL pulse, and 35% and 20% for the 200-mL pulse, respectively. Although labeling efficiency dropped with increased pulse, the 200-mL $^{13}CO_{2(gas)}$ pulse tended to raise average seedling isotope content 25% higher than the 100-mL pulse for both species ($p=0.1350$). Whole seedling excess ^{13}C content decreased with time lapsed since pulse-labeling due to respiration, exudation, tissue death, etc. Whole seedling isotope content following a 3 or 6 day chase period (mean=15.78 mg ^{13}C) averaged only 56% of that immediately following pulse-labeling (day 0, mean=27.95 mg ^{13}C) for both species ($p=0.0021$), reflecting a 44% carbon loss. All carbon isotope loss occurred during the first three days following pulse-labeling.

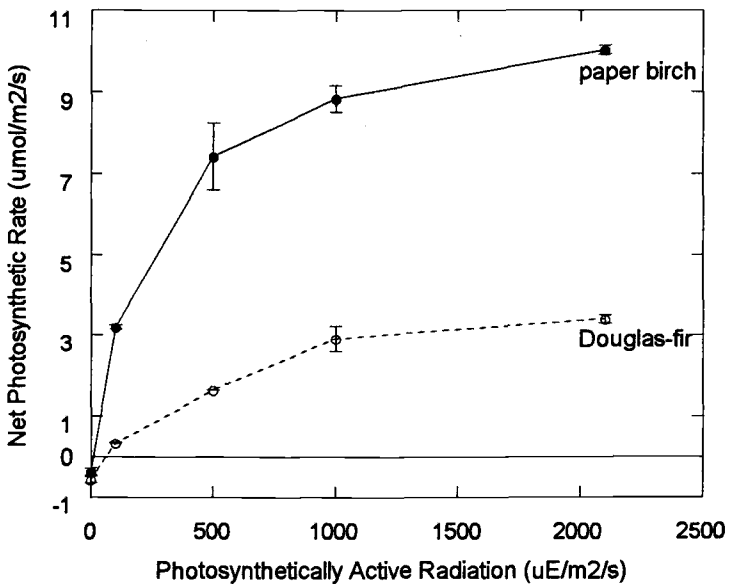

Figure 1. Net photosynthesis rate (A) vs. photosynthetically active radiation (PAR) for paper birch and Douglas-fir. Symbols represent mean ± 1 s.e. (n=3). Chi-square detected species distribution differences at p=0.0001.

The pattern of $\delta^{13}C$ (‰) among tissues varied with pulse and chase period both for paper birch and Douglas-fir (Figure 3, Table 6). The highest pulse (200-mL $^{13}CO_{2(gas)}$) resulted in greatest $\delta^{13}C$ (‰) among all tissues ($p<0.10$) averaged across both species and all chase periods. A significant interaction between pulse and species for coarse and fine roots ($p=0.0232$, $p=0.0049$) resulted from the fact that pulse had an effect on $\delta^{13}C$ of paper birch roots but not on roots of Douglas-fir. In contrast, the lack of interaction between pulse and chase for any tissue type ($p>0.10$) indicated that differences in tissue isotope contents resulting from different pulses were maintained throughout the entire 6-day chase period.

Chase period significantly influenced tissue $\delta^{13}C$ (‰) values of both species exposed to either pulse ($p=0.0001$, Figure 3, Table 6). No interactions occurred among chase, species and pulse treatments ($p>0.10$). For the 200-mL $^{13}CO_2$ treatment, $\delta^{13}C$ in paper birch foliage, stems, coarse roots and fine roots averaged 829‰, 0‰, -26‰ and -26‰, respectively, immediately following the pulse, and 182‰, 186‰, 200‰ and 198‰ 6 days later (Figure 3*b*). Douglas-fir followed the same pattern: foliage, stems, coarse roots and fine roots averaged 637‰, -14‰, -26‰ and -26‰, respectively, immediately following the 200-mL ^{13}C pulse, and 99‰, 85‰, 101‰ and 107‰ 6 days later (Figure 3*d*). These results indicate that most isotope was contained in foliage immediately following pulse-labeling, but that it was soon translocated to stems and roots resulting in an even distribution among all tissues after 6 days. Most ^{13}C translocation out of foliage occurred during the first 3 days following pulse-labeling ($p=0.0001$), but $\delta^{13}C$ values continued to increase in coarse and fine roots up to 6 days later ($p=0.0001$).

Expression of tissue isotope content on an excess mg ^{13}C rather than δ (‰) basis masked some species and pulse effects (Figure 4, Table 6). Paper birch had higher foliage, fine root and coarse root ($p<0.05$) but not stem ($p>0.10$) excess mg ^{13}C contents than Douglas-fir. Root/shoot ratio of excess mg ^{13}C content did not differ, however, between species ($p>0.10$, mean=0.49). The magnitude of the $^{13}CO_{2(gas)}$ pulse (100-mL versus 200-mL) did not influence tissue excess mg ^{13}C content ($p>0.10$) for either species. Dilution of significant species and pulse effects on tissue isotope contents by conversion from δ (‰) to excess mg ^{13}C basis may be due to cumulative error in natural abundance and biomass estimates. In contrast with pulse effects, chase period affected excess ^{13}C content of all tissue types in the same pattern as it affected $\delta^{13}C$ (‰). Average foliage excess ^{13}C content dropped from 26.92 mg immediately following pulse labeling, to 6.02 mg and 4.42 mg 3 and 6 days later ($p=0.0001$). At the same time, average stem and coarse root excess ^{13}C contents increased from 0.92 and 0.07 mg, respectively, immediately following the pulse, to 4.21 and 3.46 mg 3 days later, at which they stabilized for the remainder of the chase ($p=0.0003$, $p=0.0001$). In contrast to stems and coarse roots, fine root excess ^{13}C content continued to increase throughout the chase period ($p=0.0001$) for both species, from 0.04 mg immediately following the pulse to 2.30 mg 3 days later and 3.46 mg 6 days later ($p=0.0001$). The temporal patterns in tissue isotope content of paper birch and Douglas-fir are reflected in changing

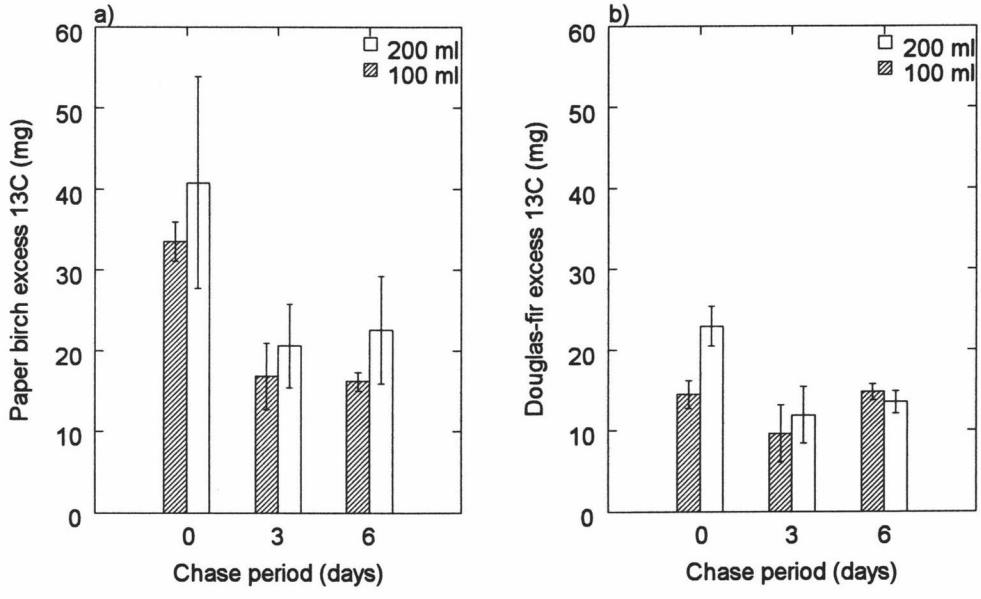

Figure 2. Whole seedling excess ^{13}C contents across pulse and chase treatments for (a) paper birch and (b) Douglas-fir. Values are means ± s.e. (n=3). ANOVA detected differences between species (p=0.0013) and chase (p=0.0021).

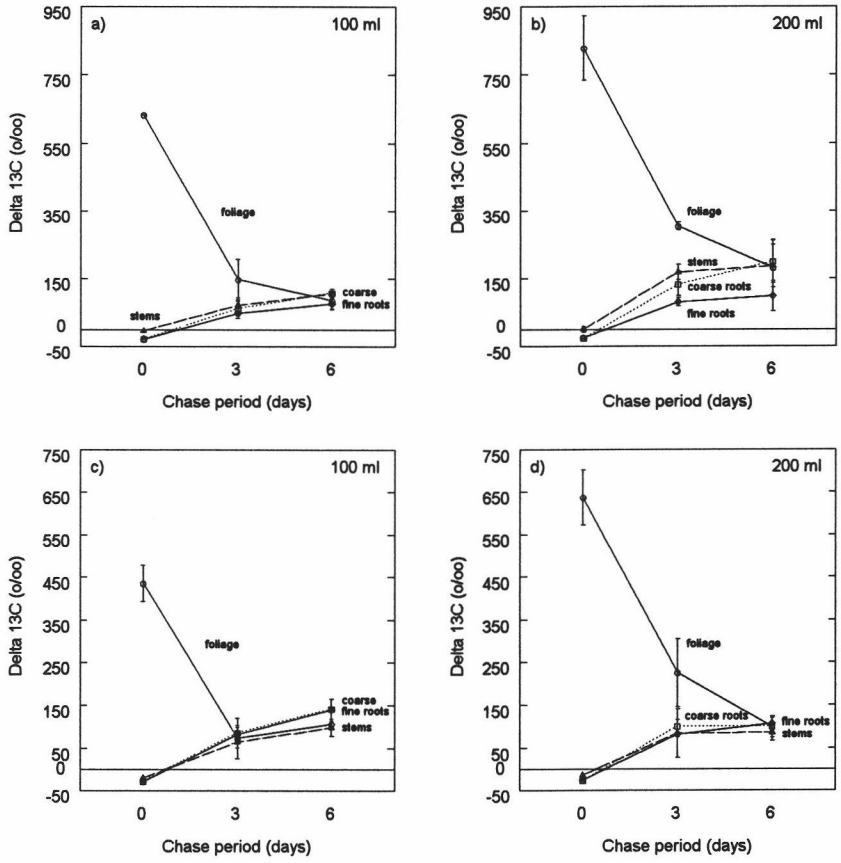

Figure 3. Tissue $\delta^{13}C$ vs chase for (a) birch 100 mL, (b) birch 200 mL, © fir 100 mL, and (d) fir 200 mL pulse. ANOVA detected differences between pulse and chase for all tissues ($p<0.10$). Values are means ± s.e. (n=3).

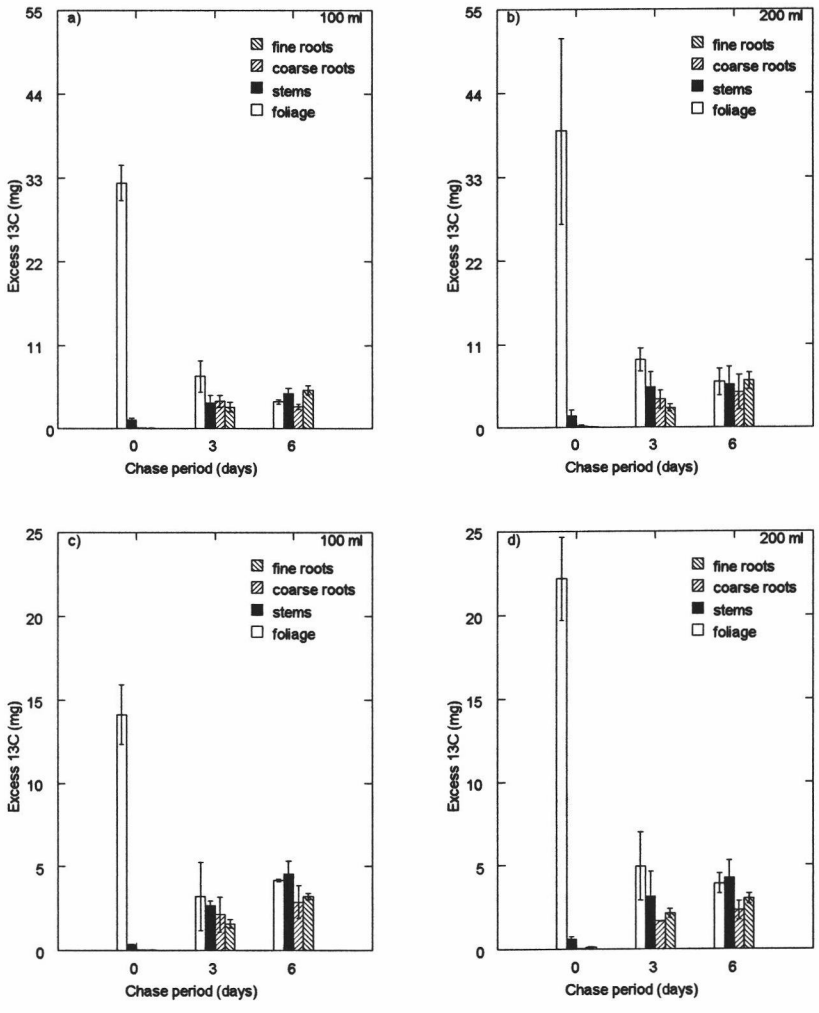

Figure 4. Tissue excess ^{13}C vs chase for (a) birch 100 mL, (b) birch 200 mL, © fir 100 mL, and (d) fir 200 mL pulse. ANOVA detected differences between chases ($p<0.05$) but not pulses. Values are means ± s.e. (n=3).

Table 6. Mean natural abundance (‰), total carbon(mg) and ^{13}C contents of paper birch and Douglas-fir seedlings. For ^{13}C contents, upper values are $\delta^{13}C$ (‰) and lower values are excess mg^{13}C. Standard errors are in parentheses. ANOVA detected differences among chase periods for all tissues ($p<0.01$). Different letters indicated significant differences ($p<0.01$) in tissue ^{13}C (‰ or mg) between chase periods. Differences among pulses were n.s. ($p>0.10$). Differences between species were significant for all tissues types ($p<0.05$) except stems ($p>0.10$).

Factor	Total	Foliage	Stems	Coarse roots	Fine roots
Natural abundance ‰					
paper birch	-	-29.3 (0.3)	-30.3 (0.3)	-29.7 (0.9)	-29.3 (0.3)
Douglas-fir	-	-27.7 (2.3)	-24.7 (2.9)	-27.0 (0.6)	-28.0 (0.6)
Total carbon (mg)					
paper birch	14,508 (1347)	4005 (394)	3926 (447)	3960 (434)	2617 (181)
Douglas-fir	9544 (496)	2595 (200)	3274 (166)	1734 (119)	1940 (144)
100-mL ^{13}C pulse					
paper birch					
o d	- 33.56 (2.44)a	633.0 (0.0)a 32.34 (2.31)a	-4.0 (2.9)b 1.18 (0.23)b	-27.7 (0.7)c 0.03 (0.03)b	-28.3 (0.3)c 0.00 (0.01)c
3 d	- 16.88 (4.08)b	148.0 (59.7)b 6.93 (2.06)b	72.0 (21.6)a 3.42 (0.99)a	62.7 (21.9)b 3.66 (0.79)a	47.7 (15.3)b 2.87 (0.62)b
6 d	- 16.21 (1.14)b	85.3 (25.1)b 3.57 (0.30)c	104.7 (15.2)a 4.64 (0.68)a	108.7 (11.8)a 2.90 (0.35)a	77.0 (15.5)a 5.10 (0.60)a
Douglas-fir					
o d	- 14.49 (1.69)a	436.7 (42.7)a 1 4.14(1.77)a	-20.33 (0.9)b 0.33 (0.05)b	-27.0 (0.6)c 0.04 (0.02)b	-28.7 (1.2)c 0.00 (0.03)c
3 d	- 9.61 (3.53)b	73.0 (47.0)b 3.22 (2.02)b	64.3 (13.5)a 2.68 (0.27)a	87.0 (16.9)b 2.14 (1.02)a	82.3 (16.7)b 1.58 (0.25)b
6 d	- 14.81 (0.98)b	107.3 (28.7)b 4.17 (0.08)b	98.7 (20.4)a 4.55 (0.78)a	143.3 (3.5)a 2.89 (0.98)a	140.0 (25.0)a 3.21 (0.20)a
200-mL ^{13}C pulse					
paper birch					
o d	- 40.80 (13.07)a	829.0 (95.7)a 39.01 (12.18)a	0.0 (7.6)b 1.57 (0.78)b	-26.0 (1.5)c 0.16 (0.11)b	-26.0 (0.0)c 0.06 (0.02)c
3 d	- 20.63 (5.15)b	304.0 (11.2)b 8.99 (1.52)b	168.7 (22.5)a 5.34 (2.07)a	132.3 (33.0)b 3.72 (1.22)a	81.0 (12.1)b 2.59 (0.46)b
6 d	- 22.60 (6.64)b	181.7 (83.3)b 6.04 (1.79)b	186.0 (62.9)a 5.69 (2.36)a	200.3 (62.7)a 4.69 (1.12)a	197.7 (44.2)a 6.18 (2.27)a
Douglas-fir					
o d	- 22.94 (2.41)a	637.3 (64.4)a 22.17 (2.49)a	-14.0 (3.79)b 0.63 (0.15)b	-26.3 (0.3)c 0.05 (0.01)b	-25.7 (1.5)c 0.10 (0.05)c
3 d	- 11.92 (3.50)b	225.3 (79.2)b 4.94 (2.03)b	84.3 (57.8)a 3.15 (1.50)a	101.0 (14.6)b 1.68 (0.02)a	81.0 (5.1)b 2.15 (0.28)b
6 d	- 13.53 (1.36)b	98.7 (25.4)b 3.92 (0.60)c	85.3 (19.1)a 4.25 (1.03)a	101.0 (5.5)a 2.33 (0.56)a	107.3 (12.4)a 3.03 (0.31)a

root/shoot ratios of excess ^{13}C, which climbed from 0.00 immediately following the pulse to 0.61 three days later and 0.87 six days later (p=0.0001).

Choice of the appropriate pulse-chase regime for labeling out-planted paper birch and Douglas-fir seedlings for below-ground interspecific translocation studies is based on isotope distribution among tissue types. The optimal regime is that which results in the highest isotope content in fine roots of both paper birch and Douglas-fir. Fine root isotope content of both paper birch and Douglas-fir was greatest following the 6 day chase (p=0.0001, Figure 5). Although $\delta^{13}C$ (‰) of paper birch fine roots benefited from the higher pulse, excess mg ^{13}C fine root content of neither paper birch nor Douglas-fir differed between pulse treatments. These results indicate that the 100-mL $^{13}CO_{2(gas)}$ pulse and 6-d chase are appropriate for quantifying belowground carbon translocation between paper birch and Douglas-fir.

Interspecific carbon transfer study

Paper birch and Douglas-fir growing in association in rootboxes differed in total and tissue-specific biomass. Shoot, root and total biomass of paper birch averaged 2.7, 1.5 and 2.0 times that of associated Douglas-fir (p=0.0038, p=0.0021, p=0.0815, respectively). Root/shoot ratio averaged 0.90 and did not differ between species (p>0.10).

Isotope transferred from paper birch to associated Douglas-fir averaged 580±11 µg, which represented 4.7% of the excess ^{13}C fixed in all paper birch and Douglas-fir tissues (12,190±395 µg) in the rootbox system (Figure 6). Isotope respired by seedlings in labeled rootboxes and re-assimilated by seedlings in control rootboxes averaged 66±10 µg and 38±7 µg for paper birch and Douglas-fir, respectively. The total isotope respired and re-assimilated by photosynthesis represented 0.85% of ^{13}C fixed in the rootbox system. Assuming that isotope content of Douglas-fir in control rootboxes represents the amount of isotope respired by paper birch foliage and subsequently re-fixed by associated Douglas-fir foliage through photosynthesis, we estimate that the isotope transferred via belowground mechanisms was 542 µg (*i.e.*, total transferred - fixation by control Douglas-fir = 580 µg - 38 µg = 542 µg), or 4.4% of the isotope fixed in all tissues in the rootbox system.

Excess ^{13}C content of donor birch seedlings averaged 11,609±395 µg, of which more occurred in shoots (6,328±116 µg) than roots (5,281±302 µg, p=0.0030, Figure 7). That 45% of donor isotope that occurred in roots indicated that ^{13}C was easily translocated out of foliage to fine roots where it would be available for belowground interspecific transfer. Isotope received by Douglas-fir was evenly distributed between roots (288±15 µg) and shoots (293±17 µg, p>0.10), indicating ready translocation from roots to shoots. If we assume that isotope respired by paper birch shoots and re-fixed by Douglas-fir foliage through photosynthesis remained in foliage, we

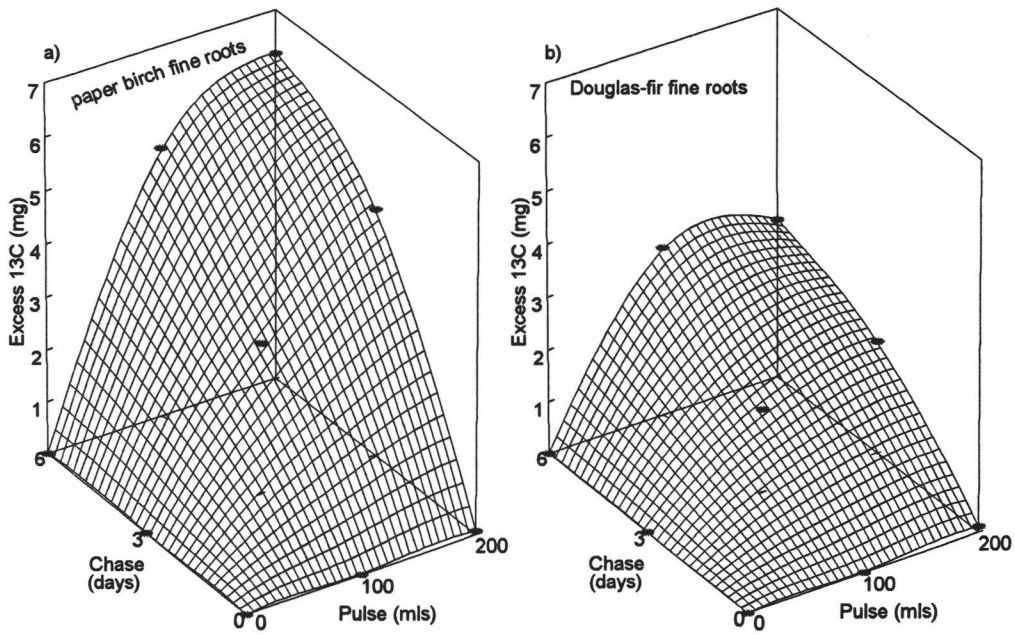

Figure 5. Fine root excess ^{13}C in response to pulse and chase treatments for (a) birch and (b) fir. ANOVA detected differences between chase ($p<0.05$) but not pulse for both species. Values are means ± s.e. (n=3).

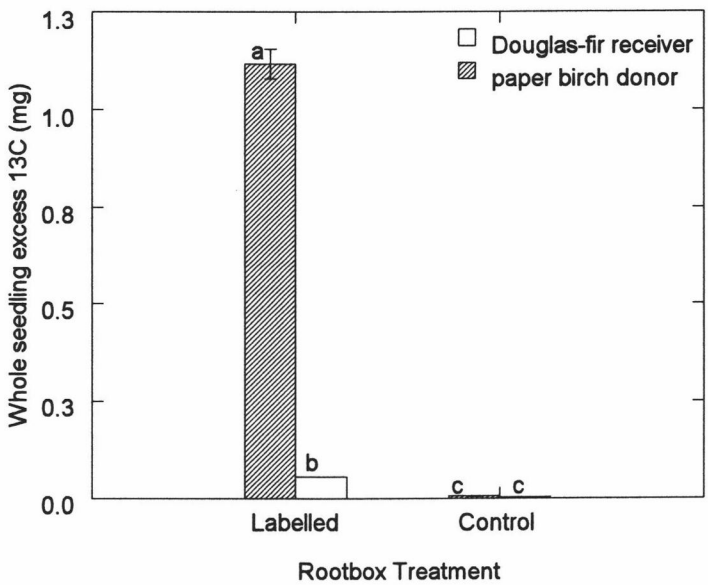

Figure 6. Whole seedling excess ^{13}C vs rootbox treatments: donor birch, receiver fir, control birch, control fir. ANOVA detected differences between treatments ($p<0.05$). Symbols are means ± s.e. (n=3).

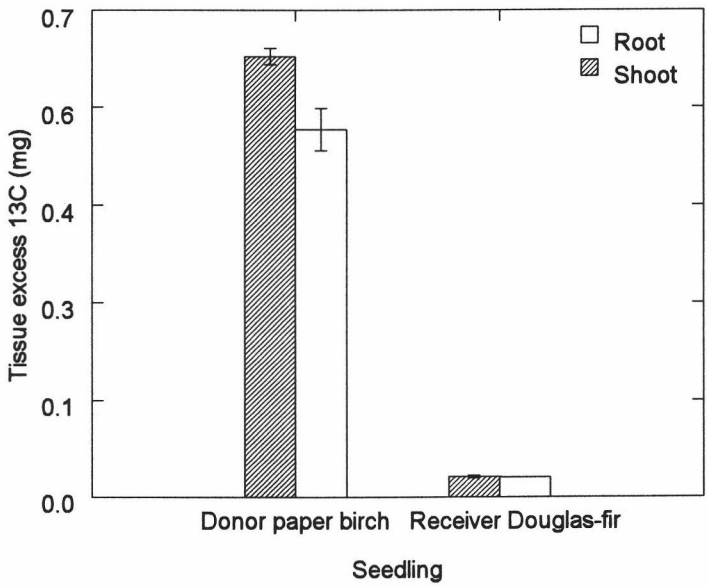

Figure 7. Tissue excess ^{13}C contents of donor paper birch and receiver Douglas-fir. ANOVA detected differences between tissues ($p<0.05$). Values are means ± s.e. (n=3).

estimate that at least 255 µg or 87% of the isotope in receiver Douglas-fir shoots arrived there by below-ground transfer and subsequent redistribution. This is a conservative estimate of belowground transfer to Douglas-fir shoots, because results of the pulse-label experiment indicate that ^{13}C fixed by Douglas-fir foliage is readily translocated to roots over 6 d. To that end, we estimate that 2.1% of the total isotope fixed by paper birch was transferred belowground and then translocated upward to Douglas-fir shoots, and 2.4% was transferred belowground and then remained in Douglas-fir roots.

Discussion

Pulse-labeling efficiency

The pulse labeling procedure resulted in $\delta^{13}C$ enrichment in paper birch and Douglas-fir foliage by over 660‰ and 460‰, respectively, ½ h following the 100-mL $^{13}CO_{2(gas)}$ pulse. Whole seedling uptake was 34 mg and 14 mg excess ^{13}C, representing pulse labeling efficiencies of 57% and 25% for paper birch and Douglas-fir, respectively. Comparable enrichment was observed following short-term $^{13}CO_{2(gas)}$ pulse labeling of *Bromus tectorum* (Svejcar *et al.* 1990), *Agropyron desertorum* (Miller and Rose 1992), and *Sorghum halepense* (Berg *et al.* 1991). High labeling efficiencies (40-50%) have also been achieved with long-term exposure to ^{14}C (Paul and Kucey 1981) or ^{13}C (Kouchi and Yoneyama 1984). Factors which affected pulse-labeling efficiency were photosynthetic discrimination against ^{13}C (Van Norman and Brown 1952, Buchanan *et al.* 1953, Craig 1954), photorespiration and respiration (Pearcy *et al.* 1987).

The greater pulse-labeling efficiency of paper birch than Douglas-fir is partly due to its higher net photosynthetic rate; leaf and whole seedling rates of paper birch were 2.2 and 13 times those of Douglas-fir, respectively. Assimilation of $^{14}CO_2$ has been correlated with net photosynthetic rates in conifers (Neilson 1977, Price *et al.* 1986, Pearcy *et al.* 1987), and ^{13}C uptake has been shown to be a better indicator of net photosynthetic rates than ^{14}C (e.g., Van Norman and Brown 1952) . Differences in paper birch and Douglas-fir leaf net photosynthetic rates measured in this study were similar to those for one-year old out-planted seedlings in the field experiments (Chapters 5 and 7). Net photosynthetic rate of measured paper birch leaves also was within the range reported for seedlings by Ranney *et al.* (1991) and for saplings by Wang *et al.* (1995) and Jurik *et al.* (1988), and that of Douglas-fir shoots was similar to 6 month nursery seedlings by Dosskey *et al.* (1990) and at the low end of the range of values reported for 30 y coastal Douglas-fir by Brix (1981) and Price *et al.* (1986). The greater pulse-labeling efficiency of paper birch than Douglas-fir may also be due to a greater photosynthate sink (for growth and respiration) generated by its larger root system; paper birch root biomass was almost twice as great and its root/shoot ratio 35% higher than Douglas-fir. Photosynthetic uptake of ^{14}C has been shown to be positively

correlated with root activity (Herold 1980, Geiger 1986) and with mycorrhiza infection (Reid *et al.* 1983, Nylund and Wallander 1989, Wang *et al.* 1989, Dosskey *et al.* 1990).

Initial $\delta^{13}C$ of paper birch foliage increased from 633‰ to 829‰ ($p=0.0001$, 1.30 relative increase), and of Douglas-fir foliage from 437‰ to 637‰ ($p=0.0004$, 1.43 relative increase) when the pulse was increased from 100- to 200-mL $^{13}CO_2$. Chamber $^{12+13}CO_2$ concentration was not directly monitored in this study; however, we estimate it was 350 and 400 µL L^{-1} for the 100- and 200-mL pulses, respectively, representing a 15% difference in concentration. Elevated atmospheric CO_2 concentrations have been shown to result in relative photosynthetic enhancement of 1.28 for *Betula pendula* (700 versus 350 µL CO_2 L^{-1}, Pettersson and McDonald 1992) and 1.32 for Douglas-fir (640 versus 340 µL L^{-1}, Hollinger 1987). Although the estimated difference in CO_2 concentration between pulses in our study was smaller than in those examined by Pettersson and McDonald (1992) and Hollinger (1987), the enhanced $^{13}CO_2$ uptake rates were comparable. When foliar isotope content was expressed as mg excess ^{13}C, however, differences between pulses were not significant ($p>0.10$). In spite of the tendency toward increased ^{13}C uptake, the lower pulse labeling efficiency of the 200- compared to 100-mL $^{13}CO_2$ pulse for both paper birch (57% compared with 35%) and Douglas-fir (25% compared with 20%) indicated an inability, particularly for paper birch, to fully exploit atmospheric CO_2 enrichment. This restricted capacity to use additional CO_2 in photosynthesis is reportedly due to limitations in light harvesting, electron transport to NADPH, ATP synthesis, Calvin cycle capacity, and end-product synthesis (Gunderson and Wullschleger 1994).

Intraspecific carbon allocation

During the first 3 days following pulse labeling, 44% of the assimilated ^{13}C (based on the whole seedling content ½ h post-labeling) had been lost on average by paper birch and Douglas-fir species to root and shoot respiration, root exudation, tissue death, etc. This loss was comparable to estimates of whole tree maintenance respiration by beech (Larcher 1983) and pine (Kinerson 1977), as well as whole plant respiration by barley (Gordon *et al.* 1977). It was slightly greater than the 29% loss 1 d following ^{13}C labeling of *Bromus tectorum* (Svejcar *et al.* 1990) and 35% loss 4 d following ^{14}C labeling of *Vicia faba* (Paul and Kucey 1981), possibly due to greater maintenance respiration demands of the older conifer tissues.

Distribution of isotope among tissues changed dramatically over the course of the chase period for both paper birch and Douglas-fir. Over 95% of the fixed isotope occurred in foliage immediately following the pulse, of which 75% was translocated to stem and root tissues within 3 d. In other studies, most translocation of fixed carbon isotope out of foliage occurred within 1 d of pulse-labeling herbs and grasses (Gordon *et al.* 1977, Svejcar *et al.* 1990, Miller and Rose 1992). Isotope content of paper birch and Douglas-fir stems and coarse roots did not change after 3 d, but

continued to increase in fine roots through 6 d following the pulse. This change in isotope distribution among tissues is most succinctly described by the change in isotope root/shoot ratio from 0.00 immediately following the pulse to 0.61 three days later and 0.87 six days later. Miller *et al.* (1989) found that root/shoot ratio of ^{14}C activity in *Pinus ponderosa* seedlings decreased from 0.85 to 0.76 between four and nine days following pulse-labeling, presumably due to re-translocation and respiration. In contrast to tree seedlings, isotope distribution among grass roots and shoots stabilized at root/shoot ratio of approximately 0.6 one day following pulse labeling with ^{13}C (Svejcar *et al.* 1990, Miller and Rose 1992). Since tissue allocation patterns with paper birch and Douglas-fir were independent of pulse, 100 mL $CO_{2(gas)}$ was considered adequate for examining intraspecific carbon allocation patterns.

The continual change in ^{13}C distribution throughout the chase indicated that carbon allocation among tissues should be compared between paper birch and Douglas-fir after 6 days. After 6 days, paper birch allocated 49% (average 9.5 mg) and Douglas-fir 41% (average 5.8 mg) of fixed isotope to roots. The proportion of isotope allocated to roots equaled the proportion of seedling biomass in roots for both species, suggesting that the additional photosynthate allocated to paper birch roots was used mainly for their growth respiration and maintenance respiration. Of the total isotope allocated to roots after 6 d, an average of 60% (5.6 mg) went to fine roots of paper birch and 55% (3.1 mg) to fine roots of Douglas-fir. In forest plantations in the southern interior of B.C., shoot growth (aboveground biomass accumulation) of paper birch far exceeds that of neighboring Douglas-fir (Simard and Vyse 1992). Assuming allometric relations and carbon allocation patterns measured in this study apply to seedlings growing in mesic field conditions, we expect in the field that paper birch root systems are larger, have greater potential for gathering nutrients and water, and are a greater source of energy for microbial activity (through exudation and turnover) than those of Douglas-fir. The higher throughput of photosynthate from foliage to fine roots in paper birch relative to Douglas-fir seedlings may mean a greater potential for carbon export to the rhizosphere microbial community, extramatrical hyphae of mycorrhizal fungi, soil and/or neighboring plants. The relative potential of paper birch and Douglas-fir to export carbon belowground is expected to change with age, however, because Douglas-fir biomass accumulation surpasses that of paper birch at approximately 30 years in mixed stands in the southern interior of B.C. (Simard and Vyse 1994).

Measurement of belowground ^{13}C transfer between associated paper birch and Douglas-fir requires that a significant amount of isotope is translocated from foliage to fine roots of donor plants. Results of this study show that over 20% (3-6 mg excess ^{13}C) of seedling isotope was translocated to fine roots of both species following either a 100-mL or 200-mL $CO_{2(gas)}$ pulse and 6 day chase, representing rapid and substantial isotope enrichment of fine roots. Interplant translocation of ^{14}C has been detected with considerably lower (<0.001 mg) isotope concentrations in tissues of donor plants by Hirrel and Gerdemann (1979), Francis and Read (1984), Finlay and

Read (1986), Duddridge *et al.* (1988), and Alpert *et al.* (1991). Consequently, we consider the fine root isotope contents achieved in this study adequate for detecting export of labeled photosynthate from either species to neighboring seedlings. Because differences in fine root isotope content between the 100-mL and 200-mL $CO_{2(gas)}$ pulse were insignificant, a 100-mL pulse was considered sufficient for carbon transfer studies.

Interspecific carbon transfer

Pulse-labeling paper birch in rootboxes resulted in substantial isotope transfer to neighboring Douglas-fir through above- and below-ground mechanisms. The amount of isotope transferred represented 4.7% of the excess ^{13}C fixed by paper birch, of which 82% was transferred via belowground mechanisms and 18% was transferred by aboveground mechanisms (respiration by birch foliage and stems and subsequent re-fixation by Douglas-fir foliage). The amount transferred via different belowground mechanisms was not possible to distinguish because roots of paper birch and Douglas-fir were intimately mingled in this experiment. Potential interspecific belowground transfer pathways include root grafts, mycorrhizal linkages, or soil pools of exudate, sloughed tissue, and respired $CO_{2(gas)}$. Root grafts are not common between different species (Newman 1988), and none were evident between paper birch and Douglas-fir through careful examination of intermingled root systems. Similarly, anapleurotic uptake of $^{13}CO_{2(gas)}$ by paper birch or Douglas-fir roots was not detectable in this study. However, in a bioassay using the same soil and seed source as this experiment, nursery-grown paper birch and Douglas-fir seedlings were shown to share seven ectomycorrhizal morphotypes in common over 90% of their root tips (Chapter 2). Those results suggest there is potential for interconnection and carbon transfer between species by ectomycorrhizal fungi. In this study, it is not possible to estimate the importance of direct ectomycorrhizae-mediated ^{13}C transfer relative to indirect transfer via paper birch root exudates or sloughed root and fungal material in the soil pool.

The percentage of ^{13}C assimilated by paper birch that was transferred belowground to neighboring Douglas-fir was approximately half the ^{14}C found transferred from floating clonal *Eichhornia crassipes* parents to connected offspring ramets (Alpert *et al.* 1991). Transport of photosynthate between connected ramets is widespread in clonal plants (Hutchings and Bradbury 1986), and Alpert *et al.* (1991) considered the 10% transfer they measured among connected *E. crassipes* ramets considerable. In contrast, the 4.4% of ^{13}C labeled assimilate transferred from paper birch to Douglas-fir was substantially greater than the amount of ^{14}C shown by Read and others to be transferred between plants via mycorrhizal fungi. For example, transfer of ^{14}C from *Plantago lanceolata* to shaded *Festuca ovina* via vesicular arbuscular mycorrhizae represented only 0.1% of the labeled carbon in the donor (Francis and Read 1984). Finlay and Read (1986) showed that 0.2-1.0% of donor *Pinus contorta* ^{14}C-labeled assimilate was transferred to

mycorrhizal-connected receiver *P. contorta*, whereas only 0.02% was transferred between non-mycorrhizal seedlings. Read *et al.* (1985) also reported interspecific ^{14}C transfer between *Betula pubescens* and *Pinus sylvestris* or *Pinus contorta* when connected by ectomycorrhizae, although they did not show amounts. The benefit to receiver plants of mycorrhiza-mediated carbon transfer is controversial partly because of the small amounts transferred in studies by Read and others (Newman 1988). The large amount of photosynthate transported between connected ramets of clonal plants has been shown, however, to increase their survivorship and growth (see Hutchings and Bradbury 1986). The proportion of assimilated carbon transferred from paper birch to Douglas-fir was comparable to that of receiver ramets, suggesting survivorship and growth benefits also may be gained by Douglas-fir. These benefits may result from both the carbon (or organic nutrient) gain by Douglas-fir as well as the requisite loss by paper birch.

The amount of ^{13}C transferred from paper birch to Douglas-fir was more similar to mycorrhizae-mediated transfer of ^{15}N between *Alnus glutinosa* and *Pinus contorta* (Arnebrant *et al.* 1993) than of mycorrhizae-mediated ^{14}C transfer described in the above studies. Arnebrant *et al.* (1993) found that 5-15% of $^{15}N_{(gas)}$ fixed by the *A. glutinosa-Frankia* association was transferred to *P. contorta* via ectomycorrhizal connections. They also detected transfer of ^{14}C from alder to pine, which they suggested was translocated through mycelium as the carbon skeleton of amino acids biosynthesized in alder nodules. Several others provide evidence that carbon is translocated through mycelium and into connected plants in combination with nitrogen as free amino acids (Martin *et al.* 1988, Abuzinadah and Read 1989, Smith and Smith 1990). The amount of nitrogen and carbon translocated between plants presumably depends on source-sink relationships and concentration gradients between soil and interconnected plants (Read *et al.* 1985, Newman 1988, Bethlenfalvay *et al.* 1991, Arnebrant *et al.* 1993). In this study, whole seedling net photosynthetic rates of paper birch were estimated as 13 times that of Douglas-fir. In addition, paper birch foliar nitrogen concentration (Chapter 5) was approximately double that of Douglas-fir. These interspecific carbon and nitrogen differences suggest that a source-sink relationship exists between paper birch and Douglas-fir that may be sufficiently strong to influence the direction and extent of interplant carbon and nitrogen transfer.

Carbon isotope transferred via belowground mechanisms was about evenly distributed between roots (56%) and shoots (44%) of Douglas-fir. Of the total ^{13}C assimilated by paper birch, 2.4% was transferred belowground to roots and 2.1% to shoots of Douglas-fir. Distribution of isotope among Douglas-fir receiver tissues was similar to that among shoots and roots of receiver *E. crassipes* ramets (Alpert *et al.* 1991). However, mycorrhizal-mediated transfer of ^{14}C has resulted in <1-14% of transferred ^{14}C reaching shoots (Francis and Read 1984, Finlay and Read 1986, Read *et al.* 1985). The beneficial significance of the small amount of ^{14}C received by shoots has been questioned by Newman (1988). In this study, however, the substantial proportion of assimilated carbon stored in receiver roots may have significance to Douglas-fir survival under

stressful conditions (e.g., drought or shade), and that transferred to shoots may supplement photosynthetic carbon or nitrogen available for shoot growth. Carbon isotope in Douglas-fir foliage more likely translocated there in free amino acids along a nitrogen concentration gradient rather than as carbohydrate along a carbon concentration gradient, because fully developed leaves are usually strong sinks for nitrogen, and are sources rather than sinks for carbon (Pearcy *et al.* 1987).

The pulse-labeling procedure worked well for examining intraspecific carbon allocation and Interspecific carbon transfer between paper birch and Douglas-fir. Considerable isotope was translocated to fine roots of both species following a 100-mL $^{13}CO_{2(gas)}$ pulse and 6-d chase. The considerable amount of isotope translocated to fine roots facilitated quantification of carbon transfer from pulse-labeled paper birch to receiver Douglas-fir growing in rootboxes. The results of the carbon transfer study emphasize the importance of belowground carbon transfer from paper birch to Douglas-fir, but do not prove that equal amounts of isotope were transferred in the reverse direction from Douglas-fir to paper birch, i.e., that net transfer occurred (Newman 1988).

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Chapter 4

Reciprocal Translocation of Carbon Isotopes between Ectomycorrhizal *Betula papyrifera* and *Pseudotsuga menziesii*

Abstract

Uptake and translocation of carbon was studied in laboratory rootboxes containing six-month-old ectomycorrhizal (EM) *Betula papyrifera* (Marsh) and *Pseudotsuga menziesii* (Mirb. Franco) seedlings growing in individual, root-restrictive (28µm pore size) pouches filled with field soil. Bioassays showed that the two species shared seven EM morphotypes in common over 90% of their root tips (Chapter 2), indicating potential for EM hyphal connections and interspecific carbon transfer. Interspecific carbon transfer was examined by reciprocal labeling with the stable isotope ^{13}C and the radioactive isotope ^{14}C. One seedling was exposed to $^{13}CO_{2(gas)}$ and its neighbor to $^{14}CO_{2(gas)}$ for 1 h, and the amount in donor and receiver seedlings examined after 6 d. Amount of carbon transferred directly through EM connections versus indirectly through the soil pool was examined by comparing rootboxes where interconnecting hyphae were left intact versus where they were severed immediately prior to labeling.

Transfer between paper birch and Douglas-fir was bi-directional, but there was on average a net carbon gain by Douglas-fir ($p=0.10$). Net carbon transfer to Douglas-fir represented on average 4% of the total isotope ($^{13}C + {}^{14}C$) assimilated by the rootbox system, 10% of the isotope assimilated by Douglas-fir, and 7% of that assimilated by paper birch. Mean values for net transfer were approximately three times greater where hyphae were connected than severed; however, differences were not significant ($p=0.28$). Net transfer from paper birch to Douglas-fir might be explained by a concentration gradient resulting from a 10-fold greater whole seedling net photosynthetic rate and two-fold greater foliar nitrogen concentration of paper birch than Douglas-fir.

Gross (total bi-directional) transfer represented 29% of the total isotope assimilated in the rootbox system. Unidirectional gross transfer from paper birch to Douglas-fir was 50% greater where hyphae were unsevered than where severed ($p=0.15$), but severing hyphae had no effect on gross transfer in the opposite direction ($p>0.15$). These results suggest that paper birch received all of its isotope from Douglas-fir indirectly through the soil pool, whereas Douglas-fir may have received isotope from paper birch directly through interconnecting fungi in addition to that via the soil pool.

Introduction

The low host specificity for arbuscular mycorrhizal (AM) fungi and many ectomycorrhizal (EM) fungi can result in physical hyphal connection between host plants (Molina *et al.* 1992), which has been demonstrated using ^{14}C-labeling and macro-autoradiography (Brownlee *et al.* 1983, Francis and Read 1984, Finlay and Read 1986). One of several possible consequences of hyphal interconnections is direct transport of carbon, minerals, or water between plants. Interconnecting mycorrhizal fungi have been demonstrated to facilitate interplant transfer of the isotope tracers $^{3}H_2O$ (e.g., Duddridge *et al.* 1980), ^{14}C (e.g., Francis and Read 1984), ^{32}P (e.g., Newman and Eason 1993), and ^{15}N (e.g., Arnebrant *et al.* 1993). The receiver plant could benefit from this transfer by obtaining mineral nutrients from the fungus whose carbon was supplied by the donor plant, and/or by directly obtaining carbon or organic nutrients via mycorrhizal links from the donor plant (Newman 1988). Other possible consequences of hyphal interconnections include: greater or more rapid infection of seedlings that grow into contact with established plants, transfer of nutrients from dying to living roots thus by-passing the soil pool, and alteration of the balance of plant-plant interactions (Newman 1988, Miller and Allen 1992).

Although interplant transfer has been demonstrated, direct facilitation via hyphal links is controversial. The debate centers on whether the extent of net transfer of materials from one plant to another is sufficiently large to significantly affect plant survival or growth (Newman 1988, Miller and Allen 1992). Several problems are as follows. Isotope transfer has been very small (^{14}C) or very slow (^{32}P) in most studies. For example, *(i)* only 0.1% of donor ^{14}C was transferred from *Plantago lanceolata* to shaded *Festuca ovina* via AM (Francis and Read 1984), *(ii)* 0.2-1.0% of donor ^{14}C was transferred between *Pinus contorta* seedlings, with more in shade than sun (Read *et al.* 1985), *(iii)* rate of ^{32}P transfer between mycorrhizal-connected *Lolium perenne* was too slow to affect their nutrient status (Newman and Eason 1993), and *(iv)* mycorrhizae influenced N status of corn (*Zea mays*) more through improved P uptake than through direct ^{15}N transfer from the neighboring legume, *Glycine max* (Hamel and Smith 1992). In contrast to these examples, however, Arnebrant *et al.* (1993) found that 5-15% of $^{15}N_{(gas)}$ fixed by the *A. glutinosa-Frankia* association was transferred to *P. contorta* via ectomycorrhizal connections, and that approximately 20% of the nitrogen found in pine was derived from N_2-fixation.

Another problem is that net transfer from one plant connected to another has yet to be proven; i.e., that gain in material by one plant exceeds that of its connected neighbor. Invariably one plant has been fed an isotope and its neighbor assessed a few days later, thereby quantifying unidirectional transfer. Although Arnebrant *et al.* (1993) quantified only one-way transfer of ^{15}N from N_2-fixer *Alnus glutinosa* to *Pinus contorta*, they suggest that the large amounts transferred implies net transfer. For ^{14}C-labeled photosynthate, Jakobsen (1991) suggests that net or bi-directional transfer can only be determined by comparing the results of reciprocal labeling in two

parallel experiments. If net transfer does occur, its significance could be evaluated by comparing net amount transferred with the receiver plants' gain by photosynthesis (Newman 1988).

Net transfer can be expected to occur only if connected plants differ in some way, such as net photosynthetic rate, nutrient status, or N_2-fixing capability, that establishes a source-sink relationship (Read *et al.* 1985, Newman 1988, Bethlenfalvay *et al.* 1991, Arnebrant *et al.* 1993). For example, *(i)* Read *et al.* (1985) established a photosynthate concentration gradient by shading *Festuca* seedlings connected to mature *Plantago* plants via AM hyphae, and showed that over six times more ^{14}C was transferred to shaded than unshaded seedlings, *(ii)* ^{15}N transfer via ecto- or arbuscular mycorrhizal hyphae has been shown to occur from nodulated N_2-fixing to non-N_2-fixing plants, which clearly differ in tissue nitrogen contents (Bethlenfalvay *et al.* 1991, Hamel and Smith 1992, Frey and Schuepp 1992, Arnebrant *et al.* 1993), and *(iii)* fertilization of donor plants increased mycorrhizal-mediated ^{32}P transfer to unfertilized receiver plants (Eissenstat 1990, Ritz and Newman 1986). Although a source-sink relationship existed between connected plants in these one-way labeling studies, whether it influenced transfer is questionable in light of studies that show ^{14}C and ^{32}P transfer also occurs between connected plants of the same species, size, age, and nutrient status i.e., with no apparent source-sink relationship to influence transfer (Read *et al.* 1985, Ritz and Newman 1986). Miller and Allen (1992) argue, however, that extent of hyphal linkages could also influence extent of transfer, even where leaf sinks do not occur.

The present study addresses questions regarding the extent and significance of net transfer between plants that share common ectomycorrhizal fungi. Net transfer was studied in rootboxes containing ectomycorrhizal paper birch (*Betula papyrifera*) and Douglas-fir (*Pseudotsuga menziesii*) seedlings growing in individual, root-restrictive pouches filled with field soil. The two species were shown to share in common seven ectomorphotypes over 90% of their root tips in a bioassay using the same plant and soil material as the present study (Chapter 2), and careful visual examination indicated that root zones of neighboring seedlings in rootboxes were interconnected by an extensive hyphal network at the time of labeling. Higher foliar nitrogen concentration (Chapter 5) and net photosynthetic rate (this chapter) of paper birch than Douglas-fir indicated that carbon and nitrogen source-sink relationships occurred between the two species. Net transfer was studied following reciprocal exposure of one plant to the radioactive carbon isotope ^{14}C and the other to the stable carbon isotope ^{13}C.

The objective of our study was to quantify the gross and net amounts of carbon isotope translocated belowground between paper birch and Douglas-fir seedlings. We hypothesize that isotope translocation is bi-directional, but that more is translocated from paper birch to Douglas-fir than visa versa (*i.e.*, positive net transfer to Douglas-fir) because of concentration gradients created by differences in net photosynthetic rates and foliar nitrogen concentration.

Methods

Site description

Soil was collected from the same site as that used for the field labeling experiments described in Chapter 5. The study site is located within the Clearwater Forest District, Kamloops Forest Region, of south-central British Columbia. It occurs within the Thompson variant of the Moist Warm Interior Cedar Hemlock biogeoclimatic subzone (Lloyd *et al.* 1991). The subzone is characterized by warm, moist summers and cold, snowy winters, with mean temperatures of 19°C in July and -6°C in January, and mean annual precipitation of 670 mm, of which 290 mm falls as rain during the growing season (Environment Canada 1980). The submesic-mesic site is a flat terrace (0-5% slope) at 700 m elevation, just above the North Adams River valley bottom. The soil is a Humo-Ferric Podzol (Canadian Soil Survey Committee 1978), formed over a granitic alluvial blanket. The soil surface layers (to 50 cm) are sandy loam to loamy sand, with coarse fragment content less than 10%.

The original mixed forest of Douglas-fir (*Pseudotsuga menziesii*), paper birch (*Betula papyrifera*), western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), lodgepole pine (*Pinus contorta*), western white pine (*Pinus monticola*), and trembling aspen (*Populus tremuloides*) was clearcut in 1987 and planted to Douglas-fir in 1988. Due to high incidence of *Armillaria ostoyae* root disease among planted seedlings, the site was mechanically de-stumped in 1991 to reduce inoculum load, and re-planted in the spring of 1992 to Douglas-fir, paper birch and western redcedar. Areas between the planted seedlings were quickly occupied by native hardwoods (primarily paper birch and trembling aspen (*Populus tremuloides*)), shrubs (primarily raspberry (*Rubus spectabilis*) and thimbleberry (*Rubus parviflorus*)), and forbs (primarily spreading dogbane (*Apocynum androsaemilfolium*) and fireweed (*Epilobium angustifolium*)).

Soil collection and preparation

In late July 1993, soil was collected from the area (approximately 0.25 ha) where the field labeling experiments were conducted (Chapter 5). Mineral soil was collected to 15 cm depth from five sample points randomly located between pairs of Douglas-fir and paper birch seedlings. The five samples were composited to make one sample. The composite sample was placed in plastic bags, set on ice in a cooler, and then transported to the laboratory, where it was immediately sieved to 4 mm, homogenized, and mixed (3:1 by volume) with perlite. The sample was split and one portion used for the bioassay (Chapter 2) and the other for the present study. Beyond soil collection, research was conducted at Oregon State University, Corvallis, Oregon.

Preparation and planting of rootboxes

A modification of the root-mycocosm design described by Rygiewicz *et al.* (1988) was used to construct 12 rootboxes. Each acrylic plastic rootbox was 22 cm high, 20 cm wide and 3 cm thick, with a single root chamber. Two Nitex membrane (Tetko Inc., New York) pouches 20 cm high, 10 cm wide and 3 cm thick, were fit snugly side-by-side in the root chamber of each rootbox (Figure 8). The membrane pore size was 28µm, which has been shown to restrict root but not hyphal penetration (Neufeld *et al.* 1989). Each pouch was filled with the 3:1 soil:perlite rooting medium. Douglas-fir seeds were planted in one and paper birch in the other pouch of each rootbox.

Douglas-fir seeds were surface sterilized and stratified by soaking first in 30% H_2O_2 for 2 min. and then in 3% H_2O_2 for 5 h, rinsed with distilled water, and dried at room temperature for 24 h. The same procedure was used for paper birch, except seed was soaked in aerated 10% H_2O_2 for 15 min. Five seeds of one species were planted per pouch on August 1, 1993. Sterile silica sand was spread over Douglas-fir seeds to ½ cm depth, and over paper birch seeds to a bare covering, to stabilize the surface and minimize mortality due to damping-off fungi. The rootbox faceplates were wrapped in aluminum foil to ensure darkness and reduce evaporation, and tilted at approximately 45° to promote hyphal entrainment along the lower faceplate.

Seedlings were grown in the greenhouse under high intensity light (280 µmol m^{-2} s^{-1}) with a 16h/8h light/dark cycle and temperatures ranging from 24°C (light) to 18°C (dark). Rootboxes were watered daily and relocated monthly to reduce environmental differences. After four weeks, seedlings were thinned to one per pouch. After five months, each pouch received 50 mL of Peters solution (20:20:20 N-P-K) to maintain seedling growth. When seedlings were six months old, hyphae were severed in a randomly selected subset of rootboxes, after which all seedlings were immediately labeled with carbon isotopes. At the time of treatment, an extensive network of mycorrhizal mycelium had grown out of the membrane pouches and spread over the upper and particularly lower faceplates of the rootboxes. Visual examination indicated that root zones of Douglas-fir and paper birch seedlings in each rootbox were interconnected by this network of mycelium.

Individual seedlings of paper birch and Douglas-fir also were grown for measurements of net photosynthetic rate, and ^{13}C and ^{14}C natural abundance ($mg^{13}C_{na}$). The seedlings were grown in leach tubes using the same rooting medium, planting procedures and greenhouse conditions described above. Three replicate seedlings of each species were used for net photosynthesis measurements and four for natural abundance determinations.

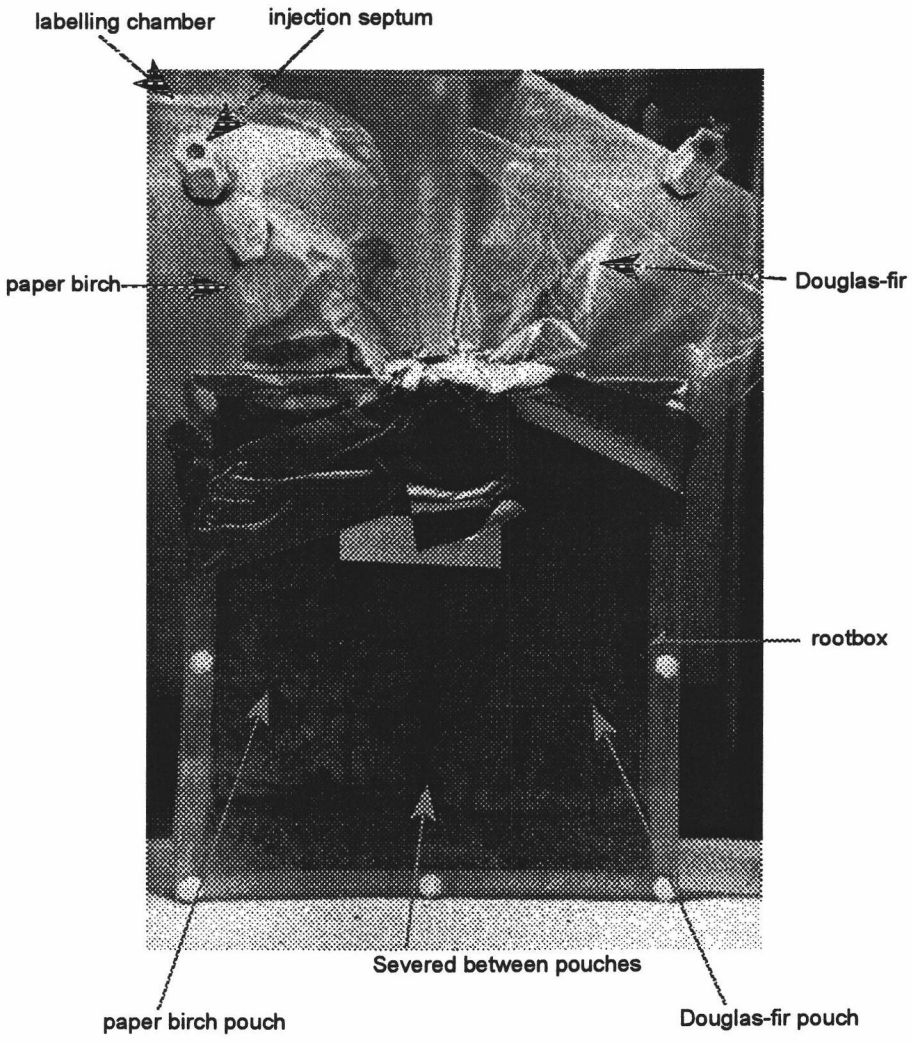

Figure 8. Rootbox and labelling chambers. Paper birch and Douglas-fir were grown in separate pouches permeable (28µm) to hyphae but not roots. Labelling chambers were sealed over shoots.

Study design

The rootbox experiment consisted of two "severing" treatments and two labeling schemes in a 2 x 2 factorial set of treatments. The treatments were replicated three times in a completely randomized design (12 experimental units). The two severing treatments were imposed in order to distinguish between interspecific isotope translocation via ectomycorrhizal fungi, and that through other pathways (respired $CO_{2(gas)}$ as well as root/fungal exudates and sloughed root/fungal cells in irrigation water allowed to pass between pouches). In one treatment (Unsevered), the mycelial network connecting the root zones of paper birch and Douglas-fir seedlings was left intact, and in the other treatment (Severed) the hyphae at the pouch interface were severed immediately prior to labeling. For the Severed treatment, the lower faceplate of the rootbox was carefully removed, the hyphae connecting the root zones of paper birch and Douglas-fir seedlings severed by running a blade between the pouches, and the faceplate then replaced. Isotope labeling was conducted within 10 min. of imposing this treatment.

The paper birch and Douglas-fir seedlings in each rootbox were pulse-labeled with different isotopes: one with ^{13}C and the other with ^{14}C. This approach enabled detection of the carbon isotope which was received by one seedling from the other. Seedlings pulse-labeled with a particular isotope are referred to as "donors", and those which received that same isotope are referred to as "receivers". Due to possibilities of differences in isotopic discrimination between ^{13}C and ^{14}C, two labeling schemes were applied to each severing treatment. For three replicates per severing treatment, paper birch (PB) was labeled with ^{14}C and Douglas-fir (DF) with ^{13}C (labeling scheme called 14PB-13DF) . For the other three replicates, the reciprocal scheme was applied, where paper birch was labeled with ^{13}C and Douglas-fir with ^{14}C (13PB-14DF).

^{13}C and ^{14}C labeling procedures

Seedlings were labeled with ^{13}C and ^{14}C using 1-h pulse and 6-d chase periods. The 6-d chase was appropriate for maximum isotope translocation to fine roots (Chapter 3). The pulse and chase periods occurred under high intensity lights inside a fumehood. A 1 m x 1 m steel frame light stand was constructed inside the fumehood to support a 90 cm x 30 cm x 8 cm open-topped, circulating acrylic plastic water bath at approximately 80 cm height. Six high intensity lamps were wired 5 cm above the bath, which kept seedling temperatures near 25°C, and light diffusion plates taped to the bottom of the bath to diffuse the direct beam. The light intensity under the water bath was 1000 $\mu E\ m^{-2}\ s^{-1}$, which is near saturation light intensities for paper birch and Douglas-fir (Chapter 3). The steel frame was large enough to pulse four rootboxes at once, or one replicate of each treatment.

Immediately prior to labeling the seedlings in a rootbox, the seedling shoots and rooting medium were separately sealed using plastic Saran Wrap® and duct tape. Each shoot was then sealed inside flexible, air-tight, 5 mil thick x 15 cm wide x 15 cm tall, fluoropolymer gas sampling bags (Teflon Brand, Chemware). Each sampling bag was fit with a silicone septum in a polypropylene housing, for injections with a hypodermic needle. Moments before injections, the bags were completely sealed by screwing on the septum housing. The shoot of one partner seedling was then pulse labeled for 1 h with 50 mL of $^{13}CO_{2(gas)}$ (^{13}C, 99%, equivalent to 2.25 mmol, 29.25 mg ^{13}C). At the same time, the shoot of the other partner seedling was pulse labeled for 1 h with $^{14}CO_{2(gas)}$ released from 50 µCi (1.85 MBq, equivalent to 13.22 µg ^{14}C) $Na_2{}^{14}CO_3$ with lactic acid. Then after 1 h, the labeling bags were removed to release residual $^{13}CO_{2(gas)}$ and $^{14}CO_{2(gas)}$. All rootboxes were labeled within 4 h of each other (12:00 h-16:00 h), six hours before the end of the photoperiod (22:00 h).

Rootboxes remained inside the fumehood for the 6-d chase period. During that period, a 16 h/8 h light/dark cycle was maintained, with a light intensity of approximately 300-400 µE m^{-2} s^{-1}. Air respired from seedlings was allowed to escape through the fumehood. Seedlings were watered daily with an eyedropper inserted into a small port in the sealant separating the shoots from the rooting medium.

At the end of the chase period, seedlings were harvested and separated into four tissue fractions: leaves, stems, coarse roots (>1 mm diameter), and fine roots (<1 mm diameter). At the same time, individual paper birch and Douglas-fir seedlings grown for natural abundance determinations were harvested and separated into the four tissue fractions. The tissues were ovendried at 80°C for 48 h, weighed, and then ground to 20 mesh in a Wiley mill. Ground tissue samples (1 mg) were analyzed for ^{13}C abundance using a Europa Scientific ANCA mass spectrometer, and then combusted in a sample oxidizer and ^{14}C counted via liquid scintillation. Samples also were analyzed for total C (%) by combustion.

Net photosynthetic rate

Net photosynthetic rate and specific leaf area were measured on three replicate individual seedlings of paper birch and Douglas-fir. Net photosynthetic rate was measured at PAR of 1000 µE m^{-2} s^{-1}, the light intensity under which seedlings were pulse-labeled in the rootbox experiment. For each paper birch seedling, a single attached, fully developed leaf located approximately 1/3 from the top of the crown was randomly sampled. For each Douglas-fir, a lateral branch were randomly sampled from the top whorl. Net photosynthetic rate was measured four times per sample leaf or branch using a portable closed CO_2 gas analyzer (Li-Cor 6200, Lincoln, Nebraska). Light intensity (1000 ± 50 µE $m^{-2}s^{-1}$), air temperature (21 ± 1°C), relative humidity (16 ± 1%), and initial CO_2 concentration (385 ppm) were monitored at the same time to ensure consistency among samples.

Leaves were immediately harvested and leaf area (one side) measured using a leaf area meter (Li-Cor 3100, Lincoln, Nebraska). Biomass was measured after leaves were oven-dried at 80°C for 48 h. Specific leaf area ($cm^2\ g^{-1}$) was calculated as the ratio of leaf area to corresponding leaf weight.

Specific leaf area (individual seedlings) and foliar biomass (rootbox seedlings) were used to estimate total leaf area of rootbox seedlings. Net photosynthetic rate (individual seedlings) was then applied to total leaf area estimates for estimations of whole seedling net photosynthetic rates in the rootboxes.

Gross and net carbon transfer

Sample $\delta^{13}C$ (‰) and ^{14}C (Bq) values were converted to a common unit, milligrams C isotope, for gross and net transfer calculations as well as other comparisons. The conversions for ^{13}C and ^{14}C were based on procedures described by Boutton (1991) and Warembourg and Kummerow (1991), respectively. The tissue $\delta^{13}C$ values first were converted to the absolute isotope ratio ($^{13}C/^{12}C$) of the sample (R):

$$R_{sample}=^{13}C/^{12}C=[(\delta^{13}C/1000)+1] \times R_{standard}$$

where $R_{standard}=0.0112372$, the international PDB standard. The fractional abundance (A) of ^{13}C relative to $^{13}C+^{12}C$ was then related to R by the equation:

$$A=^{13}C/(^{13}C+^{12}C)=R/(R+1).$$

Fractional abundance and total carbon content (mg) of the sample were used to calculate mg ^{13}C of the sample:

$$mg\,^{13}C_{sample} = A \times [^{13}C+^{12}C]\ (mg).$$

The enrichment level of the sample ($mg^{13}C_{sample}$) in excess of natural abundance ($mg^{13}C_{na}$) was calculated as:

$$\text{excess } mg^{13}C_{sample}=mg^{13}C_{sample}-mg^{13}C_{na}.$$

Excess mg^{13}C of the tissue (excess $mg^{13}C_{tissue}$) was calculated as the product of excess $mg^{13}C_{sample}$ (per mg of sample) and tissue biomass (mg). Finally, excess mg^{13}C of the whole plant (excess $mg^{13}C_{plant}$) was determined by summing the excess $mg^{13}C_{tissue}$ of the four tissue types.

Conversion of $Bq^{14}C$ to $mg^{14}C$ was based on the batch specific activity (λ) of $Na_2{}^{14}CO_3$, λ=1.96 GBq/mmol (Amersham Canada). First, radioactivity of a sample (Bq or dps) was expressed per mg C ($Bq^{14}C_{mgC}$):

$$Bq^{14}C_{mgC}=Bq^{14}C_{sample}/mgC_{sample}.$$

Radioactive units (Bq) were converted to $mols^{14}C$ using λ, and then $mg^{14}C$ using the molecular weight of ^{14}C ($mw^{14}C$):

$$mols^{14}C_{mgC}=Bq^{14}C_{mgC}/\lambda,$$

$$mg^{14}C_{mgC}=mols^{14}C_{mgC}/mw^{14}C.$$

Excess $mg^{14}C_{mgC}$ of a sample was calculated by subtracting natural abundance ($Bq^{14}C_{na}$) values from sample values. Excess $mg^{14}C$ of the tissue (excess $mg^{14}C_{tissue}$) was calculated as the product of excess $mg^{14}C_{mgC}$ and tissue carbon (mgC_{tissue}):

$$\text{excess } mg^{14}C_{tissue}=\text{excess } mg^{14}C_{mgC} \times mgC_{tissue}.$$

As for ^{13}C, excess $mg^{14}C$ of the whole plant (excess $mg^{14}C_{plant}$) was determined by summing the excess $mg^{14}C_{tissue}$ of the four tissue types.

Gross and net transfer calculations were based on whole plant levels of excess isotope which was received from the partner donor plant in a rootbox. For the labeling scheme 14PB-13DF, paper birch received ^{13}C from Douglas-fir while Douglas-fir received ^{14}C from paper birch. Similarly, for the reciprocal labeling scheme 13PB-14DF, paper birch received ^{14}C from Douglas-fir while Douglas-fir received ^{13}C from paper birch. Gross transfer was the sum of isotope received by both species in a rootbox:

Gross transfer=isotope received by DF + isotope received by PB.

For example, gross transfer (GT) in a rootbox subject to the labeling scheme 14PB-13DF was calculated as:

$$GT_{14PB\text{-}13DF}=\text{DF excess } mg^{14}C_{plant} + \text{PB excess } mg^{13}C_{plant}$$

Net transfer was calculated as the difference between isotope received by Douglas-fir and that received by paper birch in a rootbox:

Net transfer=isotope received by DF - isotope received by PB.

For example, net transfer (NT) in a rootbox subject to the labeling scheme 14PB-13DF was calculated as:

$$NT_{14PB\text{-}13DF} = \text{DF excess mg}^{14}C_{plant} - \text{PB excess mg}^{13}C_{plant}.$$

Positive net transfer indicates that a greater amount of isotope was received by Douglas-fir than by paper birch, and negative net transfer indicates the opposite. Gross and net transfer also were expressed as proportions of isotope fixed by (a) Douglas-fir, (b) paper birch, and © the rootbox system (sum of Douglas-fir and paper birch).

Statistical analysis

Data were analyzed using SAS (SAS Institute Inc.). Net photosynthetic rate of individual seedlings was compared between species using *t*-tests. For seedlings subject to the rootbox experiment, data was pooled over all treatments to compare total biomass and isotope content between species using *t*-tests. Species biomass and isotope contents were then compared between severing treatments using *t*-tests. Biomass and isotope contents were compared among tissues within a species using one-factor analysis of variance (ANOVA).

The effects of labeling scheme (14PB-13DF versus 13PB-14DF) and severing treatment (Unsevered versus Severed) on net transfer were detected by two factor ANOVA in a completely randomized design (n=3). Significant ($p<0.05$) effects of labeling scheme were removed by applying a correction factor to excess $mg^{14}C$ on a treatment x species x tissue basis. The correction factor (CF) was the species-specific ratio of excess $mg^{13}C_{tissue}$ to excess $mg^{14}C_{tissue}$ measured in the reciprocal labeling schemes of the same severing treatment. For example, excess $mg^{13}C$ received by Douglas-fir fine roots measured in the labeling scheme, 13PB-14DF, was divided by excess $mg^{14}C$ received by Douglas-fir fine roots measured in the reciprocal labeling scheme, 14PB-13DF, of the same severing treatment. The treatment-species-tissue-specific CF values were averaged over the three replicates per labeling scheme. The corrected excess $mg^{14}C$ values (excess $mg^{14}C$ * CF) were analogous to excess $mg^{13}C$-equivalent values. The same result is derived whether ^{14}C values are corrected to ^{13}C-equivalent values or, conversely, ^{13}C values are corrected to ^{14}C-equivalent values because the latter is simply the reciprocal of the former. Using the corrected excess $mg^{14}C$ values (*i.e.*, ^{13}C-equivalent values), data were subjected to *t*-tests for pairwise comparisons of net transfer between Unsevered and Severed treatments (n=6). Relationships between CF and excess $mg^{14}C$ were explored using simple regression.

Results

Seedling biomass and net photosynthesis

Paper birch seedlings were twice as large as neighboring Douglas-fir in the rootboxes (Table 7, Figure 9). Total root biomass of paper birch was 2.2 times that of Douglas-fir, which was reflected in a higher root/shoot ratio. Leaf net photosynthetic rate (PAR=1000 $\mu E\ m^{-2}\ s^{-1}$) of paper birch was also was over twice that of Douglas-fir (Table 7). Paper birch leaves had higher specific leaf area than Douglas-fir, which reflects their larger surface area and thinner leaf structure. Integration of leaf net photosynthetic rate, specific leaf area, and foliage biomass revealed that whole seedling leaf area was approximately five times larger and whole seedling net photosynthetic rate over 10 times greater for paper birch than Douglas-fir. Foliar nitrogen concentration of paper birch was almost double that of Douglas-fir growing on the site where soil was collected for the present study (Chapter 5).

Isotopic composition of paper birch and Douglas-fir

Donors

Pulse-labeling resulted in total ^{13}C and ^{14}C contents of 3.37 mg and 2.67 µg per donor seedling, respectively, with no differences ($p>0.10$) between species or severing treatments (Table 8). This represented average pulse-labeling efficiencies of 11.5% for ^{13}C and 20.2% for ^{14}C. Within each species, isotope content differed among tissues ($p<0.10$), particularly because of high variance among Douglas-fir seedlings. Paper birch and Douglas-fir seedlings pulsed with ^{13}C translocated over 70% to roots, 85-90% of which occurred in fine roots at the end of the chase period ($p<0.10$, Figure 10*a*). Consequently, pulse-labeling with ^{13}C resulted in favorable isotope distribution for detection of carbon translocation to neighboring seedlings, whether through ectomycorrhizal fungi or other mechanisms. In contrast, seedlings pulsed-labeled with ^{14}C retained most of the isotope in their foliage and stems ($p<0.01$, Figure 10*b*). Pulse-labeled paper birch roots contained only 6% of whole seedling ^{14}C content, whereas Douglas-fir roots contained (30% of total in fine roots). For seedlings pulse-labeled with ^{14}C, more isotope was better positioned for belowground export from Douglas-fir than from paper birch. The differences in ^{13}C and ^{14}C distributions among tissues of pulse-labeled paper birch and Douglas-fir indicate that metabolic discrimination along the translocation pathway from foliage to fine roots differed between isotopes.

Table 7. Net photosynthetic rates, specific leaf area and biomass of six-month-old paper birch and Douglas-fir seedlings. Values are means and standard errors (parentheses). T-test detected differences between paper birch and Douglas-fir at ** $p<0.01$ and * $p<0.10$, d.f.=11 (d.f.=2 for specific leaf area).

Characteristic	Paper birch	Douglas-fir	p-value
Leaf net photosynthetic rate (μmol m^{-2} s^{-1}) at PAR 1000 μE m^{-2} s^{-1}	6.48 (0.68)	2.92 (0.32)	0.000**
Specific leaf area (cm^2 g^{-1})	131.3 (6.4)	51.1 (0.2)	0.000**
Total biomass (g)	1.70 (0.10)	0.83 (0.06)	0.000**
Root/shoot ratio	2.18 (0.19)	1.77 (0.13)	0.091*
Foliage biomass (g)	0.44 (0.04)	0.22 (0.02)	0.000**
Whole seedling leaf area (cm^2)	57.8 (4.7)	11.2 (0.8)	0.000**
Whole seedling net photosynthetic rate (mmol s^{-1})	374.5 (30.0)	32.1 (2.4)	0.000**
Foliar nitrogen concentration (%)[1]	2.31 (0.03)	1.38 (0.02)	0.000**

[1] from Chapter 7

Table 8. Total isotope contents of paper birch and Douglas-fir seedlings. Values are means and standard errors (brackets). T-test detected differences between paper birch and Douglas-fir at *$p \leq 0.10$, d.f.=11.

Whole seedling isotope content	Paper birch	Douglas-fir	p-value
^{13}C pulsed-labeled (mg)	3.66 (1.47)	3.08 (1.04)	0.77
^{14}C pulse-labeled (μg)	2.99 (0.11)	2.36 (0.59)	0.34
Total (^{13}C+^{14}C) pulse-labeled (mg)	3.66 (1.47)	3.08 (1.03)	0.77
^{13}C received (mg)	1.75 (0.39)	2.91 (0.52)	0.10*
^{14}C received (μg)	0.10 (0.09)	0.15 (0.04)	0.66
Total (^{13}C+^{14}C) received (mg)	1.75 (0.39)	2.91 (0.52)	0.10*

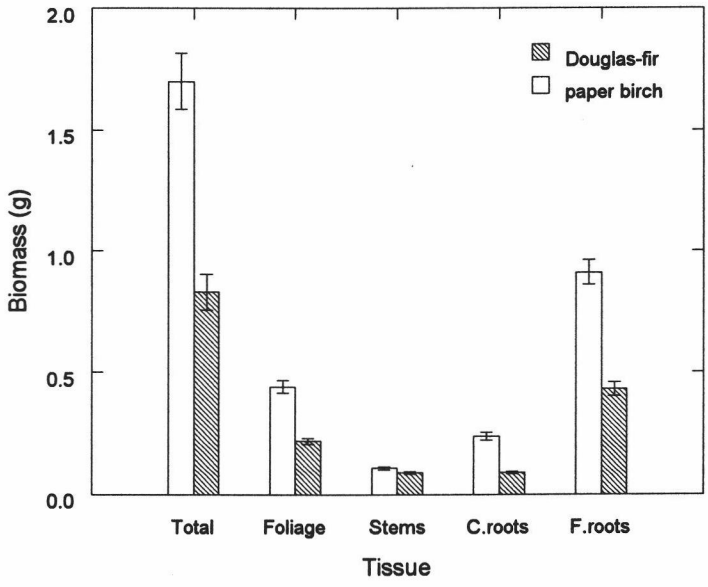

Figure 9. Total and tissue biomass of paper birch and Douglas-fir seedlings grown in rootboxes. Biomass differed among tissues for both species (p=0.0001, df=11).

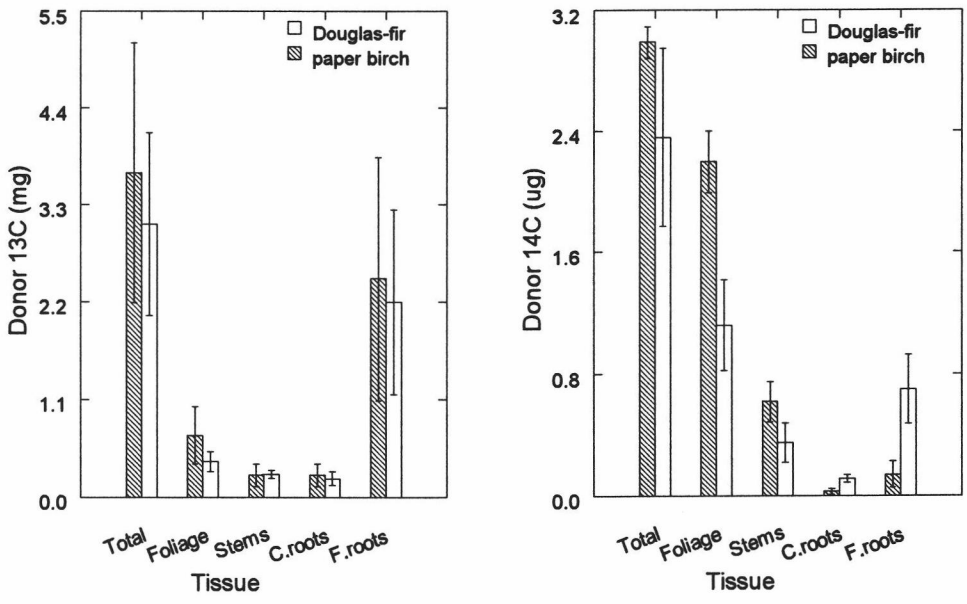

Figure 10. Total and tissue (a) ^{13}C and (b) ^{14}C contents in donor Douglas-fir and paper birch seedlings. Isotope contents differed among tissues for both species ($p<0.10$, d.f.=11).

Receivers

Douglas-fir seedlings received more total ($^{13}C+^{14}C$) isotope from neighboring paper birch than paper birch received from neighboring Douglas-fir ($p=0.10$, Table 8). Douglas-fir received 66% more ^{13}C ($p=0.10$) and 50% more ^{14}C than paper birch received from its neighbor, although the difference in received-^{14}C between species was not significant ($p>0.10$). The distribution of ^{13}C and ^{14}C among tissues of receiver seedlings varied between species. Most transferred ^{13}C remained in the roots of Douglas-fir ($p<0.01$), but was evenly distributed among all tissues within paper birch ($p>0.10$, Figure 11*a*). Whereas paper birch on average translocated 60% of received ^{13}C from the fine roots to upper tissues, Douglas-fir translocated only 33%. In both species, however, over 20% of received ^{13}C occurred in foliage. Transferred ^{14}C did not differ among tissues of either species ($p>0.10$, Figure 11*b*), mainly due to high variance in Douglas-fir stems and paper birch foliage. The amount of ^{14}C that reached paper birch and Douglas-fir foliage averaged 30% of the total received. These results suggest that ^{14}C and ^{13}C were readily translocated to all tissues of receiver seedlings, except ^{13}C in Douglas-fir.

Relationship between ^{13}C and ^{14}C fractionation

Differences in amount of isotope pulsed and metabolic fractionation between ^{13}C and ^{14}C resulted in net transfer differences dependent on labeling scheme ($p<0.01$, data not shown). For example, whole seedling contents of ^{14}C were at least 2 orders of magnitude lower than of ^{13}C, resulting in significant differences in net transfer (net transfer=isotope received by DF - isotope received by PB) between the labeling schemes, 14PB-13DF and 13PB-14DF. The correction factor (CF) eliminated the "effect of labeling scheme" and allowed estimation of net transfer. The relationship between tissue ^{14}C content and CF for donor and receiver plants is shown in Figure 12 ($R^2=0.73$, $p<0.01$, $n=32$). The smallest fractionation of ^{14}C relative to ^{13}C (i.e., lowest CF values) occurred in foliage of donor seedlings, and the greatest (i.e., highest CF values) in coarse and fine roots of receiver seedlings. This trend indicates a compounding of $^{13}C/^{14}C$ fractionation along the entire translocation pathway, from donor foliage to receiver tissues. The range in CF values ranged approximately between 25,000 and 500,000 for receiver root tissues and between only 3,000 and 155,000 for pulse-labeled root tissues, which reflects: (a) greater ^{13}C and ^{14}C variability in receiver than donor seedlings, and (b) stronger fractionation of ^{14}C relative to ^{13}C during isotope transfer between seedlings (e.g., through the soil pool or connecting mycorrhizal hyphae) than isotope translocation within seedlings.

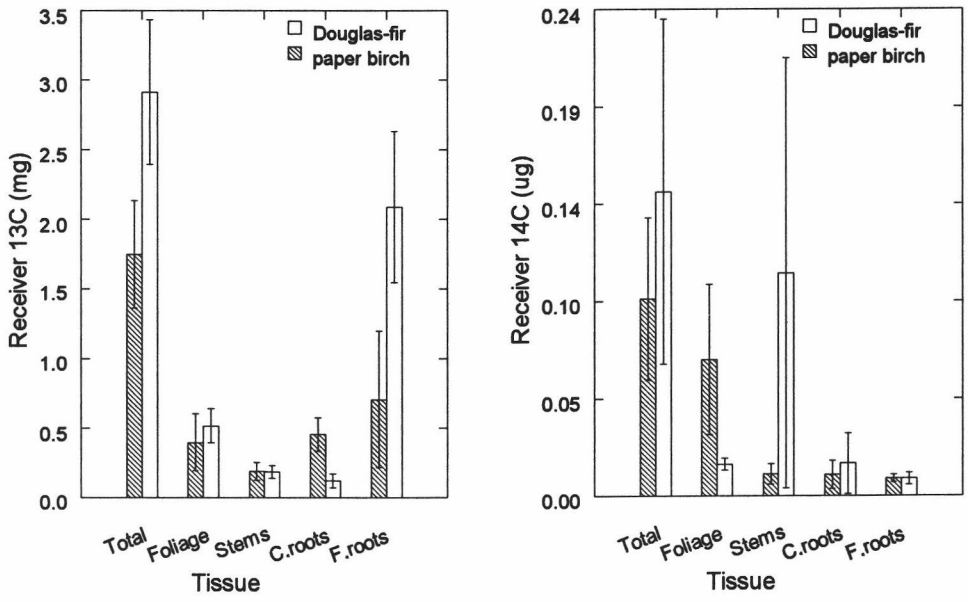

Figure 11. Total and tissue (a) ^{13}C and (b) ^{14}C received by fir and birch. Isotopes did not differ among tissues for either species ($p>0.10$, d.f.=11) except ^{13}C received by Douglas-fir ($p<0.01$).

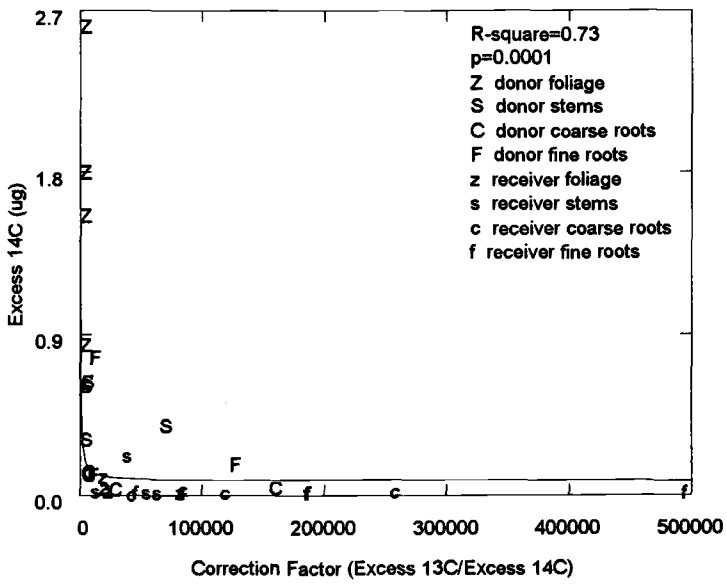

Figure 12. Tissue ^{14}C content versus CF for donor and receiver seedlings. Letters represent tissue type. Regression equation is y=0.083+481.866(1/x).

Effect of hyphal severing on gross and net transfer

Total isotope (^{13}C + ^{14}C) received by Douglas-fir from paper birch was 50% greater in Unsevered than in Severed rootboxes (p=0.15, Table 9, Figure 13). Severing had no effect, however, on total isotope received by paper birch from Douglas-fir (p>0.15). These trends suggest that hyphae facilitated isotope transfer from paper birch to Douglas-fir, but not in the opposite direction. Gross transfer (total isotope transferred in rootbox system) was unaffected by severing (p>0.15), and represented on average 29% of the total isotope fixed in the rootbox system.

Net transfer (calculated with CF-corrected ^{14}C values) was positive in both severing treatments, indicating greater isotope received by Douglas-fir than paper birch. In other words, there was net transfer of isotope from paper birch to Douglas-fir, regardless of mode of transfer (through hyphal connections or other mechanisms). Net transfer to Douglas-fir averaged almost three times greater where hyphae were left intact than where they were severed; however, because of high variance, the difference between severing treatments was not significant (p>0.15). Net transfer represented on average 4% of the total isotope fixed in the rootbox system, 10% of isotope fixed by Douglas-fir, and 7% of that fixed by paper birch.

Table 9. Net and gross transfer in severing treatments. Values are means and standard errors (parentheses). T-test detected differences between treatments at *p=0.15.

Transfer	Unsevered	Severed	p-value
^{14}C content **not** corrected with CF			
Transfer to Douglas-fir			
^{13}C (mg)	3.48 (0.63)	2.34 (0.34)	0.15*
^{14}C (µg)	0.24 (0.21)	0.07 (0.03)	0.47
Total ^{13}C+^{14}C (mg)	3.48 (0.63)	2.34 (0.34)	0.15*
Transfer to paper birch			
^{13}C (mg)	1.75 (0.18)	1.74 (0.47)	0.99
^{14}C(µg)	0.07 (0.02)	0.13 (0.09)	0.55
Total ^{13}C+^{14}C (mg)	1.75 (0.18)	1.74 (0.47)	0.99
Gross transfer (birch+fir)	5.24 (1.14)	4.09 (0.96)	0.27
^{14}C content corrected with CF			
Net Transfer (NT_{CF})	1.73 (0.84)	0.60 (0.49)	0.28

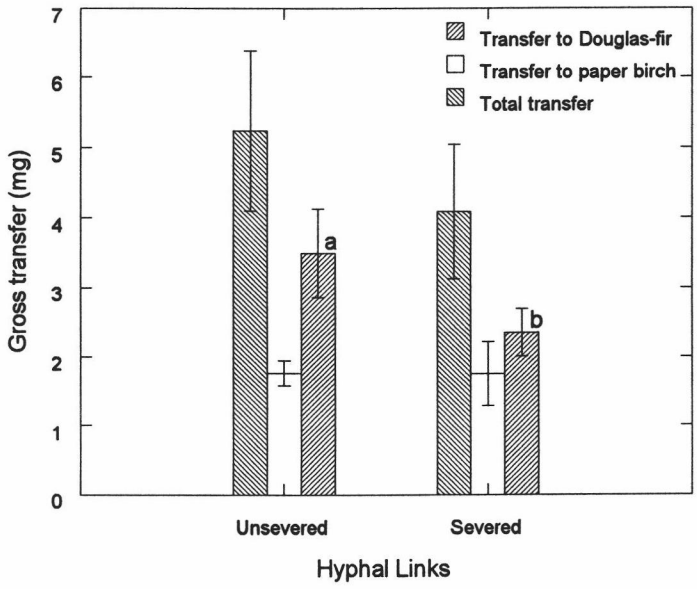

Figure 13. Isotope transferred between birch and fir (total transfer, p=0.27), transferred to birch alone (p=0.99), and transferred to fir alone (p=0.15) in Unsevered and Severed treatments. Means denoted by different letters differ at p=0.15.

Discussion

Net carbon transfer

Transfer of carbon between paper birch and Douglas-fir was bi-directional, but Douglas-fir received on average 1.7 times as much isotope from paper birch than in the reverse direction, indicating net transfer from paper birch to Douglas-fir. This, to our knowledge, is the first report of net carbon transfer between plants. Whether carbon was translocated directly through ectomycorrhizal fungi or indirectly through the soil pool remains equivocal, however, for two reasons. First, the presence and functional status of hyphal connections between paper birch and Douglas-for were not rigorously tested using autoradiography (similar to Francis and Read 1984). Hyphal connections probably occurred, however, because *(i)* paper birch and Douglas-fir shared in common seven ectomorphotypes over 90% of their root tips (Chapter 2), *(ii)* multiple hyphal connections between paper birch and Douglas-fir root zones were visible to the naked eye prior to labeling, and *(iii)* given the wide host range of many ectomycorrhizal fungi, hyphal connections between plants are considered likely quite common (Newman 1988).

Second, the difference in net carbon transferred between unsevered rootboxes (*i.e.,* directly through ectomycorrhizal connections) versus severed rootboxes (*i.e.,* indirectly through the soil carbon pool) was not significant ($p=0.28$). Net transfer averaged almost three times greater where hyphae were left intact than where they were severed immediately prior to pulse labeling, however this large difference did not approach statistical significance because of high variability among replicates. These results suggest that hyphae can facilitate carbon transfer from paper birch to Douglas-fir; however, it is a highly variable process that requires considerably more replication than we used in our experiment. Alternatively, some severed hyphae may have anastomosed and partially reconnected paper birch and Douglas-fir roots during the 6-day chase period. This is possible considering growth rates of *Thelephora terrestris* and *Laccaria proxima* mycelial fans originating from sitka spruce roots have been measured at 1-4 mm/day in field soils in mid-summer, rates which are comparable to those in greenhouse conditions (Coutts and Nicoll 1990). Finlay and Read (1986) also measured rates of mycelial spread and strand extension of *Suillus bovinus* and *Pitholithus tinctorius* from *P. contorta* seedlings at 9-16 $cm^2\ d^{-1}$ 2-4 $mm\ d^{-1}$, respectively. Given that root pouches were in close contact, results from these previous studies suggest that radiating hyphae from each plant could have at least partially anastomosed and formed interconnecting strands during the 6 d chase. The greater variation in net transfer in the severed (c.v.=82%) than intact treatment (c.v.=49%) may reflect variable rates and extent of re-connection.

Net carbon transfer from paper birch to Douglas-fir represented on average 4% of the total isotope fixed in the rootbox system, 10% of isotope fixed by Douglas-fir alone, and 7% of that fixed

by paper birch alone. Net carbon transfer in the present study exceeds one-way mycorrhizal-mediated ^{14}C transfer measured in previous studies (Hirrel and Gerdemann 1979, Francis and Read 1984, Finlay and Read 1986, Read *et al.* 1985), where ^{14}C found in receiver plants usually represented <1% of that found in interconnected donor plants. The amount of net transfer is similar, however, to the 10% ^{14}C transferred from floating clonal *Eichhornia crassipes* parents to connected offspring ramets (Alpert *et al.* 1991). It is also similar to the 5-15% transfer of $^{15}N_{2(gas)}$ fixed by the *Alnus glutinosa-Frankia* association to *Pinus contorta* through ectomycorrhizal connections (Arnebrant *et al.* 1993).

The direction and extent of inter-plant transfer is thought to be influenced by source/sink relationships between plants, such as those established by differences in net photosynthetic rate, nutrient status, or capability of fixing atmospheric N_2 (e.g., Read *et al.* 1985, Newman 1988, Bethlenfalvay *et al.* 1991, Alpert *et al.* 1991, Arnebrant *et al.* 1993). This is supported by the present study, in which whole seedling net photosynthetic rate of paper birch was estimated as 10 times that of neighboring Douglas-fir both during labeling (PAR=1000 $\mu E\ m^{-2}\ s^{-1}$) and the chase (PAR=400 $\mu E\ m^{-2}\ s^{-1}$, estimate based on light response curves shown in Chapter 3). In general, leaves of deciduous tree species have potential for higher photosynthetic rates than conifers, which reflect differences in the diffusion pathway for CO_2 (Waring and Schlesinger 1985). Net photosynthesis values for paper birch fell within the range reported by Wang *et al.* (1995) and Ranney *et al.* (1991), and those for Douglas-fir were similar to rates reported by Dosskey *et al.* (1990). Paper birch foliar nitrogen concentration also was approximately 2 times that of Douglas-fir (Chapter 5). Foliar nitrogen concentration has a well-known effect on net photosynthetic rates (e.g., Brix 1981, Pearcy *et al.* 1987, Wang *et al.* 1995). Whether plant size differences alone can influence source-sink relations has been questioned (Newman 1988); however, in this study paper birch biomass was double and root/shoot ratio 1.23 times that of neighboring Douglas-fir. The combined differences in net photosynthetic rate, foliar nitrogen concentration, and biomass between paper birch and Douglas-fir indicate that strong carbon and nitrogen source-sink relationships existed between the two species, and suggest a mechanism for net transfer from paper birch to Douglas-fir.

Gross carbon transfer

Although there was a 10% net carbon gain by Douglas-fir through transfer, a substantial amount was also received by Douglas-fir which was balanced by transfer of carbon back to paper birch. Gross transfer (total bi-directional transfer between paper birch and Douglas-fir) represented on average 29% of the total isotope assimilated in the rootbox system. This substantial belowground movement of carbon between seedlings, without unrecoverable loss to the soil carbon pool, is indicative of a tightly-linked, highly interactive seedling-soil system (Perry *et al.*

1989). Bi-directional gross transfer averaged 30% greater where hyphae were unsevered than severed; however, as with net transfer, differences between treatments were not significant (p=0.27).

Unidirectional gross transfer from paper birch to Douglas-fir averaged 50% greater where hyphae were unsevered than severed (p=0.15), but severing had no effect on gross transfer from Douglas-fir to paper birch. These results indicate that there was an 85% probability that hyphal connections facilitated isotope transfer from paper birch to Douglas-fir, and that there was no facilitation in the opposite direction. A possible explanation is that paper birch received all of its transferred carbon indirectly through the soil pool, whereas Douglas-fir received carbon directly through interconnecting fungi in *addition* to the soil pool. The direction and extent of carbon transferred via hyphae (unsevered treatment) should reflect the carbon/nutrient concentration gradient between seedlings, because interconnecting hyphae are believed to function as static conduits for nutrient transport governed by relative sink strength of their plant hosts (Miller and Allen 1992). Conversely, direction and extent of carbon transferred through the soil pool (severed treatment) should depend more on the individual demand of each species for organic nutrients (paper birch higher than Douglas-fir, based on foliar nutrient contents). Based on competition theory, seedlings with greater capacity for growth (paper birch > Douglas-fir) will have greater capacity to take up nutrients and hence deplete the soil nutrient pool to a greater extent (Tilman 1988). The small net carbon gain by Douglas-fir where hyphae were severed would then most likely have resulted from partial hyphal re-connection during the chase period.

We estimate that gross transfer averaged 29% of the total isotope assimilated in the rootbox system. The lack of difference between severed and unsevered rootboxes precludes distinction of amount transferred through ectomycorrhizal hyphae versus the soil pool. Possible pathways of indirect carbon transfer through the soil pool include *(i)* fungal and root exudate, *(ii)* respired CO_2, *(iii)* leakage from interconnecting hyphae, or *(iv)* sloughed fungal and root cells. Our evaluation of these pathways are as follows. *(i)* The portion of photosynthate exuded into the rhizosphere has been estimated in some studies as small, ranging between <1% and 4% (Paul and Kucey 1981, Miller *et al.* 1989, Jakobson and Rosendahl 1990), and in other studies much higher, ranging between 10% and 40% (Whipps and Lynch 1986, Reid and Mexal 1977). Results from the present study fall within the high range of exudate estimates in other studies. *(ii)* Anapleurotic uptake of respired CO_2 by paper birch and Douglas-fir roots was not detectable in a previous rootbox experiment (Chapter 3), even though fungal and root respiration has previously been shown to represent up to 33% of photosynthate (Paul and Kucey 1981, Harris *et al.* 1985). We discount anapleurotic uptake as a significant transfer route in this study. *(iii)* Leakage of photosynthate from interconnecting hyphae has been shown as negligible by Duddridge *et al.* (1980, 1988), so was not considered an important factor in this study. *(iv)* Unfortunately little is know about rate of carbon input to the soil pool through death and decomposition of mycorrhizal

hyphae (Finlay and Söderström 1992). The short chase period in the present study, however, likely resulted in little isotope input due to decomposition of sloughed material. Based on this information, we assume that most carbon that transferred indirectly through the soil pool originated from root and fungal exudates.

Carbon has been shown to translocate through mycelium both in fungal specific carbohydrates (Martin *et al.* 1984, Söderström *et al.* 1988) and amino acids (Martin *et al.* 1988a and 1988b, Abuzinadah and Read 1989, Arnebrant *et al.* 1993, Martin and Boutton 1993). Because there is no evidence for transport of sugars from fungal cells into plant cells, it is likely that labeled assimilated is converted to amino acids in fungal tissue before it is transferred to connected plants (Smith and Smith 1990). Abuzinadah and Read (1989) showed that labeled amino acids passed from mycorrhizal fungi into the xylem sap of host plants, and that the amount transferred increased with shade. In this study, the *(i)* photosynthate and foliar nitrogen gradient between paper birch and Douglas-fir, *(ii)* similar proportion of fixed carbon transferred compared to fixed nitrogen transferred in the *Alnus-Pinus* system of Arnebrant *et al.* (1993), and *(iii)* translocation of carbon into receiver foliage (see below), together suggest that assimilated carbon was incorporated into amino acids and translocated between plants along a carbon-nitrogen gradient from paper birch to Douglas-fir.

The amount of carbon transferred between paper birch and Douglas-fir in the present study exceeded the amount transferred in previous studies. One possible reason is that the carbon-nitrogen source/sink relationship between paper birch and Douglas-fir may have been steeper than interspecific gradients in other studies, as indicated by wide differences in whole seedling net photosynthetic rates, biomass and foliar nitrogen concentrations. Another possibility is that hyphal connections were formed by more than one fungal species, conceivably providing multiple transfer pathways, whereas previous studies examined transfer through a single fungal species ("unit mycelium"). The "fungal community concept", where several hosts are interconnected by several fungal species, more likely reflects the natural condition of mycorrhizal communities than the "unit mycelium concept" (Miller and Allen 1992).

Distribution of transferred carbon in receiver tissues

The distribution of transferred isotope in receiver seedling tissues varied as to whether ^{13}C or ^{14}C was transferred. Greater discrimination against ^{14}C than ^{13}C relative to ^{12}C during photosynthesis (Van Norman and Brown 1952, Buchanan *et al.* 1953, Craig 1954) may signify additional isotope effects along metabolic pathways during translocation and transfer; consequently, we consider ^{13}C the most reliable tracer of the fate of received ^{12}C. Most received ^{13}C remained in the roots of paper birch (66%) and Douglas-fir (74%), and may indicate that roots function as sinks for storage of transferred carbon compounds. However, substantially more was

translocated into shoots of paper birch (34%) and Douglas-fir (24%) than has been observed in previous studies (usually <10%, Newman 1988). Of the isotope transferred to shoots, the highest concentration (approximately 70%) was found in foliage. This transferred isotope could either supplement carbon in photosynthate, or supplement foliar nitrogen by functioning as the carbon skeleton in amino acids. Translocation of ^{13}C from receiver roots to foliage more likely occurs along a nitrogen rather than carbon concentration gradient, since fully developed leaves are usually strong sinks for nitrogen, and are sources rather than sinks for carbon (Pearcy *et al.* 1987).

Caveats

Various caveats must be applied to our results. Because the difference in one-way carbon transfer from paper birch to Douglas-fir between severing treatments was not highly significant ($p=0.15$), the magnitude of transfer directly through ectomycorrhizal linkages versus indirectly through the soil pool remains inconclusive. Most previous experiments have attempted to distinguish between these pathways using intermingled root systems, where transfer to/from mycorrhizal seedlings was compared with transfer to non-mycorrhizal controls (e.g., Francis and Read 1984). Because this approach can lead to size differences between mycorrhizal and non-mycorrhizal seedlings, or mask the pathway of transfer, we used a system with root-free pouches which did not permit mycorrhizal roots to intermingle and also allowed comparison of plants of similar size and physiology (similar to Bethlenfalvay *et al.* 1991, Frey and Schuepp 1992). Our comparison of severed versus un-severed hyphae should have distinguished between soil pool versus mycorrhizal pathways, respectively, but hyphal re-connection during the chase may be responsible for equivocal treatment differences. This may have been avoided by daily re-cutting of hyphal connections. A second caveat arises from use of field soil inoculation to establish hyphal connection between plants. Although overlap in ectomorphotypes of paper birch and Douglas-fir was large (Chapter 2) and multiple hyphal connections between pouches were visible to the naked eye, the functional status of the hyphal connections was not tested. Finally, because interspecific isotope transfer was highly variable, $^{13}C/^{14}C$ multipliers were also highly variable, which masked estimates of treatment effects on net transfer. This problem may have been reduced by pulse-labeling seedlings with similar quantities (mols) of ^{13}C and ^{14}C, or by increasing the sample size.

Isotopic fractionation

Isotope root/shoot ratios averaged 1.43 among donor seedlings pulse-labeled with ^{13}C, and only 0.21 among those pulse-labeled with ^{14}C. Pulse-labeled Douglas-fir and paper birch readily translocated ^{13}C out of foliage into roots, but retained most ^{14}C in foliage and stems, particularly among paper birch. Fractionation of ^{14}C relative to ^{13}C steadily increased through the whole

translocation pathway of donor seedlings, from foliage through fine roots. It increased almost an order of magnitude further, en route from donor to receiver seedlings. The greater magnitude and variability in $^{13}C/^{14}C$ discrimination in receiver than donor seedlings may be indicative of *(i)* greater fractionation during metabolic processes in the pathway between seedlings (i.e., through interconnecting hyphae or through the soil pool) than within seedlings, and/or *(ii)* greater variability in the amount of ^{14}C and ^{13}C transferred due to the indirect, tortuous pathway between seedlings.

Greater fractionation of $^{14}C/^{12}C$ than $^{13}C/^{12}C$ occurs during diffusion, chemical and biological processes (O'Leary 1981, Van Norman and Brown 1952) because the lighter isotope diffuses and reacts more rapidly. Within the framework of thermodynamic theory, the relative $^{14}C/^{13}C$ kinetic isotope effect (r) due to the atomic mass difference is approximately r=2.0 (Stern and Vogel 1971). The isotope effect during photosynthesis, which in C_3 plants occurs during CO_2 diffusion and carboxylation by ribulose bisphosphate, has been estimated to range between 2 and 4, resulting in photosynthetic discrimination of 4% against ^{13}C and 8-15% against ^{14}C relative to ^{12}C (Van Norman and Brown 1952, Buchanan *et al.* 1953, Craig 1954). Fractionation by thermodynamic and kinetic processes is compounded by the series of process stages (Craig 1954), so that isotopic composition along the translocation pathway reflects isotope fractionation both in the formation and metabolism of intermediates (O'Leary 1981). Our data showed a compounding effect of $^{14}C/^{13}C$ isotope fractionation along the entire translocatory pathway, from foliage to fine roots.

Summary

These results are significant both physiologically and ecologically because of the large proportion of assimilated isotope that was exchanged between paper birch and Douglas-fir. Douglas-fir may directly benefit from the association with paper birch through improved nutrition in nutrient-poor or patchy environments, or through supplemental carbon gain during conditions of low photosynthetic potential such as shade or drought. Conversely, paper birch may benefit from the association with Douglas-fir if the direction of transfer is reversed during early spring or fall when paper birch foliage is absent. Whether transfer occurs directly through interconnecting hyphae or indirectly through the soil pool can be resolved only through further research; this experiment could be repeated, for example, with greater replication and better hyphae severing procedures. In addition, further research is needed to improve understanding of differences in isotopic discrimination between ^{13}C and ^{14}C during translocation and transfer.

If results of this study reflect the magnitude of carbon transfer in natural systems, then plant-plant interactions have more dimensions than resource competition alone, and the net effect of one species on another cannot be predicted without a better understanding of the multi-dimensionality of their relationship. Maintaining a component of paper birch in Douglas-fir plantations for mitigation of root disease (Morrison *et al.* 1981), sustaining site productivity (Sachs

and Comeau 1995) or enhancement of species diversity (e.g., Cannell *et al.* 1992) then requires consideration of interactions at the community rather than simply the individual tree level. Further research is required to evaluate the implications of carbon transfer to other ecological processes, such nutrient distribution or disease resistance, and the long-term effects of these processes under field conditions.

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Chapter 5

Shading Influences Net Carbon Transfer among Ectomycorrhizal Tree Species in the Field

Abstract

Two dual-isotope (^{14}C and ^{13}C) labeling experiments were conducted in the field in 1993 and 1994 in order to examine (a) whether carbon isotope is translocated among ectomycorrhizal (EM) *Betula papyrifera* Marsh. and *Pseudotsuga menziesii* (Mirb.) Franco and arbuscular mycorrhizal (AM) *Thuja plicata* seedlings, and (b) how shading *Pseudotsuga menziesii* affects the amount of carbon isotope transferred. A previous greenhouse bioassay showed that *Betula papyrifera* and *Pseudotsuga menziesii* shared seven EM morphotypes in common over 90% of their short root tips (Chapter 2), indicating potential for EM hyphal connections and interspecific carbon transfer. Out-planted *Thuja plicata* formed only AM and *Betula papyrifera* and *Pseudotsuga menziesii* formed only EM, so *Thuja plicata* was used to signal isotope transferred indirectly between seedlings via the soil pool. One-year-old *Betula papyrifera*, *Pseudotsuga menziesii* and *Thuja plicata* seedlings were planted 50 cm apart in isosceles triangles (∴) on a mesic site in south-central British Columbia in the spring of 1993. One and two-years later, three shading levels (100%, 50% and 5% ambient sunlight) were applied to *Pseudotsuga menziesii* three to six weeks prior to labeling, then *Betula papyrifera* and *Pseudotsuga menziesii* were subject to reciprocal labeling treatments with $^{13}CO_{2(gas)}$ and $^{14}CO_{2(gas)}$. After 9 d, all seedlings were harvested, separated into four tissue types, and the tissues analyzed for ^{14}C and ^{13}C content.

Transfer between *Betula papyrifera* and *Pseudotsuga menziesii* was bi-directional, but there was a net carbon gain by *Pseudotsuga menziesii* in the 100% ambient sun treatment in 1993, and in all three shading levels in 1994. In 1993, net transfer to *Pseudotsuga menziesii* in 100% ambient sun represented 2% of the total isotope fixed by both species, 4% of the isotope assimilated by *Betula papyrifera*, and 7% of that assimilated by *Pseudotsuga menziesii*. In the 50% and 5% ambient sun treatments, isotope transfer to *Pseudotsuga menziesii* was balanced by transfer to *Betula papyrifera* (*i.e.*, 0 net transfer). In 1994, net isotope transfer to *Pseudotsuga menziesii* represented on average 6% of the total isotope fixed, and was approximately two times greater in 5% than 50% or 100% ambient sun treatments. Factors which may have been important to the change in shading effect on extent and direction of net transfer between 1993 and 1994 included extent of root development, seedling physiological vigor and phenology, and micro climatic conditions. Net transfer from *Betula papyrifera* to *Pseudotsuga menziesii* coincided with whole seedling net photosynthetic rates which were 1.5 and 4.3 times greater for *Betula papyrifera* than *Pseudotsuga menziesii* in full sun and full shade, respectively, and foliar nutrient concentrations which were 1.2-6.7 times higher for *Betula papyrifera* than *Pseudotsuga menziesii*.

Gross (total bi-directional) transfer represented 4% of the total isotope assimilated by *Betula papyrifera* and *Pseudotsuga menziesii* in 1993, and 7% in 1994. Gross transfer was unaffected by shading in 1993, but was two times greater in 5% than 50% or 100% ambient sun in 1994. This was due to increased one-way transfer from *Betula papyrifera* to *Pseudotsuga menziesii*, but not in the opposite direction, suggesting that transfer was affected by changes in sink strength of *Pseudotsuga menziesii*. Transfer to *Thuja plicata* varied between isotopes and years, but was independent of donor species and shading treatments. On average, isotope transferred to *Thuja plicata* represented <1% of gross transfer between *Betula papyrifera* and *Pseudotsuga menziesii* in 1993, and 18% in 1994.

Introduction

Ectomycorrhizae have been shown to form hyphal linkages between plants (Brownlee *et al.* 1983, Finlay and Read 1986) and function as pathways for interplant transfer of carbon, nutrients and water (e.g., Read *et al.* 1985, Duddridge *et al.* 1988, Söderström *et al.* 1988, Arnebrant *et al.* 1993). Since most ectomycorrhizae have broad to intermediate host specificity (Molina *et al.* 1992), extra-matrical hyphae are ubiquitous, and roots of different plants intermingle closely, hyphal links among different ectomycorrhizal plant species are likely quite common (Newman 1988). If ectomycorrhiza-mediated nutrient transfer between plants is extensive, then it may influence patterns in plant growth and survival, and hence plant community dynamics. Nutrient transfer from *Pinus ponderosa* to *Pseudotsuga menziesii* seedlings through *Laccaria laccata*, for example, may have been the basis for enhanced *P. menziesii* growth and foliar nutrient content in species mixtures versus monocultures (Perry *et al.* 1989).

Ectomycorrhizal links between plants have been directly observed in transparent containers in the laboratory (Read *et al.* 1985), but not in the field because of their delicate structure and microscopic size (Newman 1988). However, carbon transfer has been observed between ectomycorrhizal trees both in the laboratory (e.g. Francis and Read 1984, Finlay and Read 1986) and in the field (Read *et al.*1985). In the field, Bjorkman (1960) injected ^{14}C into stems of spruce and pine trees and later detected it in nearby *Monotropa*, and Read *et al.* (1985) labeled 35-year-old *Pinus contorta* trees with $^{14}CO_{2(gas)}$ and detected it in neighboring ectomycorrhizal but not arbuscular mycorrhizal plants. Other field experiments have also shown that the radio-isotopes of mineral nutrients applied to one ectomycorrhizal plant can be detected soon afterward in neighboring plants (e.g., Woods 1970). Additional indirect evidence comes from seedlings which, when established in the same soil or close to existing plants, form the same type of mycorrhizal fungus, implying contact with existing mycelium and hence formation of links (e.g., Amaranthus and Perry 1989, Deacon and Fleming 1992). None of these studies have quantified the extent of

interspecific nutrient transfer in the field, however, leaving in question the ecological significance of the transfer phenomenon.

Two fundamental issues underlie controversy over ecological significance of mycorrhizal-mediated carbon and nutrient transfer (Newman 1988, Miller and Allen 1992). First, net transfer from one plant to another has yet to be proven; *i.e.*, that gain in carbon or nutrients by one plant exceeds that of its neighbor. Invariably one plant has been fed an isotope and its neighbor assessed a few days later, thereby quantifying unidirectional transfer (Hirrel and Gerdemann 1979, Duddridge *et al.* 1980, Chiariello *et al.* 1982, Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986, Newman and Ritz 1986, Ritz and Newman 1986, Eissenstat 1990, Bethlenfalvay *et al.* 1991, Hamel and Smith 1991 and 1992, Frey and Schuepp 1992, Arnebrant *et al.* 1993, Newman and Eason 1993). Jakobsen (1991) suggests that net or bi-directional transfer can only be determined by comparing the results of reciprocal labeling in two parallel experiments. Second, providing net transfer does occur, whether it is sufficiently large to significantly affect plant survival or growth is unknown. Studies which have quantified one-way transfer of ^{14}C and ^{32}P have generally shown isotope transfer as very small. For example, less than 2% of donor ^{14}C has been shown to transfer between linked ectomycorrhizal or arbuscular mycorrhizal plants, with more in shade than sun (Francis and Read 1984, Read *et al.* 1985). In addition, rates and magnitude of ^{32}P transfer between arbuscular mycorrhizal-connected grasses or herbs has been shown as too slow or too small to significantly affect their nutrient status (Newman and Eason 1990, Eissenstat 1990, Eissenstat and Newman 1990, Newman and Eason 1993). In contrast with carbon and phosphorus transfer, there is growing evidence for significant amounts of nitrogen transfer from actinorrhizal to non-nodulated plants via ectomycorrhizal links. Arnebrant *et al.* (1993), for example, found that 5-15% of $^{15}N_{(gas)}$ fixed by the *A. glutinosa-Frankia* association was transferred to *P. contorta* via ectomycorrhizal connections, and that approximately 20% of the nitrogen found in pine was derived from N_2-fixation. Studies of N transfer from legumes to monocots, such as grass or corn, by arbuscular mycorrhizal links have had variable results; some have shown no evidence of hyphal transfer (e.g., Hamel *et al.* 1991), whereas others indicate small amounts of ^{15}N transfer (van Kessel *et al.* 1985, Bethlenfalvay 1991, Hamel and Smith 1991 and 1992, Frey and Schuepp 1992). In review of published evidence, both Newman (1988) and Miller and Allen (1992) suggest that neither issue, occurrence nor extent of net transfer, have been adequately addressed in either the laboratory or field.

Net transfer can be expected to occur if neighboring plants differ in some way, such as net photosynthetic rate, nutrient status, or N_2-fixing capability, that establishes a source-sink relationship (Read *et al.* 1985, Newman 1988, Bethlenfalvay *et al.* 1991, Arnebrant *et al.* 1993). In several transfer studies, source-sink concentration gradients have been established or strengthened for *(i)* photosynthate by shading receiver seedlings (Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986), *(ii)* nitrogen by using a nodulated N_2-fixing donor plant and non-nodulated receiver

plant (Bethlenfalvay *et al.* 1991, Hamel and Smith 1992, Frey and Schuepp 1992, Arnebrant *et al.* 1993), and *(iii)* phosphorus by fertilization of donor plants (Eissenstat 1990, Ritz and Newman 1986). For example, *(i)* over six times more ^{14}C was transferred from mature *Plantago* plants to shaded than to unshaded *Festuca* seedlings (Francis and Read 1984), and *(ii)* the greatest amount of ^{14}C was transferred from *P. contorta* to artificially shaded *P. contorta* neighbors in the field (Read *et al.* 1985) and laboratory (Finlay and Read 1986). These authors hypothesized that diffusion occurred through connecting mycorrhizal fungi along a source-sink gradient, from illuminated plants with high concentrations (source) of assimilate in roots to shaded plants with low concentrations (sink) of assimilate in roots.

This study addresses the extent and significance of net transfer between out-planted tree seedlings which share ectomycorrhizal fungi in common. The effect of shading Douglas-fir (*Pseudotsuga menziesii*) on net transfer between paper birch (*Betula papyrifera*) and Douglas-fir was examined among one- and two-year-old seedlings in the field. Paper birch and Douglas-fir were chosen for this study for several reasons. First, paper birch and Douglas-fir are commonly associated in wet-belt forest ecosystems in the southern interior of British Columbia (Simard and Vyse 1992). Douglas-fir commonly grows beneath the canopy of paper birch both as a cohort in juvenile stands, and as new regeneration in older stands. Second, the two species were shown to share in common seven ectomorphotypes over 90% of their root tips in a bioassay using the same plant and soil material as the present study (Chapter 2), suggesting potential for mycorrhizal connections. Third, paper birch has higher foliar nitrogen concentration and net photosynthetic rates (this chapter) than neighboring Douglas-fir, indicating that carbon and nitrogen source-sink relationships occur between the two species. This gradient was further strengthened by artificial shading of Douglas-fir. Net transfer between paper birch and Douglas-fir was studied following reciprocal exposure of one seedling to the radioactive carbon isotope ^{14}C and the other to the stable carbon isotope ^{13}C.

The objectives of this study were to determine in the field: (a) whether carbon isotope is translocated among ectomycorrhizal paper birch and Douglas-fir and arbuscular mycorrhizal western redcedar (*Thuja plicata*), and (b) how shading Douglas-fir affects the amount of carbon isotope translocated. We hypothesize that bi-directional translocation would occur between paper birch and Douglas-fir, but that there would be positive net transfer from paper birch to Douglas-fir, and that positive net transfer would increase with increased shading of Douglas-fir. In addition, we expect that carbon isotope transfer between either paper birch or Douglas-fir and western redcedar would be minimal due to absence of mycorrhizal connections.

Methods

Site description

The study site is located within the Clearwater Forest District, Kamloops Forest Region, of south-central British Columbia. It occurs within the Thompson variant of the Moist Warm Interior Cedar Hemlock biogeoclimatic subzone (Lloyd *et al.* 1991). The subzone is characterized by warm, moist summers and cold, snowy winters, with mean temperatures of 19°C in July and -6°C in January, and mean annual precipitation of 670 mm, of which 290 mm falls as rain during the growing season (Environment Canada 1980). The submesic-mesic site is a flat terrace (0-5% slope) at 700 m elevation, just above the North Adams River valley bottom. The soil is a Humo-Ferric Podzol (Canadian Soil Survey Committee 1978), formed over a granitic alluvial blanket. The soil surface layers (to 50 cm) are sandy loam to loamy sand, with coarse fragment content less than 10%.

The original mixed forest of Douglas-fir, paper birch, western redcedar, western hemlock (*Tsuga heterophylla*), lodgepole pine (*Pinus contorta*), western white pine (*Pinus monticola*), and trembling aspen (*Populus tremuloides*) was clearcut in 1987 and planted to Douglas-fir in 1988. Due to high incidence of *Armillaria ostoyae* root disease among planted seedlings, the site was mechanically de-stumped in 1991 to reduce inoculum load. This operation resulted in removal of all original seedlings, so the site was not planted when our study was initiated.

Seedling establishment

Interspecific translocation of carbon isotopes was tested on groups of out-planted ectomycorrhizal paper birch and Douglas-fir and arbuscular mycorrhizal western redcedar seedlings, where western redcedar served as a marker for indirect isotope movement through the soil pool (e.g., as root or fungal exudate, respired $CO_{2(gas)}$, sloughed root or fungal cells) and as a control for isotope respired and re-assimilated by foliage of paper birch and Douglas-fir neighbors.

One-year-old nonmycorrhizal seedlings were shovel-planted in early June 1992, before the onset of a growing season soil water deficit. Forty groups of three seedlings each (one Douglas-fir, one paper birch and one western redcedar) were established over a 0.25 ha area. The three seedlings within a group were arranged in an isosceles triangle (∴), with 0.5 m sides (interplant distance). Each group was encircled with an 0.5 m wide metal sheet buried approximately 30 cm in the ground. The purpose of the encircling metal sheet was to contain soils and plant material contaminated with carbon isotopes, to partially entrain seedling root growth toward the center of the group, and to minimize human traffic through the groups. Each group was carefully hand-weeded

prior to the labeling experiments to minimize isotope contamination of non-experimental plant material.

Two labeling experiments were conducted on separate groups of seedlings: one experiment in 1993 (seedlings out-planted for one year) and the other in 1994 (seedlings out-planted for 2 years). Each seedling group represented an experimental unit. Twenty-four seedling groups were randomly selected from the available 40 for the 1993 experiment, and 15 groups randomly selected from the remainder for the 1994 experiment.

Study design

The 1993 field experiment consisted of three Douglas-fir shade treatments and two labeling schemes in a 3 x 2 factorial set of treatments, which were randomly applied to seedling groups. The treatments were replicated four times in a completely randomized design (24 seedling groups). The three Douglas-fir shade treatments were: 100SUN (100% ambient sunlight), 50SUN (approximately 50% of ambient sunlight) and 5SUN (approximately 5% of ambient sunlight). Douglas-fir was shaded with cone-shaped tents made of either a single layer of light (50SUN) or double layer of heavy (5SUN) shade cloth fastened to 1-m-tall steel rod tee-pee frames. The shade tents were placed over Douglas-fir seedlings on June 24,1993, 3 weeks prior to pulse-labeling on July 14, 1993. Each of the three shade treatments were applied to eight seedling groups.

After the 3 week shading period, the paper birch and Douglas-fir seedlings in each group were labeled with different isotopes: one with ^{13}C and the other with ^{14}C. This approach enabled detection of the carbon isotope which was received by one seedling from the other. Seedlings pulse-labeled with a particular isotope are referred to as "donors", and those which received that same isotope are referred to as "receivers". Due to possibilities of differences in isotopic discrimination between ^{13}C and ^{14}C, two labeling schemes were applied to each shade treatment. For four replicates per shade treatment, paper birch (PB) was labeled with ^{14}C and Douglas-fir (DF) with ^{13}C (labeling scheme called 14PB-13DF) . For the other four replicates, the reciprocal scheme was applied, where paper birch was labeled with ^{13}C and Douglas-fir with ^{14}C (13PB-14DF).

In the 1994 field experiment, seedling groups received the same shade treatments as 1993, except that shade tents were placed over Douglas-fir 6 weeks prior to pulse labeling (shade tents were placed over Douglas-fir on June 24, 1994, and pulse-labeling applied August 4, 1994). In 1994, each of the three shade treatments were applied to five replicate groups of seedlings in a completely randomized design. Due to shortage of replicate groups, only one labeling scheme was applied: 14PB-13DF.

Environmental conditions

Environmental conditions of the clearcut site were monitored continuously during the 1993 and 1994 growing seasons (May-September) using a CR10 datalogger (Campbell Scientific), at a location approximately 200 m from the experimental area. The datalogger monitored rainfall, solar irradiance, air and soil temperature, and soil water using a 1-h time step, and stored minimum, maximum and average values. Maximum values were used to compare environmental conditions between growing seasons, since they best represent daytime growing conditions for seedlings. The maximum values were averaged over the three labeling days in 1993 and two labeling days in 1994 (see Figure 14). At a single location, rainfall was collected in a tipping bucket rain-gauge, air temperature and relative humidity monitored at 2 m height in a Stevenson screen, and solar irradiance at 2 m height on top of the screen. Temperature was monitored at 0.15 m depth in the mineral soil (average of six locations) and 0.15 m height above the ground surface using thermocouples, and soil water was recorded at 0.15 m depth in the mineral soil using gypsum soil moisture blocks.

Soil and foliar nutrients

Soil and tissue analyses were conducted at the British Columbia Ministry of Forests Research Laboratory, Victoria, B.C., using procedures adapted from Carter (1993) and Kalra and Maynard (1991).

Mineral soil samples were collected two days prior to pulse-labeling in July, 1993. Samples were collected to 15 cm depth approximately 30 cm outside the isosceles triangle of each seedling group. They were air dried and sieved to 2 mm fraction. Total N and C were determined simultaneously on milled samples using a Leco CHN-600 elemental analyzer. Exchangeable K, Ca, and Mg were analyzed on a Technicon Auto-analyzer following extraction with neutral ammonium acetate (Morgan's method). Exchangeable P was determined colorimetrically by UV/visible spectrophotometer following extraction with Bray-P1 extractant. Total extractable S was determined on an ARL 3560 ICP spectrometer following extraction with ammonium chloride. Soil pH was measured in a 1:2 mixture of soil and water.

Foliage was sampled from paper birch and Douglas-fir in each seedling group on the same date as mineral soils were sampled. Current year foliage was collected from the top ⅓ of paper birch crowns and from lateral branches in the top whorl of Douglas-fir crowns. Ovendried foliage was ground to 40 mesh in a Wiley mill. Samples (1 mg) were digested in nitric acid and H_2O_2, and analyzed for Ca, K, Mg, P, S, Fe, Cu, Mn, Zn and B on an ARL 3560 ICP spectrometer. Nitrogen content was determined by elemental analysis of the combustion type on a Leco CHN-600.

Root colonization by ecto- and arbuscular mycorrhizae

Five seedlings each of paper birch, Douglas-fir and western redcedar were harvested from the experimental area in the fall of 1994 and examined for arbuscular mycorrhizal and ectomycorrhizal colonization. Roots were cleared, their pigment removed with 10% H_2O_2, and stained in a solution of 0.05% trypan-blue in lactic acid according to methods modified from Philips and Hayman (1970). Among roots <1 mm diameter, the percent of root length *(i)* nonmycorrhizal, *(ii)* infected with ectomycorrhiza, *(iii)* infected with arbuscular mycorrhizae (using on AM hyphae as the criterion), and *(iv)* infected with arbuscular mycorrhizae (using vesicles as the criterion), was determined by use of microscopy and the gridline intercept method of Giovannetti and Mosse (1980).

Net photosynthetic rate and light availability

Net photosynthetic rate and incident photosynthetically active radiation (PAR) of paper birch, Douglas-fir and western redcedar current year foliage were measured one day prior to pulse-labeling in both 1993 and 1994. In both years, all seedlings were measured in each replicate group in the three shade treatments. In the shade treatments, net photosynthetic rate of Douglas-fir was measured under the shade cloth tents.

Measurements were made between 1100 and 1400 hours on July 13, 1993 and August 3, 1994. The sky was uniformly overcast during the measurement period in 1993, and sunny in 1994. Two attached, fully developed leaves of paper birch were randomly sampled from the outmost and top 1/3 of the seedling crown. For Douglas-fir and western redcedar, two attached lateral branches were randomly sampled from the top whorl. All sample leaves represented current year's foliage, and all had developed prior to imposition of the shading treatments (*i.e.*, sun leaves). Leaf net photosynthetic rate and PAR were measured using a portable open CO_2 gas analyzer (LCA-2, Analytical Development Corp., Hoddeson, England). The leaves were harvested, pressed and dried flat, and leaf area (one side) later measured using a leaf area meter (Li-Cor 3100, Lincoln, Nebraska). Biomass was measured after leaves were oven-dried at 80°C for 48 h. Specific leaf area ($cm^2\ g^{-1}$) was calculated as the ratio of leaf area to corresponding leaf weight. Specific leaf area, foliar biomass of harvested seedlings, and leaf net photosynthetic rate were used to estimate whole seedling leaf area and whole seedling net photosynthetic rate.

^{13}C and ^{14}C labeling procedures

Seedlings were pulse-labeled with ^{13}C and ^{14}C using 2 h pulse and 9 d chase periods. A 6-d chase was determined appropriate for isotope translocation to fine roots in the laboratory (Chapter 3), and 9 d was used in this study to compensate for suboptimal climatic conditions. All isotope pulses were administered between 1000 and 1600 hours under minimum PAR of 800 μE m^{-2} s^{-1} for high labeling efficiency. PAR of 800 μE m^{-2} s^{-1} represents approximately 80% of saturation light intensity for paper birch and Douglas-fir (Chapter 3). In the 1993 experiment, pulse-labeling procedures were carried out over a 3-d period (July 14-16, 1993) under average PAR of approximately 900 μE m^{-2} s^{-1}. One replicate of the six shade*labeling scheme treatments was pulsed on July 14, two replicates on July 15, and one replicate on July 16. In 1994, pulse labeling procedures were carried in 2 d (three replicates on August 4 and 2 replicates on August 5) under average PAR of 1300 μE m^{-2} s^{-1}.

Shade tents were removed from Douglas-fir in the 50SUN and 5SUN treatments during the 2 h pulse period for maximum labeling efficiency. Each paper birch and Douglas-fir seedling was sealed inside flexible, air-tight, 5 mil thick x 60 cm wide x 90 cm tall, fluoropolymer gas sampling bags (Teflon Brand, Chemware). The sampling bags were supported in an expanded, upright position by tomato frames which encircled the seedlings. The bags were sealed around the root collar and legs of the tomato frames using duct tape. Each sampling bag was fit with a silicone septum in a polypropylene housing for injections with a hypodermic needle. Moments before injections, the bags were completely sealed by screwing on the septum housing. The shoot of one partner seedling was then pulsed with 200 mL of 99% $^{13}CO_{2(gas)}$ (107.42 mg ^{13}C). At the same time, the shoot of the other partner seedling was pulsed with $^{14}CO_{2(gas)}$ released from 200 μCi (7.4 MBq, 52.86μg) $Na_2{}^{14}CO_3$ with lactic acid. The ratio of pulsed mg^{13}C/mg^{14}C was 2032. After 2 h, unused $^{14}CO_{2(gas)}$ remaining in ^{14}C-labeling bags was forced through soda lime traps using compressed $N_{2(gas)}$. The traps consisted of 50 cm x 1 cm tygon tubing lightly packed with soda lime, and were attached at one end to the sealed sampling bag and plugged at the other end with a perforated rubber stopper. Once the ^{14}C-labeling bag was flushed, both labeling bags in a group were quickly removed. The shade tents were immediately replaced in the 50SUN and 5SUN treatments, and remained in place throughout the 9-d chase period. All seedling groups within a replicate were labeled within 1 h of each other.

At the end of the 9-d chase period, seedlings were carefully harvested and separated into four tissue fractions: leaves, stems, coarse roots (>1 mm diameter), and fine roots (<1 mm diameter). At the same time, four unlabeled seedlings of each species located outside the sheet metal barricades were harvested for natural abundance determinations (*i.e.,* isotope controls). Root systems were harvested by *(i)* loosening soil to below the rooting depth, *(ii)* carefully excavating the entire, intact root system of the seedling group, and *(iii)* separating roots by species.

Foliar, stem and root tissues were immediately separated in the field, and roots later separated into coarse and fine fractions in the laboratory following washing with tap water. The tissues were ovendried at 80°C for 48 h, weighed, and then ground to 20 mesh in a Wiley mill. Ground tissue samples (1 mg) were analyzed for ^{13}C abundance using a Europa Scientific ANCA mass spectrometer, and then combusted in a sample oxidizer and ^{14}C counted via liquid scintillation. Samples also were analyzed for total C (%) by combustion.

Gross and net carbon transfer

Sample $\delta^{13}C$ (‰) and ^{14}C (Bq) values were converted to a common unit, milligrams C isotope, for gross and net transfer calculations as well as other comparisons. The conversions for ^{13}C and ^{14}C were based on procedures described by Boutton (1991) and Warembourg and Kummerow (1991), respectively. The tissue $\delta^{13}C$ values first were converted to the absolute isotope ratio ($^{13}C/^{12}C$) of the sample (R):

$$R_{sample}=^{13}C/^{12}C=[(\delta^{13}C/1000)+1] \times R_{standard}$$

where $R_{standard}=0.0112372$, the international PDB standard. The fractional abundance (A) of ^{13}C relative to $^{13}C+^{12}C$ was then related to R by the equation:

$$A=^{13}C/(^{13}C+^{12}C)=R/(R+1).$$

Fractional abundance and total carbon content (mg) of the sample were used to calculate mg ^{13}C of the sample:

$$mg^{13}C_{sample} = A \times [^{13}C+^{12}C] \text{ (mg)}.$$

The enrichment level of the sample ($mg^{13}C_{sample}$) in excess of natural abundance ($mg^{13}C_{na}$) was calculated as:

$$\text{excess } mg^{13}C_{sample}=mg^{13}C_{sample}-mg^{13}C_{na}.$$

Excess mg^{13}C of the tissue (excess $mg^{13}C_{tissue}$) was calculated as the product of excess $mg^{13}C_{sample}$ (per mg of sample) and tissue biomass (mg). Finally, excess mg^{13}C of the whole plant (excess $mg^{13}C_{plant}$) was determined by summing the excess $mg^{13}C_{tissue}$ of the four tissue types.

Conversion of $Bq^{14}C$ to $mg^{14}C$ was based on the batch specific activity (λ) of $Na_2{}^{14}CO_3$, λ=1.96 GBq/mmol (Amersham Canada). First, radioactivity of a sample (Bq or dps) was expressed per mg C ($Bq^{14}C_{mgC}$):

$$Bq^{14}C_{mgC}=Bq^{14}C_{sample}/mgC_{sample}.$$

Radioactive units (Bq) were converted to $mols^{14}C$ using λ, and then $mg^{14}C$ using the molecular weight of ^{14}C ($mw^{14}C$):

$$mols^{14}C_{mgC}=Bq^{14}C_{mgC}/(\lambda),$$

$$mg^{14}C_{mgC}=mols^{14}C_{mgC}/mw^{14}C.$$

Excess $mg^{14}C_{mgC}$ of a sample was calculated by subtracting natural abundance ($Bq^{14}C_{na}$) values from sample values. Excess $mg^{14}C$ of the tissue (excess $mg^{14}C_{tissue}$) was calculated as the product of excess $mg^{14}C_{mgC}$ and tissue carbon (mgC_{tissue}):

$$\text{excess } mg^{14}C_{tissue}=\text{excess } mg^{14}C_{mgC} \times mgC_{tissue}.$$

Finally, as for ^{13}C, excess $mg^{14}C$ of the whole plant (excess $mg^{14}C_{plant}$) was determined by summing the excess $mg^{14}C_{tissue}$ of the four tissue types.

Pulse-labeling efficiency was defined as the ratio of excess mg isotope contained in pulsed seedling tissues at harvest to total mg isotope injected into the chamber.

Gross and net transfer calculations were based on whole plant levels of excess isotope which was received from the partner plant in a seedling group. For the labeling scheme 14PB-13DF, paper birch received ^{13}C from Douglas-fir while Douglas-fir received ^{14}C from paper birch. Similarly, for the reciprocal labeling scheme 13PB-14DF, paper birch received ^{14}C from Douglas-fir while Douglas-fir received ^{13}C from paper birch. Gross transfer was the sum of isotope received by both species in a seedling group:

$$\text{Gross transfer=isotope received by DF + isotope received by PB.}$$

For example, gross transfer (GT) in a seedling group subject to the labeling scheme 14PB-13DF was calculated as:

$$GT_{14PB\text{-}13DF}=\text{DF excess } mg^{14}C_{plant} + \text{PB excess } mg^{13}C_{plant}$$

Net transfer was calculated as the difference between isotope received by Douglas-fir and that received by paper birch in a seedling group:

Net transfer=isotope received by DF - isotope received by PB.

For example, net transfer (NT) in a seedling group subject to the labeling scheme 14PB-13DF was calculated as:

$$NT_{14PB\text{-}13DF} = DF \text{ excess mg}^{14}C_{plant} - PB \text{ excess mg}^{13}C_{plant}.$$

Positive net transfer indicates that a greater amount of isotope was received by Douglas-fir than by paper birch, and negative net transfer indicates the opposite. Gross and net transfer also were expressed as proportions of isotope fixed by (a) Douglas-fir, (b) paper birch, and © the seedling group (sum of Douglas-fir, paper birch and western redcedar).

Statistical analysis

Data were analyzed separately by experiment year using SAS (SAS Institute Inc.). Species and shade treatments were compared in terms of seedling biomass, net photosynthetic rate and isotope content using two-factor analysis of variance (ANOVA). Species and shade treatments were then compared separately using one-factor ANOVAs. Where significant differences ($p<0.10$) occurred, multiple comparisons were made among treatments using the Waller-Duncan-Bayes multiple range test.

In the 1993 experiment, the effects of labeling scheme (14PB-13DF and 13PB-14DF) and shade treatments (100%, 50% and 5% of ambient sunlight) on net transfer were detected by two-factor ANOVA (n=4). Significant ($p<0.10$) effects of labeling scheme were removed by applying a correction factor to excess mg^{14}C on a treatment x species x tissue basis. The correction factor (CF) was the species-specific ratio of excess mg$^{13}C_{tissue}$ and excess mg$^{14}C_{tissue}$ measured in the reciprocal labeling schemes of the same shade treatment. For example, excess mg^{13}C in Douglas-fir fine roots measured in the labeling scheme 13PB-14DF, was divided by excess mg^{14}C in Douglas-fir fine roots measured in the reciprocal labeling scheme 14PB-13DF, of the same shade treatment. The treatment-species-tissue-specific CF values were averaged over the four replicates per labeling scheme. The corrected excess mg^{14}C values (excess mg^{14}C * CF) were considered excess mg^{13}C equivalents. The same result is derived whether ^{14}C values are corrected to ^{13}C-equivalent values or, conversely, ^{13}C values are corrected to ^{14}C-equivalent values, because the latter is simply reciprocal of the former. Using the corrected excess mg^{14}C values, data were subjected to one-factor ANOVA for comparisons among shade treatments (n=8). Where

significant differences ($p<0.10$) occurred, multiple comparisons were made among treatments using the Waller-Duncan-Bayes multiple range test. Relationships between excess $mg^{13}C$ and excess $mg^{14}C$ were explored using simple regression.

The CFs and regression relationships derived with 1993 data were used to correct excess $mg^{14}C$ measured in the 1994 experiment. The effects of shade treatments on seedling and system variables were then examined using one-factor ANOVA, and means separated using the Waller-Duncan-Bayes multiple range test.

Results

Microclimate and soil properties

Soil water potential was lower during the pulse and chase periods in 1993 than 1994, indicating greater water availability to seedlings in 1994 (Figure 14*a*). Beginning at the pulse on July 14-16, 1993, soil water potential dropped from -3.30±0.23 MPa to -5.50±0.25 MPa until it was rapidly replenished to -0.34±0.00 MPa by rain after day 6 of the chase period. In contrast, soil water potential remained higher than -3 MPa during the entire pulse-chase period between August 3 and 13, 1994, varying only between -3.00±0.06 MPa and -1.76±0.01 MPa. Photosynthetically active radiation (PAR) during the pulse was also higher in 1994 than 1993, averaging 510 and 350 $\mu mol\ m^{-2}\ s^{-1}$ respectively (Figure 14*b*). Higher PAR during the pulse partially accounts for greater pulse-labeling efficiency in 1994 than 1993. PAR remained higher during the first day of the chase in 1994 ($520\pm13 \mu mol\ m^{-2}\ s^{-1}$) than 1993 ($296\pm74 \mu mol\ m^{-2}\ s^{-1}$), which may have been an important factor to the difference in extent of gross isotope transfer between years. For the remainder of the chase period, average PAR was similar between years.

Air temperature (15 cm height) during the pulse and first day of the chase was the same in 1993 and 1994, averaging 20±2°C (Figure 14*c*). It dropped to 14±1°C on the third day of the chase in 1994 due to a 2-day rainfall of 20 mm, and then quickly climbed over 19°C for the remainder of the chase period. In contrast, air temperature remained above 19°C during the entire chase period in 1993. Patterns in soil temperature (5 cm depth) tracked those of air temperature (Figure 14*d*). Soil temperature during the pulse and chase periods varied approximately between 17.5 and 21.5°C in 1993, and between 18 and 22.5°C in 1994. Soil temperature during the pulse and chase days one and six through nine, was 2 to 4°C greater in 1994 than 1993, but was similar during chase days 2 to 5. Higher soil water availability, PAR and soil temperature combined to create a

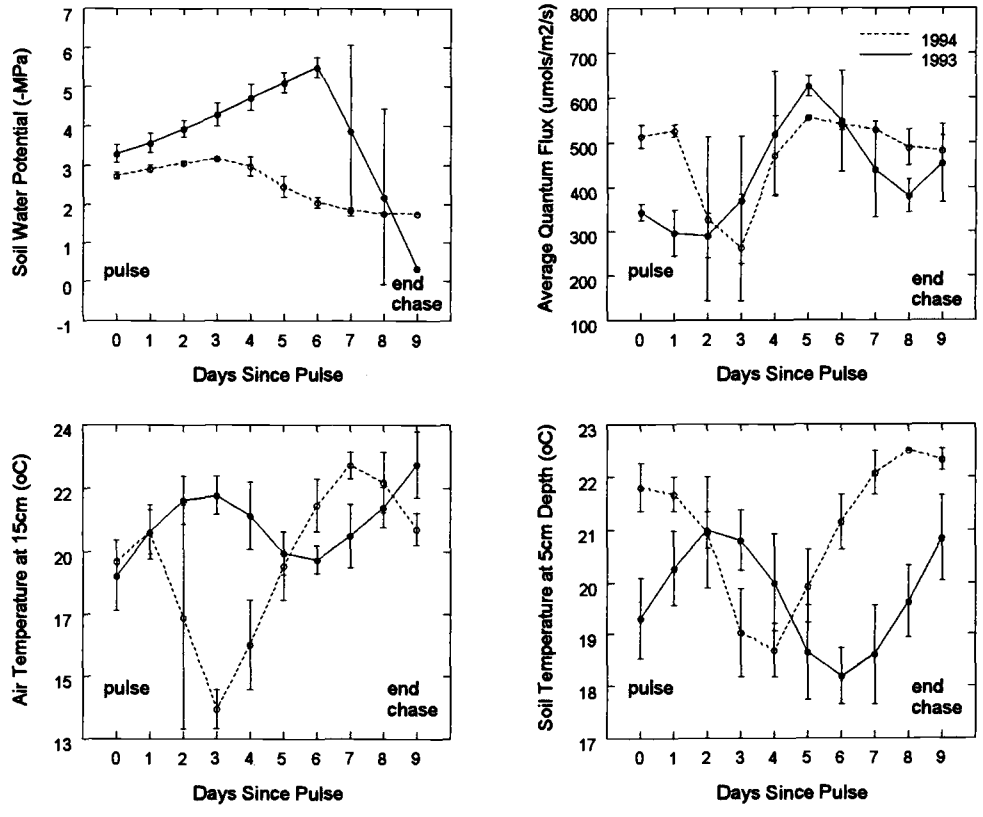

Figure 14. (a) Soil water potential (0.15 m depth), (b) PAR (2 m height), © air temperature (0.15 m height), and (d) soil temperature (0.15 m depth) during the isotope pulse and chase periods in 1993 and 1994.

potential for greater photosynthetic rates during the pulse and majority of the chase period in 1994 than 1993.

Soil nutrient concentrations and pH were not affected by shading treatments ($p>0.10$), which was expected because shading was applied to small areas (0.125 m^2) and over a short time period (4 weeks). Soils were low in total N (0.11±0.00%) and C (1.89±0.10%) (Russell 1973, Barber 1984), a result of extensive surface soil displacement during the de-stumping operation in 1992, but the low C:N ratio (17.0± 0.1) suggests that the little nitrogen remaining may have been readily available to seedlings (Stevenson 1986). Physical protection of organic matter in aggregates renders C:N ratio an unreliable indicator of nitrogen availability, however (Borchers and Perry 1992); a better measure of availability is mineralizable N, which was not measured in our study. The soils also had high pH (6.25±0.02) for conifer soils, which is favorable for availability of N, S, P, K, Ca and Mg (Pritchett 1979). Exchangeable Ca (740.2±38.9 ppm), exchangeable Mg (47.3±2.5 ppm), exchangeable K (104.0±3.8 ppm), and total S (0.013±0.001%) concentrations were moderate compared with other soils in North America (Barber 1984, Stevenson 1986, Schlesinger 1991).

Seedling characteristics

Paper birch seedlings were only 70% and 50% the biomass of Douglas-fir seedlings in 1993 and 1994, respectively (Figures 15*a* and 15*b*). Biomass differences measured in 1993 were primarily due to observed size differences between species at time of planting in 1992. The gap between paper birch and Douglas-fir biomass increased from 1993 to 1994 due to grazing of paper birch by deer during the spring of 1994. Western redcedar control seedlings were the same size as paper birch in 1993, but grew rapidly to become approximately the same size as Douglas-fir in 1994. Biomass distribution among tissues was similar between paper birch and Douglas-fir in 1993; roots accounted for 37-40% and foliage for 28-30% of total biomass (Figure 15*a*). Western redcedar also allocated 36% of its total biomass to roots, but over 39% to foliage in 1993. In 1994, root biomass of Douglas-fir and paper birch accounted for 30 and 33%, and stem biomass accounted for 38 and 44% of total biomass, respectively (Figure 15*b*). Douglas-fir foliage remained 32% but paper birch foliage dropped to 23% of total biomass due to deer browsing. Because of its poor foliage condition but robust root system, root/shoot ratio was greater for paper birch (0.44) than Douglas-fir (0.38) ($p<0.10$). Western redcedar foliage accounted for 45% and roots only 26% of total biomass, resulting in a low root/shoot ratio (0.33) in 1994. High foliage biomass partially explains the rapid growth of western redcedar between 1993 and 1994.

Leaf net photosynthetic rates were measured the day prior to commencement of pulse-labeling, which was a uniformly cloudy day in 1993 (average PAR=175 $\mu E\ m^2\ s^{-1}$) and a sunny day

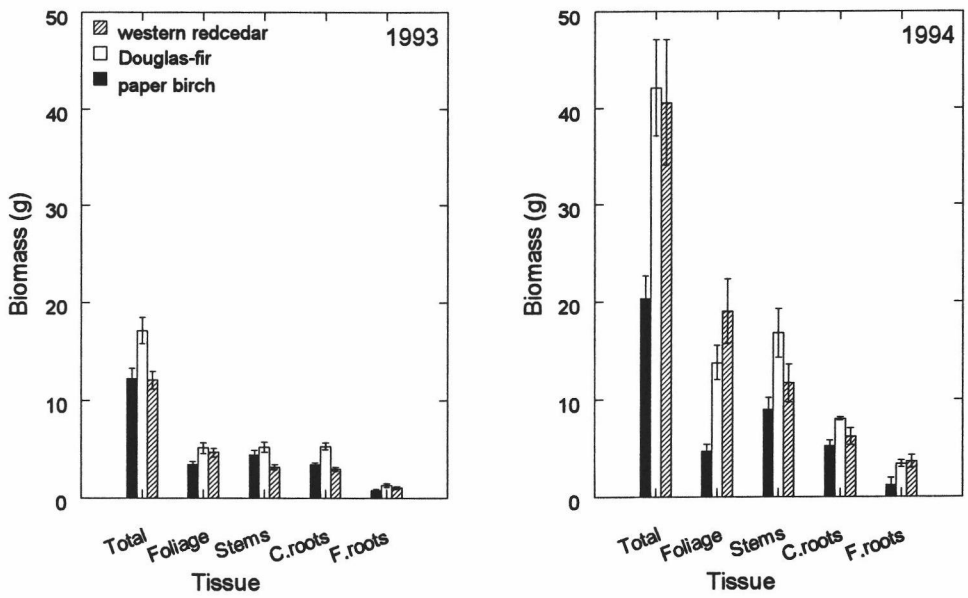

Figure 15. Biomass allocation to foliage, stems, coarse roots and fine roots of paper birch, Douglas-fir and western redcedar in (a) 1993 and (b) 1994.

in 1994 (average PAR=1330 μE m^2 s^{-1}). In 1993, leaf net photosynthetic rates in ambient (unshaded) light intensity (PAR=175 μE m^2 s^{-1}) were the same for paper birch and Douglas-fir, which in turn were double those of western redcedar (p=0.0004, Table 10, Figure 16). Leaf net photosynthetic rates of Douglas-fir decreased with increased shading (Figures 17*a*, 18*a*). Although both shading treatments resulted in significant PAR reductions (p=0.0001), Douglas-fir leaf net photosynthetic rate was affected only by decreasing PAR from 175 (100SUN) to 75 μE m^2 s^{-1} (50SUN) in 1993 (p=0.0235). As expected, shading Douglas-fir had no effect on net photosynthetic rates of either paper birch or western redcedar. In spite of the low foliar biomass of paper birch, its higher specific leaf area than either Douglas-fir or western redcedar (p=0.0001) resulted in significantly higher whole seedling leaf area (p=0.0001) and whole seedling net photosynthetic rates in the full light treatment (PAR of 175 μE m^2 s^{-1}, p=0.0001). Whole seedling net photosynthetic rates of paper birch averaged 1.5, 1.7 and 2.9 times that of Douglas-fir at PAR of 175, 75 and 15 μE m^2 s^{-1}, respectively, and 3.9 times that of western redcedar at PAR of 175 μE m^2 s^{-1}.

In 1994, leaf net photosynthetic rates in ambient (unshaded) light intensity (PAR=1330 μE m^2 s^{-1}) of paper birch were 1.7 times that of Douglas-fir and 2.2 times that of western redcedar (p=0.0001, Table 10, Figure 16). As in 1993, leaf net photosynthetic rates of Douglas-fir decreased with increased shading (Figures 17*b*, 18*b*). Specifically, Douglas-fir leaf net photosynthetic rates was cut by 60% when PAR was decreased from 1330 to 60 μE m^2 s^{-1} (p=0.10). Whole seedling leaf area did not differ among species (p>0.10), but whole seedling net photosynthetic rate in full light was 1.6 times greater for paper birch than either Douglas-fir or western redcedar (p=0.0523). Whole seedling net photosynthetic rates of paper birch averaged 1.5, 1.2 and 4.3 times that of Douglas-fir at PAR of 1330, 250 and 60 μE m^2 s^{-1}, respectively.

Foliar nutrient concentrations were greater for paper birch than Douglas-fir for all elements tested (p=0.0001, Table 11). The ratio of paper birch to Douglas-fir ranged from 1.5-1.7 for the macronutrients (listed in increasing order) S, N, and P, from 1.2 to 3.0 for the major cations K, Mg and Ca, and from 1.7 to 6.7 for the micronutrients B, Cu, Fe, Mn and Zn.

Mycorrhizal colonization

Fifteen seedlings (five Douglas-fir, five paper birch, five western redcedar) were examined for percent root length colonized by arbuscular mycorrhizae and ectomycorrhizae (Table 12). Although unconventional, expressing ectomycorrhiza abundance as a percent of root length is useful for woody angiosperms because there are substantial numbers of roots that have hartig net and mantle not associated with tips (Jones *et al.* 1990, 1991), and for western redcedar because there are no short roots. Douglas-fir and paper birch roots were 59±6% nonmycorrhizal and 39±7% ectomycorrhizal, with no differences between species (p>0.10). Neither vesicles,

Table 10. Net photosynthetic rates, specific leaf area and foliage biomass of one- and two-year-old paper birch, Douglas-fir and western redcedar seedlings. Values are means and standard errors (in parentheses). ANOVA detected differences among species at **p<0.01 and *p<0.05.

Characteristic	paper birch	Douglas-fir	cedar	p-value
1993 (1 year old)				
Leaf A[1] (μmol m^{-2} s^{-1}) 100% (175 μE m^{-2} s^{-1}) 50% (75 μE m^{-2} s^{-1}) 5% (15 μE m^{-2} s^{-1})	 1.02a - -	 1.21 (0.16)a 0.81 (0.13) 0.69 (0.08)	 0.51b - -	 0.0004**
Specific leaf area (cm^2 g^{-1})	105.75 (5.53)a	39.96 (2.45)b	37.90 (0.97)b	0.0001**
Foliage biomass (g)	3.25 (0.29)b	5.17 (0.48)a	4.72 (0.44)a	0.0026**
Whole seedling leaf area (cm^2)	344a	207b	179b	0.0001**
Whole seedling A (μmol s^{-1}) 100% (175 μE m^{-2} s^{-1}) 50% (75 μE m^{-2} s^{-1}) 5% (15 μE m^{-2} s^{-1})	 0.035 (0.003)a - -	 0.023 (0.002)b 0.021 (0.005) 0.012 (0.002)‡ ‡p=0.0001	 0.009 (0.00)c - -	 0.0001**
1994 (2 years old)				
Leaf A ((μmol s^{-1}) 100% (1330 μE m^{-2} s^{-1}) 50% (250 μE m^{-2} s^{-1}) 5% (60 μE m^{-2} s^{-1})	 4.44 (0.18)a - -	 2.61 (1.02)b 2.52 (0.56) 1.06 (0.21)	 1.98 (0.34)b - -	 0.0001**
Foliage biomass (g)	4.76 (0.72)b	13.76 (1.74)a	19.01 (3.31)a	0.0002**
Whole seedling leaf area (cm^2)	503 (76)	550 (70)	720 (125)	0.2384
Whole seedling A (μmol s^{-1}) 100% (1330 μE m^{-2} s^{-1}) 50% (250 μE m^{-2} s^{-1}) 5% (60 μE m^{-2} s^{-1})	 0.22 (0.03)a	 0.14 (0.03)b 0.18 (0.037) 0.05 (0.009)‡ ‡p=0.0188	 0.14 (0.03)b	 0.0523*

[1] 'A' is net photosynthetic rate.

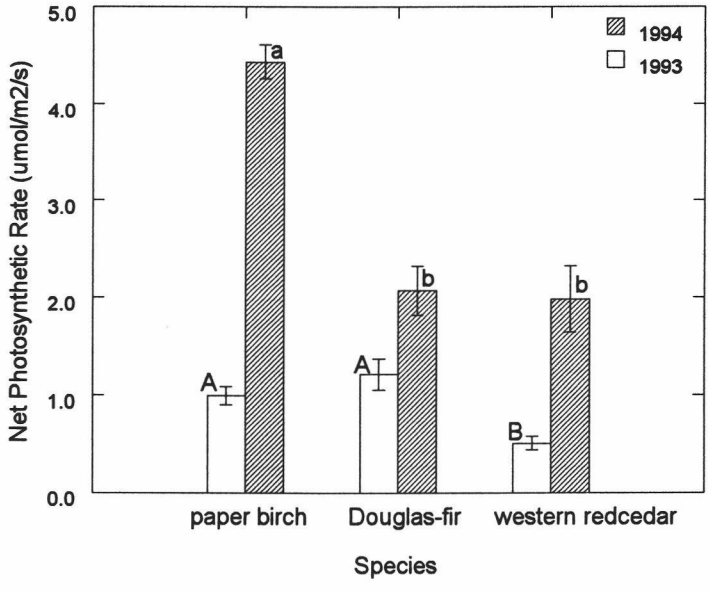

Figure 16. Leaf net photosynthetic rates of birch, fir and cedar. Upper and lower case letters refer to 1993 and 1994 means, respectively. Means denoted by the same letter do not differ significantly (p=0.05).

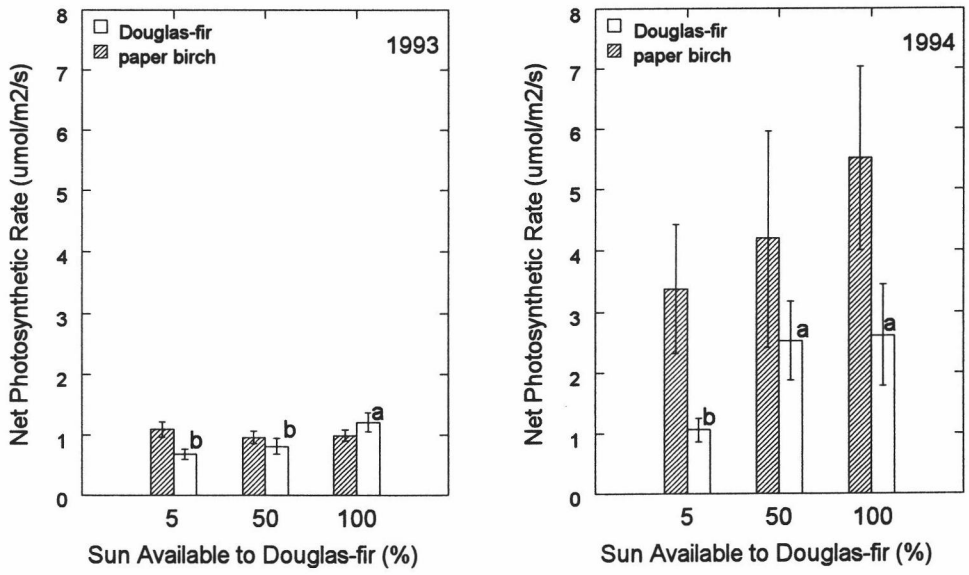

Figure 17. Leaf net photosynthetic rates of paper birch and Douglas-fir in 5%, 50% and 100% ambient sun in (a) 1993 and (b) 1994. Douglas-fir means denoted by the same letter do not differ significantly (p=0.05).

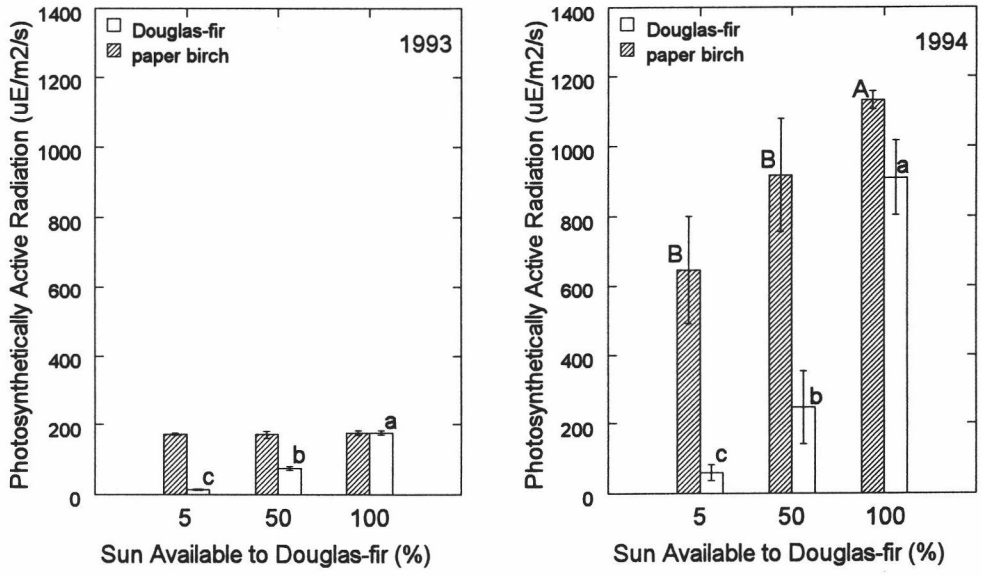

Figure 18. PAR on birch and fir in 5%, 50%, and 100% ambient sun in (a) 1993 and (b) 1994. Upper and lower case letters refer to birch and fir, respectively. Means denoted by the same letter do not differ significantly (p=0.05).

Table 11. Mean foliar nutrient concentrations of one-year-old paper birch and Douglas-fir grown in field for carbon transfer experiments. Values are means and standard errors (in parentheses). ANOVA detected differences between species for all nutrients.

Foliar nutrient	paper birch	Douglas-fir	Ratio PB/DF	p-value
Nitrogen (%)	2.312 (0.031)	1.376 (0.022)	1.68	0.0001
Phosphorus (%)	0.359 (0.008)	0.209 (0.004)	1.72	0.0001
Sulfur (%)	0.133 (0.002)	0.092 (0.002)	1.45	0.0001
K (%)	1.070 (0.014)	0.881 (0.017)	1.21	0.0001
Mg (%)	0.237 (0.004)	0.095 (0.002)	2.49	0.0001
Ca (%)	0.785 (0.015)	0.266 (0.007)	2.95	0.0001
Fe (ppm)	147.25 (9.98)	57.81 (1.78)	2.55	0.0001
Mn (ppm)	696.20 (60.55)	215.69 (19.39)	3.23	0.0001
B (ppm)	35.50 (0.93)	21.35 (0.72)	1.66	0.0001
Cu (ppm)	13.04 (1.07)	5.95 (0.14)	2.19	0.0001
Zn (ppm)	197.66 (7.93)	29.68 (1.03)	6.66	0.0001

Table 12. Percent root length of Douglas-fir, paper birch and western redcedar seedlings with ectomycorrhizal or arbuscular mycorrhizal colonization.

Species	Non-mycorrhizal	Ectomycorrhizal	Arbuscular mycorrhizal with hyphae	Arbuscular mycorrhizal with vesicles
Douglas-fir	57±11	43±11	0	0
Paper birch	61±7	34±8	0	0
redcedar	54±8	0	41±6	5±1

arbuscules nor hyphae of arbuscular mycorrhizal fungi occurred in either paper birch or Douglas-fir. Western redcedar roots were 54±8% nonmycorrhizal and 41±6% arbuscular mycorrhizal, of which 5±1% had vesicles visible. Ectomycorrhizae were absent from roots of western redcedar.

Seedling isotopic composition

Donors

In 1993, pulse-labeling efficiency of ^{13}C and ^{14}C averaged 15.1% and 8.7% respectively for labeled paper birch, and 14.3% and 7.4% respectively for labeled Douglas-fir. Paper birch was slightly more efficient at assimilating either carbon isotope than Douglas-fir, and both species were almost twice as efficient at assimilating ^{13}C than ^{14}C. In 1994, pulse-labeling efficiency increased to 20.5% for ^{13}C-labeled Douglas-fir, and 17.6% for ^{14}C-labeled paper birch.

In 1993, pulse-labeling resulted in average whole seedling ^{13}C and ^{14}C contents of 17.23 mg and 4.25 µg, respectively (Table 13, Figure 19), with no differences between species ($p>0.10$). Total isotope fixed by donor Douglas-fir was greater in the shade treatments than in full sun, and total isotope fixed by donor paper birch was greater when its Douglas-fir neighbor was in partial and full sun than when the neighbor was in full shade ($p<0.10$, Table 14). In 1994, whole seedling isotope contents were generally higher, with ^{13}C content of pulse-labeled Douglas-fir averaging 20.49 mg, and ^{14}C content of pulse-labeled paper birch averaging 9.10 µg. Total ^{14}C fixed by paper birch did not vary among shading treatments ($p>0.10$), but more ^{13}C was fixed by Douglas-fir in full shade than full sun ($p=0.09$)

Table 13. Total isotope contents of paper birch, Douglas-fir and western redcedar seedlings. Values are means and standard errors (in parentheses).

Whole seedling isotope content	paper birch	Douglas-fir	cedar
1993			
^{13}C (mg) pulse-labeled	17.69 (2.07)	16.76 (2.11)	-
^{14}C (µg) pulse-labeled	4.60 (0.73)	3.90 (0.58)	-
^{13}C (mg) received	0.573 (0.122)	0.798 (0.324)	0.00000
^{14}C (µg) received	0.007 (0.003)	0.013 (0.005)	0.00014 (0.00004)
1994			
^{13}C (mg) pulse-labeled	-	20.49 (2.52)	-
^{14}C (µg) pulse-labeled	9.10 (1.04)	-	-
^{13}C (mg) received	1.45 (0.21)	-	0.26 (0.15)
^{14}C (µg) received	-	0.14 (0.05)	0.09 (0.01)

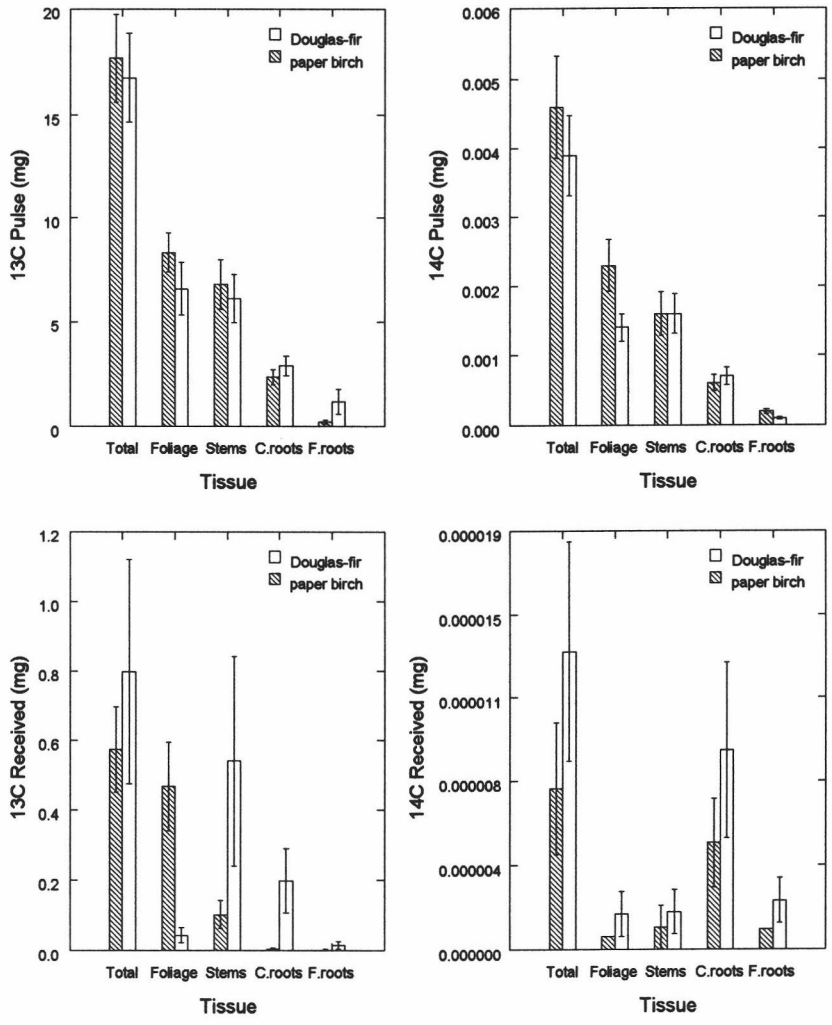

Figure 19. Tissue isotope contents of **DONORS** labeled with **(a)** ^{13}C and **(b)** ^{14}C, and **RECEIVERS** labeled with **(d)** ^{13}C and **(e)** ^{14}C in 1993 (year 1).

Table 14. Gross and net transfer among Douglas-fir shading treatments in 1993 and 1994. Values are means and standard errors (in parentheses). Values are based on 1993 correction factors unless otherwise indicated. Units are 'mg ^{13}C equivalents' unless otherwise indicated.

Transfer	5% Ambient Sun	50% Ambient Sun	100% Ambient Sun	p
1993 Experiment				
System isotope $_{content\ CF}$	31.84 (4.51)	43.89 (2.85)	31.75 (4.43)	0.11
Isotope fixed by DF	19.40 (3.36)b	21.74 (1.91)b	10.86 (1.42)a	0.01*
Isotope fixed by PB	12.50 (2.19)a	22.21 (2.93)b	20.93 (3.34)b	0.05*
Gross Transfer (DF+PB)	0.97 (0.29)	1.19 (0.20)	2.13 (0.59)	0.35
GT/System Isotope (%)	2.7%	2.6%	6.9%	0.23
Transfer to PB/ isotope fixed by DF (%)	1.9%b	2.9%b	6.6%a	0.07*
Transfer to DF/ isotope fixed by PB (%)	6.5%	2.4%	8.5%	0.51
Net Transfer (DF-PB)	0.04 (0.19)	-0.13 (0.20)	0.76 (0.44)	0.10*
NT/System Isotope (%)	0.1%	0.3%	2.4%	0.11
NT/DF Isotope (%)	0.0%b	0.7%b	6.6%a	0.07*
NT/PB Isotope (%)	0.2%	0.4%	3.9%	0.32
1994 Experiment				
^{14}C values NOT corrected				
System isotope content (mg)	29.82 (4.45)	21.92 (3.09)	14.11 (5.67)	0.09*
^{13}C fixed by DF (mg)	29.81 (4.45)a	21.91 (3.09)ab	14.10 (5.67)b	0.09*
^{14}C fixed by PB (µg)	11.21 (1.51)	8.97 (2.11)	7.60 (1.75)	0.39
Gross Transfer (DF+PB, mg)	1.80 (0.36)	1.33 (0.29)	1.24 (0.44)	0.53
^{14}C received by DF (µg)	0.23 (0.05)a	0.12 (0.04)b	0.07(0.03)b	0.00**
^{13}C received by PB (mg)	1.80 (0.36)	1.33 (0.29)	1.24 (0.44)	0.53
GT/System Isotope (%)	6.0%	6.1%	8.8%	0.17
^{14}C corrected with 1993 CF				
Gross Transfer (DF+PB)	32.19 (4.20)a	15.68 (2.94)b	11.30 (2.34)b	0.01**
Net Transfer (DF-PB)	28.60 (4.67)a	13.21 (3.32)b	8.64 (1.99)b	0.00**
^{14}C corrected with 1993 CF adjusted for change in labeling efficiency				
Gross Transfer (DF+PB)	22.06 (2.88)a	10.87 (2.04)b	7.98 (1.65)b	0.01**
Net Transfer (DF-PB)	18.47 (3.00)a	8.40 (2.14)b	5.32 (1.28)b	0.01**

Distribution of ^{13}C among seedling tissues was similar between species and years, although Douglas-fir translocated less to roots in 1994 than 1993 (Figures 19a and 20*a*). Paper birch in 1993 had at the end of the chase period translocated on average 15% of pulse-labeled ^{13}C to roots, 9% of which occurred in fine roots. Douglas-fir in 1993 and 1994 had translocated on average 24% and 9% of pulse-labeled ^{13}C to roots, 29% and 39% of which occurred in fine roots, respectively. Both species retained 39-57% of ^{13}C isotope in foliage. Distribution of ^{14}C among seedling tissues was similar between species in 1993, but paper birch translocated relatively less to roots in 1994 (Figures 19*b* and 20*a*). Douglas-fir in 1993 had at the end of the chase periods translocated on average 21% of pulse-labeled ^{14}C to roots, 23% of which occurred in fine roots. Similarly, paper birch in 1993 and 1994 had translocated on average 17% and 10% of pulse-labeled ^{14}C to roots, of which 25% and 23% occurred in fine roots, respectively. Paper birch retained more ^{14}C in foliage than Douglas-fir (36%) in both 1993 (50%) and particularly 1994 (65%).

Receivers

Douglas-fir received on average 39% more ^{13}C and 86% more ^{14}C from neighboring paper birch than paper birch received from neighboring Douglas-fir in 1993, however replicates varied widely and differences between receiving species were not significant ($p=0.52,0.26$, Table 13, Figure 19*c* and 19*d*). Distribution of ^{14}C among tissues of receiving seedlings was similar between species; paper birch and Douglas-fir retained 78-81% of the isotope in roots and translocated only 9-12% to foliage. In contrast, ^{13}C was translocated more readily out of roots and into stems and foliage. Paper birch retained <1% in roots and translocated 82% to foliage, while Douglas-fir retained 27% in roots, and translocated 68% to stems. The same trend occurred in 1994; Douglas-fir retained 58% of received ^{14}C in its roots and translocated 20% to foliage, whereas paper birch retained only 12% of ^{13}C in its roots and translocated 82% to foliage. Results from both 1993 and 1994 indicate that ^{13}C is translocated more readily than ^{14}C among tissues of receiver seedlings.

Western redcedar control seedlings received isotope from neighboring pulsed paper birch and Douglas-fir in both 1993 and 1994, indicating some isotope was transferred between plants indirectly via the soil pool (Table 13). Gross transfer to western redcedar represented <1-18% of gross bi-directional transfer between paper birch and Douglas-fir; the amount varied between isotopes and between years, but not among shading treatments. The average ^{14}C content of western redcedar in 1993 was 0.14± 0.04 ηg, which represented on average 1.3±0.4% of total ^{14}C transferred by either Douglas-fir or paper birch. There was no difference in amount of ^{14}C cedar received from either paper birch or Douglas-fir ($p>0.10$). In contrast, ^{13}C content of cedar in 1993 on average did not exceed natural abundance levels. In 1994, ^{14}C content of western redcedar was 0.09± 0.01 μg, which represented on average 64±20% of ^{14}C transferred by paper birch, and

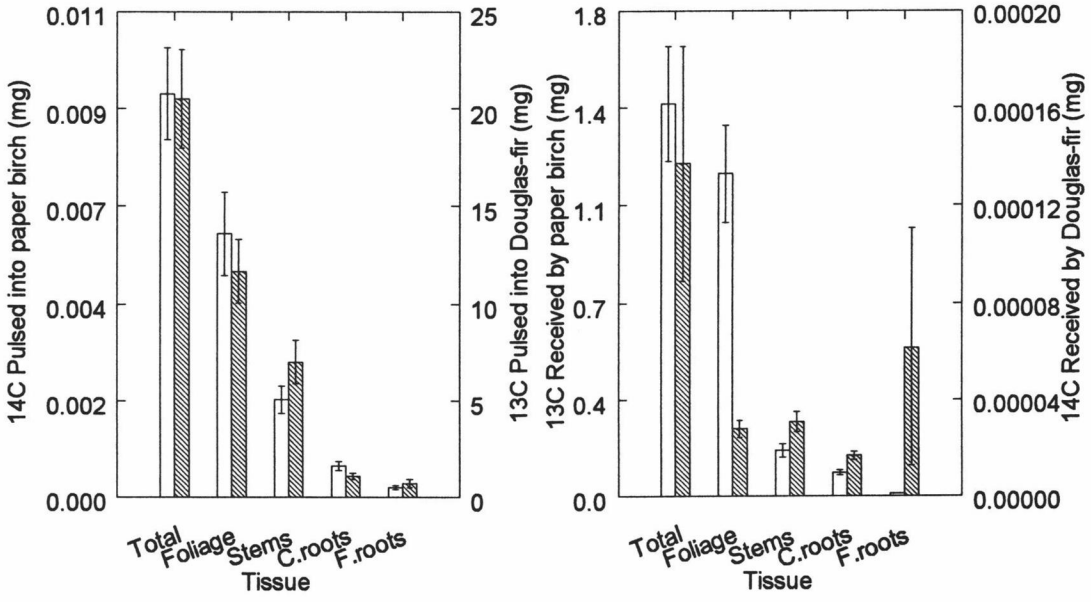

Figure 20. Mean ^{13}C and ^{14}C content of foliage, stems, coarse roots and fine roots of paper birch and Douglas-fir (a) donor and (b) receiver seedlings in 1994 (year 2). Open and cross-hatched bars represent ^{13}C and ^{14}C, respectively.

^{13}C content was 0.26±0.15 mg, which represented only 15 ±10% of ^{13}C transferred by Douglas-fir. Western redcedar received significantly less ^{13}C from Douglas-fir than did paper birch, but received similar amounts of ^{14}C from paper birch as did Douglas-fir in 1994. The lack of consistency in amount of isotope received by western redcedar between years and isotopes suggests a complicated pattern of indirect carbon transfer through the soil pool.

Effect of shading on gross isotope transfer

Gross isotope transfer ($^{13}C+^{14}C_{CF}$, where ^{14}C converted to ^{13}C-equivalent value using correction factor derived from 1993 experiment) between paper birch and Douglas-fir did not vary among Douglas-fir shading treatments in 1993 (p=0.3480), but was approximately two times greater under full shade (5SUN) than partial (50SUN) or full sun (100SUN) in 1994 (p=0.0016, Figure 21, Table 14).

In 1993, mean gross transfer between paper birch and Douglas-fir was 1.43±0.29 mg C isotope, which represented 4.05±0.01% of the total isotope pulsed into the system (paper birch plus Douglas-fir). Gross transfer to Douglas-fir alone represented 5.8±2.5% of total isotope pulsed into paper birch, and was not affected by the shading treatment (p>0.10). Gross transfer to paper birch alone represented 3.8±0.8% of total isotope pulsed into Douglas-fir, and was greater where Douglas-fir was in full light (6.6%) than in full shade (1.9%, p=0.0690). This apparent shading effect on percentage transferred can be explained by lower isotope content in pulse-labeled Douglas-fir in full light than partial or full shade (p=0.0700).

In 1994, gross isotope transfer to Douglas-fir alone (^{14}C) was over two times greater in full shade than partial or full ambient sun (p=0.0026). In contrast, gross isotope transfer to paper birch alone (^{13}C) did not vary among shading treatments (p>0.10). This indicates that shading of Douglas-fir affected transfer from paper birch to Douglas-fir, but it did not affect transfer in the reverse direction from Douglas-fir to paper birch. Mean gross transfer (not corrected with 1993 CF) was 1.45±0.21 mg C isotope, which represented on average 6.97±0.91% of the total isotope pulsed into the system (paper birch plus Douglas-fir).

Effect of shading on net isotope transfer

Net transfer (^{14}C converted to ^{13}C-equivalent value using 1993 CF) was affected by Douglas-fir shading treatments in both 1993 and 1994 (p=0.1000 and p=0.00, Figure 22, Table 14). In 1993, there was positive net transfer from paper birch to Douglas-fir where Douglas-fir was in full ambient sun, but zero net transfer where Douglas-fir was in full or partial shade. In full sun, net transfer from paper birch to Douglas-fir was 0.76± mg C isotope, which represented 2.4% of the total isotope pulsed into the system. Net transfer from paper birch to Douglas-fir in full sun

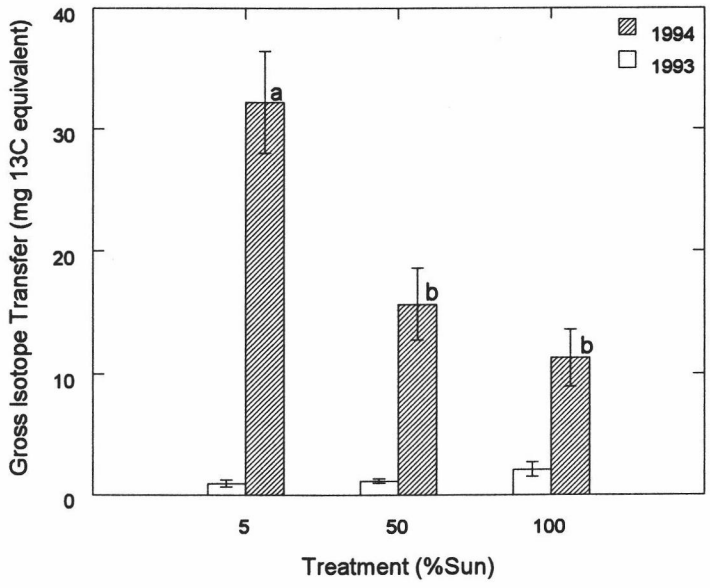

Figure 21. Gross isotope transfer between paper birch and Douglas-fir in 5%, 50% and 100% ambient sun in 1993 and 1994. Means denoted by the same letter do not differ significantly (p=0.01).

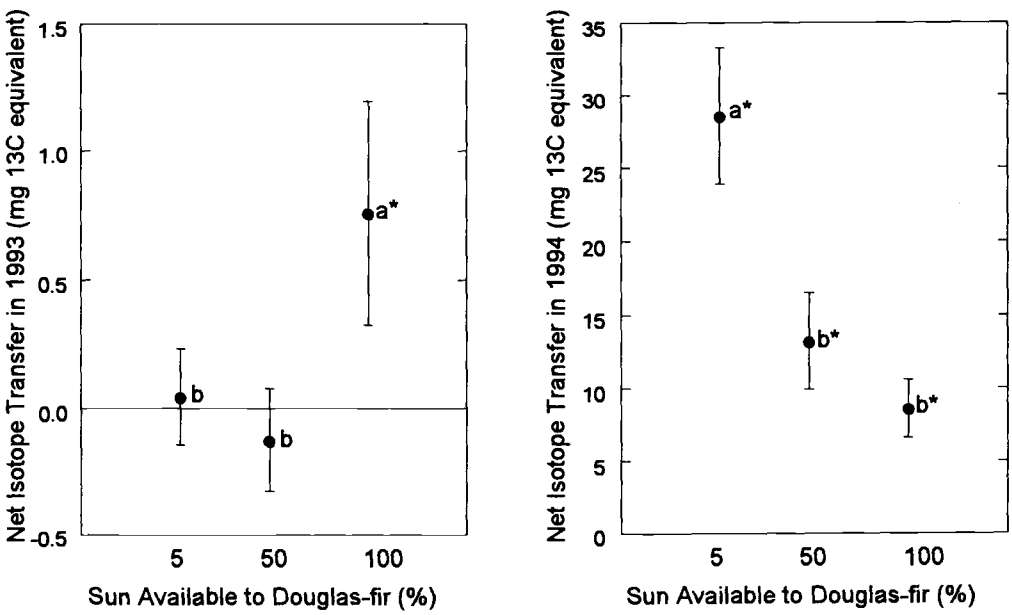

Figure 22. Gross isotope transfer between paper birch and Douglas-fir in 5%, 50% and 100% ambient sun treatments in 1993 and 1994. Means denoted by the same letter do not differ significantly (p=0.01).

represented 3.9% of total isotope pulsed into paper birch and 6.6% of the amount Douglas-fir fixed itself by photosynthesis.

In 1994, net transfer was positive (from paper birch to Douglas-fir) in all shading treatments and represented on average 6% of total isotope fixed by seedling groups. Parallel to gross transfer estimates, net transfer was over two times greater in full shade than partial or full ambient sun ($p=0.0042$).

Relationship between ^{13}C and ^{14}C fractionation

Differences in amount of isotope pulsed and metabolic fractionation between ^{13}C and ^{14}C resulted in net transfer differences dependent on labeling scheme in 1993 ($p<0.01$, data not shown). Whole seedling contents of ^{14}C were generally three orders of magnitude lower than of ^{13}C, resulting in significant differences in net transfer (net transfer=isotope received by DF - isotope received by PB) between the labeling schemes, 14PB-13DF and 13PB-14DF. The correction factor, or $^{13}C/^{14}C$ ratio for a particular treatment x species x tissue, eliminated the "effect of labeling scheme" and allowed estimation of net transfer.

The relationship between receiver and donor tissue ^{13}C and ^{14}C contents for both paper birch and Douglas-fir is shown in Figure 23, where tissue types are identified by letters. The cluster of points near the origin represent tissues which received isotope as a result of interplant transfer, and the remainder represent tissues which were pulse-labeled. There is no clear relationship between ^{13}C and ^{14}C of receiver tissues alone ($p>0.10$), suggesting interplant isotope transfer does systematically discriminate between isotopes. The linear relationship between ^{13}C and ^{14}C of pulse-labeled tissues alone is significant ($p=0.0001$, $R^2=0.32$), but is improved by including both receiver and pulse-labeled tissues in a single relationship. The linear equation $y=0.78+2778x$, where y=excess mg^{13}C and x=excess mg^{14}C of either pulse-labeled or receiving tissues, explains 51% of the variation in y ($p=0.0001$, $R^2=0.51$). The slope represents the average tissue ratio of $^{13}C/^{14}C$. Because the ratio $^{13}C/^{14}C$ pulsed into the labeling chambers was 2032, the slope of 2778 indicates that additional discrimination against ^{14}C relative to ^{13}C occurred during fixation, translocation, metabolism and interplant transfer. Some of this differential discrimination occurred during photosynthesis, as indicated by the difference in pulse-labeling efficiency between ^{13}C and ^{14}C. Pulse-labeling was approximately twice as efficient for ^{13}C than ^{14}C in 1993 for each species.

The nonlinear equation $\ln(y)=10.40+1.76\ln(x)+(0.06\ln(x))^2$, where y=excess mg$^{13}C$ and x=excess mg^{14}C of either pulse-labeled or receiving tissues, explained more variation in y ($p=0.0001$, $R^2=0.70$) than the linear equation. This equation describes lower $^{13}C/^{14}C$ ratios were tissue isotope contents are high (in leaves and stems), and higher ratios where isotope contents are low (in coarse and fine roots). It indicates that greater isotopic discrimination against ^{14}C than ^{13}C

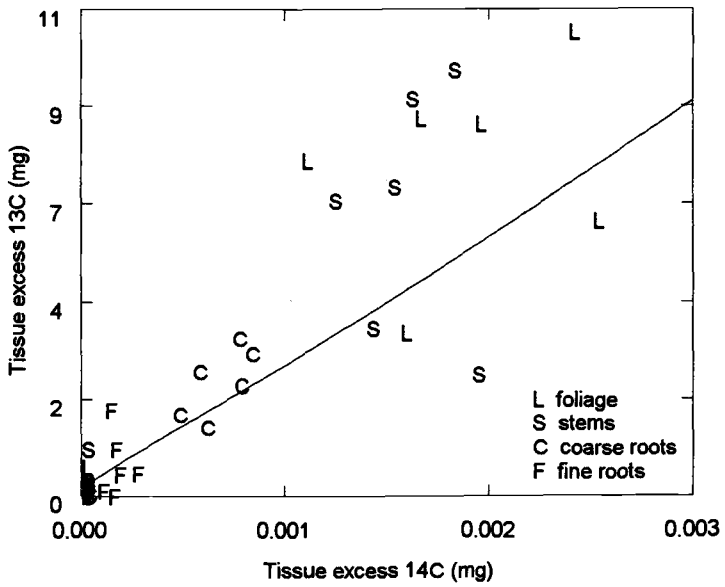

Figure 23. Relationship between tissue ^{13}C and ^{14}C contents for donor and receiver seedlings. Letters represent tissue type. Regression equation is $\ln(y)=10.40+1.76(x)+(0.06\ln(x^2))$.

occurs along the entire translocation pathway of donor and receiver seedlings, from foliage to stems to roots.

Transfer calculations in 1994 required correction of tissue ^{14}C contents with the CF ($^{13}C/^{14}C$) values derived from the 1993 experiment, since only one labeling scheme (14PB-13DF) was applied in 1994. The decrease in relative labeling efficiency of $^{13}C/^{14}C$ from 1.8 in 1993 to 1.2 in 1994, however, indicates that the 1993 CF values and regression equation would overestimate corrected ^{14}C values for 1994 (Table 14). As a result, the correction factors were used only to rank gross and net transfer among shading treatments in 1994, not for quantifying absolute amount transferred.

Discussion

Gross carbon transfer

Carbon transfer between paper birch and Douglas-fir was bi-directional, supporting Newman's (1988) assertion that results of one-way labeling studies do not necessarily prove net movement to the receiver plant. Mean gross transfer represented 4% of the total isotope pulsed into the system (paper birch and Douglas-fir) in 1993, and increased to 7% in 1994. The increase in proportion transferred from 1993 to 1994 coincided with *(i)* higher potential both for root overlap and hyphal linkages between seedlings, which was due to increased root biomass of both paper birch and Douglas-fir, as well as *(ii)* higher net photosynthetic rates of both species and more favorable environmental conditions for evapotranspiration. Gross transfer between paper birch and Douglas-fir was considerably less in the field than the 29% measured in rootboxes in the laboratory (Chapter 4). Possible reasons for greater gross transfer in rootboxes than the field include closer proximity and entrainment of root systems, as well as containment of all isotope in the narrow confines of the rootbox. As with the rootbox study, however, gross carbon transferred in the field in both years exceeded one-way mycorrhizal-mediated ^{14}C transfer measured in previous studies (Hirrel and Gerdemann 1979, Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986), where ^{14}C found in receiver plants usually represented <1% of that found in interconnected donor plants. Gross transfer in this study was more similar to the 10% ^{14}C transferred from clonal *Eicchornia crassipes* parents to offspring ramets via connecting stolons (Alpert *et al.* 1991), and the 5-15% fixed ^{15}N transferred from *Alnus gluntinosa* to associated *Pinus contorta* via ectomycorrhizal hyphal linkages (Arnebrant *et al.* 1993).

Shading of Douglas-fir had no effect on bi-directional gross transfer in 1993, but was approximately two times greater in full shade than in partial or full sun in 1994. The two-fold increase in carbon transferred in shade versus sun in 1994 was of similar magnitude to the six-fold increase in ^{14}C transferred via arbuscular mycorrhizae from *Plantago* to *Festuca* seedlings (Francis

and Read 1984), and the two- to four-fold increase in ^{14}C transferred via ectomycorrhizae between 6-month-old *Pinus* seedlings (Finlay and Read 1986) in full darkness versus full light. In our study, full shade (mean PAR=60 μE m^{-2} s^{-1}) of Douglas-fir was necessary to affect the magnitude of transfer; partial shade (mean PAR=250 μE m^{-2} s^{-1}) affected neither transfer nor mean net photosynthetic rate of Douglas-fir.

One-way gross transfer from Douglas-fir to paper birch in 1993 represented on average 4% of isotope pulsed into donor Douglas-fir, and was greater in full ambient sun (7%) than either shade treatment (2%, p=0.07). This may have resulted from the lower total isotope content of donor Douglas-fir in full sun than either shade treatment (p=0.01), rather than from a true shading effect. One-way gross transfer in the opposite direction, from paper birch to Douglas-fir, represented on average 6% of isotope assimilated by paper birch, and was not affected by shading treatment. This was similar to results of the pilot rootbox study (Chapter 3), where one-way ^{13}C transfer from paper birch to Douglas-fir represented almost 5% of that assimilated by paper birch. It was considerably lower than in the other rootbox study (Chapter 4), however, where one-way transfer to Douglas-fir represented 25% and one-way transfer to paper birch represented 15% of the total isotope assimilated by the rootbox system.

Full shading of Douglas-fir in 1994 resulted in increased one-way gross transfer from paper birch to Douglas-fir, but did not affect gross transfer in the opposite direction, from Douglas-fir to paper birch. These results are consistent with other studies showing that magnitude of transfer is affected by changes in the source-sink relationships (e.g.,Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986) . Our only measure of change in Douglas-fir sink strength with full shading (reduced light intensity) was net photosynthetic rate, which on a whole seedling basis was reduced to approximately ⅛ the rate in full or partial ambient sun. Others have also influenced extent of carbon or nutrient movement between plants by altering existing source-sink relationships through relative changes in light intensity (Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986), nitrogen status (e.g., Frey and Schuepp 1992) or phosphorus status (Ritz and Newman 1986, Eissenstat 1990) of source and sink plants.

Net carbon transfer

Net transfer of carbon isotope from paper birch to Douglas-fir occurred in both 1993 and 1994, representing the first reported measurements of net transfer between plants in the field. The extent of net transfer varied among shading treatments and between years. In 1993, positive net transfer to Douglas-fir (*i.e.*, where carbon gain by Douglas-fir>carbon gain by paper birch) occurred when Douglas-fir was fully illuminated, but not when it was partially or fully shaded (carbon gain by Douglas-fir = carbon gain by paper birch). This contradicts results of Francis and Read (1984) and Read *et al.* (1985), who found greater ^{14}C transfer to receiver roots in shade than sun, apparently

due to the greater "demand" for carbon by shaded seedlings. One possible explanation is that paper birch and Douglas-fir root systems were only weakly intermingled in 1993, and that the pattern in net transfer was more related to extent of hyphal connections than relative sink strength for carbon of associated seedlings. In that case, seedling groups in full sun may have developed more extensive roots and hyphae than in shade. Miller and Allen (1992) suggest that net transfer is influenced not only by relative sink strengths of associated plants, but also by the total number of hyphal connections, physiology of connecting fungi, and nutrient gradients in the soil. A second possible explanation is that sink strength is a function of both transpiration and carbon or nutrient demand, and that only Douglas-fir in full sun were physiologically active enough to draw a net carbon gain from paper birch through the seedling-hyphae/soil-seedling pathway. This effect may have been compounded by weak or infrequent hyphal connections between seedlings in 1993. Douglas-fir net photosynthetic rate was significantly greater in full sun than either shade treatment in 1993, and net photosynthesis has been shown as well-correlated with transpiration rates in paper birch (Wang *et al.* 1995) and Douglas-fir (Lopushinsky 1990). Greater foliar and root biomass, higher whole seedling net photosynthetic rates, and greater soil water availability in 1993 than 1994 may have enabled Douglas-fir to exceed a critical transpiration threshold in 1994 and draw net carbon from paper birch regardless of shading treatment. A third possible explanation is that Douglas-fir exported less carbon isotope to connected paper birch in the full sun treatment because it fixed on average less carbon isotope than in the two shade treatments. The lower carbon isotope fixation contradicts higher net photosynthetic rates measured in full sun than shade, for reasons which are unclear. However, carbon isotope transferred from paper birch to Douglas-fir was not balanced by transfer back from Douglas-fir possibly because less was available for export. This is unlikely given the lack of significant relationship between isotope pulsed into donor seedlings and that transferred to receiver seedlings (data not shown). In addition, differences in system (seedling group) isotope content ($p=0.11$) among shading treatments had no effect on bi-directional gross transfer ($p=0.35$) in 1993.

Net transfer from paper birch to Douglas-fir in full sun in 1993 represented approximately 2% of the total isotope fixed by paper birch and Douglas-fir in a seedling group. It represented approximately 4% of the isotope assimilated by paper birch and almost 7% of that assimilated by Douglas-fir. In 1994, we estimate that net transfer was 6% of total isotope fixed averaged over all three shading treatments. This represents a substantial carbon gain by Douglas-fir, and is similar to amounts which have been suggested to improve survival and growth of connected ramets among clonal plants (e.g., Alpert *et al.* 1991, Hutchings and Bradbury 1986). Estimates of net transfer in the field were similar to that measured in the laboratory rootbox study (Chapter 4), where net transfer to Douglas-fir represented 4% of total isotope assimilated in the rootbox system, 7% of that assimilated by paper birch and 10% of that assimilated by Douglas-fir.

In 1994, net transfer was consistently positive (*i.e.*, net carbon gain by Douglas-fir) in all three shading treatments, and was approximately two times greater in full shade than partial or full sun. This shading pattern is opposite to that observed in 1993, indicating considerable variation in net transfer with environmental conditions and seedling age. The pattern of net and gross transfer among shading treatments in 1994 corroborates results of shading experiments by Francis and Read (1984), Read *et al.* (1985), and Finlay and Read (1986). A caveat must be attached to our results, however. Relative labeling efficiency of ^{13}C versus ^{14}C decreased from approximately 1.8 in 1993 to 1.2 in 1994, indicating that the 1993 CF values and regression equation would overestimate corrected ^{14}C values for 1994. After adjusting 1993 CF values for the change in labeling efficiency between years, however, estimates of net transfer to Douglas-fir remained positive, and net and gross transfer remained in the same order of magnitude and resulted in the same treatment ranking as estimated with unadjusted 1993 CF values (Table 14).

The change in shading effect on gross and net transfer, from greatest transfer in full sun in 1993 to greatest transfer in full shade in 1994, coincided with several changes in seedling and environmental factors between years. First, root biomass within a seedling group almost doubled from 1993 to 1994, increasing potential for root-root contact and hyphal interconnections. Mycorrhizal interactions may have been too weak or infrequent in 1993 for source-sink relationships to significantly affect extent of interspecific carbon transfer. Second, soil water availability, light intensity and soil temperature were more favorable for seedling metabolism during the 1994 than 1993 experiment, which may have affected transpiration rates and amount of carbon allocated to rhizosphere symbionts. Whereas only Douglas-fir in full sun in 1993 may have been physiologically active enough to draw a net carbon gain from neighboring paper birch, all Douglas-fir were a strong sink for carbon in 1994 regardless of shading treatment. Third, the 1993 experiment was conducted in mid-July, immediately following completion of Douglas-fir shoot growth, and the 1994 experiment conducted in early August, several weeks following termination of shoot growth. Changes in carbon allocation patterns through the growing season were not measured in this study, but there was a trend toward increased in root/shoot ratio between June and August among mixtures of one-year-old paper birch and Douglas-fir seedlings on the same study site in 1994 (Wang 1995). Greater carbon allocation to roots and mycorrhizae, greater potential for interspecific root contact and hyphal connections, and greater physiological activity of seedlings in 1994 than 1993, may in combination have permitted light intensity to significantly affect source-sink relationships between paper birch and Douglas-fir, and hence influence extent of gross and net transfer in 1994.

Carbon and nutrient concentration gradients

The direction and extent of inter-plant transfer is thought to be influenced by source/sink relationships between plants, such as those established by differences in net photosynthetic rate, nutrient status, or capability of fixing atmospheric N_2 (e.g., Read *et al.* 1985, Newman 1988, Bethlenfalvay *et al.* 1991, Alpert *et al.* 1991, Arnebrant *et al.* 1993). In the present study, whole seedling net photosynthetic rate of paper birch was estimated as 1.5 times that of neighboring Douglas-fir in full sun in both 1993 and 1994. Net photosynthesis values for paper birch fell within the range reported by Wang *et al.* (1995) and Ranney *et al.* (1991), and those for Douglas-fir were similar to rates reported by Dosskey *et al.* (1990). The gradient in whole seedling net photosynthetic rate between species increased with shading of Douglas-fir, so that paper birch rates in full sun were approximately three to four times that of Douglas-fir in full shade. The decrease in net photosynthetic rate of Douglas-fir with shade may have reduced assimilate supply to its roots and thus increased the assimilate concentration gradient between paper birch and Douglas-fir roots (Finlay and Read 1986).

Paper birch also had higher foliar concentrations than Douglas-fir of all macronutrients (N, P, S), cations (K, Mg, Ca) and micronutrients (B, Cu, Fe, Mn, Zn) measured, indicating a nutrient concentration gradient between species. Paper birch was not deficient in any nutrient, but had notably high concentrations of P, Fe, Mn, and Zn compared with values published in a review by Perry (1994). Douglas-fir foliage may have been slightly deficient in N, Mg (Ballard and Carter 1985) and S, but all other nutrients fell within the normal range for temperate conifers (Perry 1994). The ratios of N to K, P, Ca and Mg indicate that neither species was limited with respect to balance of these nutrients (Ingestad 1979). Since nutrients did not appear limiting to paper birch, and some may have been in the luxury range, then some transfer to neighboring plants should not adversely affect health of paper birch. In contrast, slight nutrient deficiencies in Douglas-fir may signal a potentially important benefit of transfer from paper birch, particularly during periods of nutrient stress. Based on the Mitscherlich curve of diminishing returns, transfer of nutrients via mycorrhizal fungi from nutrient-rich paper birch would result in greater gain by nutrient-poor Douglas-fir neighbors than the relative magnitude of paper birch's requisite loss (Perry *et al.* 1992).

Nutrient demands of paper birch and Douglas-fir may be estimated from their changes in leaf area or foliar biomass from 1993 to 1994, because foliar growth is positively correlated with nutrient uptake (Smith *et al.* 1981, Perry 1994). The change in mean whole seedling leaf area of Douglas-fir (343 cm^2) between 1993 and 1994 was almost double that of paper birch (159 cm^2). Moreover, the change in foliar biomass of Douglas-fir (8.59 g) was almost six times greater than that of paper birch (1.51 g). The simplest expression of nutrient demand between 1993 and 1994 is the product of foliar biomass increment and foliar nutrient concentration, assuming the latter is constant from year to year. Using that approach, we estimated that nitrogen demand, for example,

of Douglas-fir was 3.4 times that of paper birch. The lower concentration yet higher demand for all macronutrients in Douglas-fir than paper birch together may define a source (paper birch) -sink (Douglas-fir) nutrient gradient between the two species.

Photosynthetic rate and foliar nitrogen concentration are positively correlated (Brix 1981, Pearcy *et al.* 1987, Wang *et al.* 1995), and may together influence direction and extent of net transfer between paper birch and Douglas-fir. It is possible that carbon is combined with nitrogen (and other nutrients) and transferred between connected plants as amino acids (Smith and Smith 1990). Labeled amino acids have been shown to pass directly from mycorrhizal fungi into the xylem sap of host plants (Abuzinadah and Read 1989), whereas sugars have not (Smith and Smith 1990).

Hyphal connections

Paper birch and Douglas-fir were shown to share seven ectomorphotypes in common over 90% of their root tips in a bioassay of the same soil and plant material as the current study (Chapter 2). The shared types included *Thelephora terrestris, Laccaria laccata, Cenococcum geophilum, Tuber*, e-strain, and MRA. Unique to paper birch was also *Lactarius* type, *Hebeloma* type, and *Tuber* type, and unique to Douglas-fir was *Rhizopogon vinicolor*. This large overlap in shared types suggested potential for ectomycorrhizal hyphal connections between paper birch and Douglas-fir.

Whether carbon or nutrients transfer between plants directly through mycorrhizal hyphal connections versus indirectly through the soil pool has commonly been distinguished in laboratory studies by comparing transfer from mycorrhizal with that from non-mycorrhizal plants (e.g., Francis and Read 1984, Finlay and Read 1986, Arnebrant *et al.* 1993), or by using barriers permeable to fungi but not roots (e.g., Bethlenfalvay *et al.* 1991, Frey and Schuepp 1992). These techniques are more difficult to apply in the field, particularly due to unwanted infection of non-mycorrhizal controls, although Hamel and Smith (1991, 1992) did compare ^{15}N transfer between arbuscular mycorrhizal versus non-mycorrhizal soybean and corn in field intercrops. Other field labeling studies have inferred transfer between ectomycorrhizal plants through hyphal connections based on absence of radioactivity in roots of neighboring arbuscular mycorrhizal plants (e.g., Read *et al.* 1985). In the current study, we used arbuscular mycorrhizal western redcedar seedlings to distinguish between amount of isotope transferred via the soil pool with that transferred between paper birch and Douglas-fir through ectomycorrhizal fungi. There are several potential criticisms associated with this approach. First, Douglas-fir and paper birch may form arbuscular mycorrhizae in addition to ectomycorrhizae, providing direct pathways of carbon transfer to western redcedar. Arbuscular mycorrhizae (Cazares and Smith 1992, 1995) have recently been observed in *Pseudotsuga menziesii* grown in the field and greenhouse soil bioassays. Ectendomycorrhizal and arbuscular mycorrhizal associations have been recorded for some species of *Betula* (Harley and

Harley 1987, Wilcox and Wang 1987, Molina *et al.* 1992), and ectomycorrhizae have been reported in some genera of the Cupressaceae (family to which *Thuja plicata* belongs) (Harley and Harley 1987, Molina *et al.* 1992). In the current study, however, arbuscular mycorrhizae were not observed in paper birch or Douglas-fir roots, nor were ectomycorrhizae observed in western redcedar harvested from the experimental area. Based on these observations, we assume that isotope transferred to western redcedar occurred indirectly through the soil pool. A second criticism is that isotope transferred through the soil pool to western redcedar does not necessarily reflect that transferred through the soil pool between paper birch and Douglas-fir, because the three species differ in root architecture and physiology. Root biomass of western redcedar was similar to the other species, however, particularly to paper birch in 1993 and Douglas-fir in 1994. A third criticism is that ectomycorrhizal connection between paper birch and Douglas-fir were never directly identified in the field. As a result, we cannot unequivocally determine whether labeled carbon was transferred between paper birch and Douglas-fir directly via interconnecting hyphae, or whether it leaked into the soil and then was picked up by the neighboring seedling (Newman 1988). A more conclusive method is to measure the rate of nutrient movement in the soil versus the hyphal network, which would involve enumeration of hyphal connections (Miller and Allen 1992).

The proportion of total carbon isotope ($^{13}C+^{14}C$) transferred from paper birch and Douglas-fir to western redcedar (*i.e.*, indirectly through the soil pool) relative to gross ($^{13}C+^{14}C$) transfer between paper birch and Douglas-fir (*i.e.*, directly through ectomycorrhizal connections) ranged between <1% (1993) and 18% (1994). Our estimates fall within the range of other published values, where transfer through the soil pool has been estimated to range between <1% and 20% of hyphal transfer (Hirrel and Gerdemann 1979, Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986). The amount transferred to western redcedar varied depending on the isotope (^{13}C versus ^{14}C) and year, but not on shading treatment. In 1993, western redcedar received 1.3% of gross ^{14}C and none of gross ^{13}C transferred between paper birch and Douglas-fir. In 1994, this increased to 64% of ^{14}C transferred from paper birch and 15% of ^{13}C transferred from Douglas-fir, suggesting a complicated pattern of indirect movement among species. Reasons for differences between isotopes are unclear, however differences between years may be due to greater root biomass (and overlap) among species as well as improved environmental conditions for plant metabolism in 1994 than 1993.

Possible sources of carbon which could be indirectly transferred through the soil pool include *(i)* root respired CO_2, *(ii)* carbon compounds leaked or exuded from roots, mycorrhizae and mycorrhizal hyphae, and *(iii)* dead and decomposing fungal and root cells. Our evaluation of these pathways is as follows. *(i)* Anapleurotic uptake of respired CO_2 by paper birch and Douglas-fir roots was not detectable in a previous rootbox experiment (Chapter 3), even though fungal and root respiration has previously been shown to represent up to 33% of photosynthate (Paul and Kucey 1984, Harris *et al.* 1985). Based on our rootbox study, we assume that anapleurotic uptake was

not a significant transfer pathway in this field study. *(ii)* Carbon leaked from interconnecting hyphae has been shown as negligible by Duddridge *et al.* (1980, 1988). However, photosynthate exuded into the rhizosphere has been estimated to range between <1% (Kucey and Paul 1982, Miller *et al.* 1989, Jakobson and Rosendahl 1990) and 40% of that fixed by plants (Whipps and Lynch 1986, Reid and Mexal 1977). We have no direct measurement of root and fungal exudate; however, exudate could account for the source of at least part of the isotope transferred to western redcedar. *(iii)* Little is know about rate of carbon input to the soil pool through death and decomposition of mycorrhizal hyphae (Finlay and Söderström 1992). Turnover time of mycorrhizal sheath and hyphal tissues was estimated as 1.64 and 1 year, respectively, in young Douglas-fir stands in western Oregon (Fogel and Hunt 1979). Although they may have underestimated hyphae turnover time, results from Fogel and Hunt (1979) suggest that the nine-day chase period in the present study may have been too short for significant hyphal turnover and consequent carbon isotope throughput to the soil pool. Decomposition of fine roots should be even slower than that of hyphae. Using fine root biomass and turnover rates from Vogt *et al.* (1986), fine roots in cold temperate broadleaf forests are estimated to turnover between approximately 1.4 and 2.9 years. Live fine root turnover times of mature Douglas-fir stands in western Oregon, however, have been estimated to range between 0.36 (dry site) and 0.59 (wet site) years (Santantonio and Hermann 1985). This information suggests that most carbon that transferred indirectly through the soil pool originated from root and fungal exudates, and little originated from respired CO_2 or decomposition of fine roots and hyphae.

Distribution of transferred carbon in receiver tissues

The distribution of transferred carbon in receiver seedling tissues varied greatly by isotope, but was comparatively similar between species and years. Root/shoot ratios for ^{14}C ranged between 1.2 and 3.6, indicating that most ^{14}C remained in the roots of receiver seedlings. Between 9 and 20% of received ^{14}C was translocated to foliage. In contrast, root/shoot ratios for received ^{13}C ranged between 0.01 and 0.37, indicating that most ^{13}C was translocated from roots to shoots of receiver seedlings. The amount of ^{13}C in foliage was highly variable (6-82%). Greater discrimination against ^{14}C than ^{13}C along the translocation pathway from root to shoots in receiver seedlings parallels that in donor seedlings, and may be explained by slower diffusion and reaction rates of the heavier isotope (Craig 1954, O'Leary 1981). In spite of these marked differences in isotope behavior, there was a trend in tissue isotope distribution between species. On average, root/shoot ratio of carbon isotope was 0.43 for paper birch and 1.15 for Douglas-fir, and foliage content was 45% in paper birch and only 13% in Douglas-fir. Greater translocation of received isotope to shoots, particularly to foliage, of paper birch than Douglas-fir may be explained by differences in net photosynthetic rates and, by correlation, transpiration rates (Lopushinsky 1990,

Wang *et al.* 1995). The average proportion of received carbon isotope translocated into shoots of paper birch (70%) and Douglas-fir (48%) exceeded that observed in previous carbon transfer studies (usually <10%, Newman 1988). This translocated carbon could either supplement carbon in photosynthate, or serve as the carbon skeleton for nitrogen in amino acids. Translocation of carbon from receiver roots to foliage may occur along a nitrogen concentration gradient, since fully developed leaves (as in this study) are usually strong sinks for nitrogen, and sources rather than sinks for carbon (Pearcy *et al.* 1987).

Isotope fractionation

Pulse-labeling efficiency of ^{13}C was double that of ^{14}C for both paper birch and Douglas-fir in 1993. This isotope effect corresponds with that measured during photosynthesis by Buchanan *et al.* (1953) and Craig (1954). In C_3 plants, the $^{14}C\backslash^{13}C$ isotope effect occurs during CO_2 diffusion and carboxylation by ribulose bisphosphate, and has been estimated to range between two (Buchanan *et al.* 1953, Craig 1954) and four (Van Norman and Brown 1952), resulting in photosynthetic discrimination of 4% against ^{13}C and 8-15% against ^{14}C relative to ^{12}C. Greater fractionation of $^{14}C/^{12}C$ than $^{13}C/^{12}C$ occurs during diffusion, chemical and biological processes (O'Leary 1981, Van Norman and Brown 1952) because the heavier isotope diffuses and reacts more slowly. Within the framework of thermodynamic theory, the relative $^{14}C/^{13}C$ kinetic isotope effect due to the atomic mass difference alone is approximately two (Stern and Vogel 1971).

Pulse-labeling efficiency in 1994 was two times greater for ^{14}C (paper birch) and 1.4 times greater for ^{13}C (Douglas-fir) than in 1993. Increased pulse-labeling efficiency in the second year coincided with higher foliar biomass and whole seedling net photosynthetic rate for both paper birch and Douglas-fir, and better environmental conditions (greater light, soil temperature and soil water availability) for plant metabolism. Reasons for the relative change in pulse-labeling efficiency of ^{13}C and ^{14}C are unclear.

Isotope root/shoot ratios averaged 0.24 (range 0.17-0.32) for donor seedlings pulse-labeled with ^{13}C, and 0.13 (range 0.11-0.15) for those pulse-labeled with ^{14}C, indicating greater fractionation against ^{14}C than ^{13}C through the translocation pathway from foliage and to fine roots. Isotope fractionation is compounded by the series of process stages (Craig 1954), and isotopic composition along the translocation pathway reflected fractionation in the formation and metabolism of intermediates (O'Leary 1981). The magnitude of this translocation isotope effect varied little between years or species of donor seedling.

The $^{14}C/^{13}C$ isotope effect increased an order of magnitude further en route from donor to receiver seedlings. In contrast with donor plants, where tissue ^{14}C and ^{13}C contents were positively correlated, receiver plants showed no systematic relationship between tissue isotope contents. The greater magnitude and variability in $^{14}C/^{13}C$ discrimination in receiver than donor seedlings may be

indicative of *(i)* greater fractionation during metabolic processes in the pathway between seedlings (i.e., through interconnecting hyphae or through the soil pool) than within seedlings, and *(ii)* greater variability in the amount of ^{14}C and ^{13}C transferred due to the tortuous pathway between seedlings. Our data showed a compounding effect of $^{14}C/^{13}C$ isotope fractionation along the entire translocatory pathway, from foliage in donor plants to foliage in receiver plants. Estimates of net transfer were made possible by eliminating this isotope effect using treatment-species-tissue-specific correction factors.

Summary

This study, to our knowledge, is the first report of net carbon transfer between seedlings in the field. A large proportion (4-7%) of assimilated isotope was exchanged among neighboring seedlings, with a net flux from paper birch to Douglas-fir. Isotope transferred to neighboring cedar was variable (<1-18% of gross transfer between paper birch and Douglas-fir), which suggests a complex pattern of indirect carbon transfer through the soil pool. The difference in gross transfer between EM and AM seedlings may equate to ectomycorrhizal-mediated inter-seedling transfer, but this remains equivocal since ectomycorrhizal hyphal linkages between paper birch and Douglas-fir were never directly identified in the field. Full shading doubled net transfer to Douglas-fir in 1994, which suggests that extent of movement is influenced by source-sink gradients that can be manipulated by factors such as light intensity. Differences in results between 1993 and 1994 emphasize that carbon transfer is a variable process, and that extent and direction of net transfer may be influenced by factors such as extent of root and mycorrhizal development, seedling phenology (month), and micro climatic conditions.

These results are significant both physiologically and ecologically. Douglas-fir may directly benefit from the association with paper birch through improved nutrition in nutrient-poor or patchy environments, or through supplemental carbon gain during conditions of low photosynthetic potential such as shade or drought. Conversely, paper birch may benefit from the association with Douglas-fir if the direction of transfer is reversed during early spring or fall when paper birch foliage is absent. If results of this study reflect the magnitude of carbon transfer in natural systems, then plant-plant interactions may be influenced not only by resource competition but also by mutualistic relationships among themselves and with their mycorrhizal fungi. Maintaining a component of paper birch in Douglas-fir plantations then requires consideration of interactions at the community rather than simply the individual tree level. Further research is required to evaluate the long-term ecological effects of carbon transfer under field conditions.

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Chapter 6

Effects of Soil Trenching on Occurrence of Ectomycorrhizae on *Pseudotsuga menziesii* Seedlings Grown in Mature Forests of *Betula papyrifera* and *Pseudotsuga menziesii*

Abstract

Seedlings of *Pseudotsuga menziesii* were grown for six to 16 months in Untrenched and Trenched treatments in three 90- to 120-year-old mixed forests dominated by *Betula papyrifera* and *Pseudotsuga menziesii* in the southern interior of British Columbia. Each forest was characterized by mesic conditions and low light intensity (PAR <200 $\mu E\ m^{-2}\ s^{-1}$) in the understory. The objective of the study was to evaluate the influence of overstory tree roots on *(i)* ectomycorrhizal fungal composition, richness and diversity, and *(ii)* photosynthesis and growth of understory *Pseudotsuga menziesii* seedlings. Seventeen ectomycorrhizal morphotypes were recognized on seedlings in the Untrenched treatment and nine in the Trenched treatment over the three sites. Six types occurred in both treatments, of which on average *Rhizopogon vinicolor* was 20 times more abundant and *Thelephora terrestris* six times less abundant in the Untrenched than Trenched treatment. Of types that formed strands or rhizomorphs, eight occurred in the Untrenched treatment where they occupied on average 23% of root tips, and only four occurred in the Trenched treatment over 4% of the root tips. Mean richness, diversity, and evenness of ectomycorrhizal associates per seedling were approximately twice as great in the Untrenched than Trenched treatment.

Net photosynthetic rate of *Pseudotsuga menziesii* seedlings was greater in the Untrenched than Trenched treatment in July and August, but not September 1994. Height, diameter and biomass of seedlings did not differ between treatments, but height:diameter ratio was greater in the Untrenched than Trenched treatment at time of harvest. The effect of trenching on seedling performance was attributed mainly to differences in ectomycorrhizal colonization patterns, because trenching had no effect on soil nutrient concentrations (total C, total N, NH_4-N, available N, exchangeable Ca, exchangeable Mg, exchangeable K), C:N ratio, soil pH or light availability. Nor was there any difference in soil water in August, when seedlings in untrenched plots had higher net photosynthetic rates than seedlings in trenched plots. Results suggest that influence of overstory trees and pattern of ectomycorrhiza formation are important to *Pseudotsuga menziesii* seedling performance in deeply shaded forest environments.

Introduction

The ability of mycorrhizae to contact, colonize and form hyphal connections between roots of different plants has previously been demonstrated among several tree species (reviews by Newman 1988, Miller and Allen 1992), including species in the Pinaceae and Betulaceae families (e.g., Read *et al.* 1985, Arnebrant *et al.* 1993). Field and laboratory soil bioassays have shown that Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) can share ectomycorrhizal fungi in common and potentially form hyphal linkages with several other plant species (e.g., Amaranthus and Perry 1987, Amaranthus and Perry 1989, Borchers and Perry 1990, Massicotte *et al.* 1994, Smith *et al.* 1995, Horton and Parker 1995). The low host specificity of AM and many EM fungi (Molina *et al.* 1992) has led to the hypothesis that such hyphal connection between plant species is common (Newman 1988). One advantage is that seedlings form mycorrhizae quickly, capture resources early, and are more likely to survive through the critical regeneration period in harsh environments, such as in deep shade (Miller and Allen 1992), drought (Borchers and Perry 1989), or at high elevation (Perry *et al.* 1987). Several studies show better performance, for example, among ectomycorrhizal than non-mycorrhizal planted Douglas-fir seedlings (e.g., Amaranthus and Perry 1987, Amaranthus *et al.* 1987, Villeneuve *et al.* 1991, Hunt 1992). In addition to rapid mycorrhizal infection, hyphal links to larger, established trees may benefit neighboring seedlings through direct transfer of organic nutrients (e.g., Francis and Read 1984, Finlay and Read 1986, Hamel and Smith 1992, Newman and Eason 1993, Arnebrant *et al.* 1993), increased access to inorganic nutrients or water in a greater volume of soil (Eissenstat and Newman 1990, Miller and Allen 1992), and alteration of the balance of plant-plant interactions (Grime *et al.* 1987, Newman 1988, Perry *et al.* 1989, Miller and Allen 1992, Perry *et al.* 1992).

Conifer seedlings planted near an established tree may also form a greater diversity and different ectomycorrhizal types than those planted in isolation. Borchers and Perry (1989), for example, found that Douglas-fir grown in soils collected from beneath hardwoods (tanoak, Pacific madrone or canyon live oak) formed ectomycorrhizae predominantly with two types (*Rhizopogon* sp. and *Cenococcum geophilum*), whereas seedlings grown in soils collected >4 m from hardwoods formed <½ the number of ectomycorrhizal tips with predominantly a single, unidentified brown type. Similarly, Deacon *et al.* (1983) showed that *Betula* sp. seedlings grown in soil collected 1 m from the base of a parent *Betula* sp. tree formed fewer numbers and different types of ectomycorrhizae than those grown in soil collected only 0.25 m away. Those formed in distal soils (1 m) were referred to as "early stage (ruderal)" mycorrhizae (e.g., *Laccaria* spp., *Hebeloma* spp., *Thelephora terrestris*), and were characterized by early, ready establishment from spores as well as low carbon demands, while those formed in proximal soils (0.25 m) were "late stage (*K*-selected)" mycorrhizae (e.g., *Lactarius* spp., *Russula* spp., *Amanita* spp., *Cortinarius* spp.),

characterized by later development on older trees and high carbon demands (Deacon and Fleming 1992). Access to a diversity of ectomycorrhizal inoculum is important to seedling fitness because the ectomycorrhizal fungi differ in physiology and allow seedlings to acclimate to changing environmental conditions over time (Perry *et al.* 1987, Molina and Amaranthus 1990, Molina *et al.* 1992, Allen *et al.* 1995). Different mycorrhizal fungal species have different capabilities, for example, for nutrient absorption (Dighton 1991, Molina *et al.* 1992), extracellular enzyme production (Caldwell *et al.* 1991, Dighton 1991), drought tolerance (Parke *et al.* 1983, Dosskey *et al.* 1990, Guehl *et al.* 1992), pathogen protection (Sylvia and Sinclair 1983, Chakravarty and Unestam 1986), and infection of different parts of the root system (Gibson and Deacon 1988) or different aged hosts (Bledsoe *et al.* 1982, Fleming *et al.* 1984).

The ability of residual paper birch (*Betula papyrifera* Marsh.) and Douglas-fir trees to function as reservoirs of ectomycorrhizal inoculum for neighboring Douglas-fir seedlings has not been evaluated. This study focuses on results of a field bioassay with Douglas-fir seedlings growing in untrenched and trenched plots in the understory of three 90- to 120-year-old mixed paper birch and Douglas-fir stands in the southern interior of British Columbia. Our objective was to evaluate the effects of Douglas-fir seedling root access to overstory tree roots (untrenched versus trenched) on seedling (a) ectomycorrhizal fungal composition, richness and diversity, and (b) photosynthesis and growth. The influence of trenching on patterns of colonization are discussed in relation to legacy effects, inter-plant hyphal connections, fungal succession, and plant community dynamics.

Methods

Site descriptions

Soil trenching was performed in three mature forests dominated by paper birch (*Betula papyrifera*) and Douglas-fir (*Pseudotsuga menziesii*) in the Kamloops Forest Region of the southern interior of British Columbia. Site 1 is located at the north end of Adams Lake in the Clearwater Forest District, Site 2 at Hidden Lake in the Vernon Forest District, and Site 3 at Malakwa in the Salmon Arm Forest District. The sites range in elevation from 650-750 m, and occur within the Moist Warm Interior Cedar Hemlock (ICHmw) biogeoclimatic subzone (Lloyd *et al.* 1991). The subzone is characterized by warm, moist summers and cold, snowy winters, with mean temperatures of 19°C in July and -6°C in January, and mean annual precipitation of 670 mm, of which 290 mm falls as rain during the growing season (Environment Canada 1980). Soil and vegetation characteristics of each site are given in Table 15.

Table 15. Physiographic, soil and vegetation characteristics of the three study sites.

Characteristic	Site 1	Site 2	Site 3
Location	Adams Lake	Hidden Lake	Malakwa
Biogeoclimatic variant	ICHmw3	ICHmw2	ICHmw3
Site series	04/01	01	05/01
Elevation	700 m	650 m	750 m
Slope/aspect	0-5%/east	0-10%/east	0%/flat
Soil classification	Humo-Ferric Podzol	Dystric Brunisol	Humo-Ferric Podzol
Soil texture (50 cm)	sandy loam	loam	sandy loam
Coarse fragment content (50 cm)	10%	25%	10-30%
Parent material	alluvial blanket	morainal blanket	alluvial blanket
Dominant tree species	*Pseudotsuga menziesii* *Betula papyrifera* *Tsuga heterophylla* *Thuja plicata* *Pinus monticola*	*Thuja plicata* *Tsuga heterophylla* *Betula papyrifera* *Pseudotsuga menziesii*	*Thuja plicata* *Tsuga heterophylla* *Betula papyrifera* *Pseudotsuga menziesii* *Pinus monticola*
Stand age (y)	120	100	90
Dominant understory species	*Rubus parviflorus* *Chimaphilla umbellata* *Paxistema myrisinites* *Vaccinium* spp.	*Paxistema myrisinites* *Vaccinium* spp. *Tiarella unifoliata* *Cornus canadensis*	*Streptopus amplex* *Tiarella unifoliata* *Gymnocarpium sp.* *Linnea borealis*

Study design

Two "trenching" treatments (Trenched, Untrenched) were replicated on three sites in a randomized block design, where blocks were represented by sites. The three sites (Adams Lake, Hidden Lake and Malakwa) were characterized by 90-120 year-old forests dominated by paper birch and Douglas-fir, with minor amounts of other conifer species The trenching treatments were imposed to evaluate the ability of overstory trees to serve as sources of ectomycorrhizal inoculum for Douglas-fir seedlings growing in the understory. In the Trenched treatment, a 2 m^2 (r=0.80 m) "cookie" of earth was isolated from surrounding vegetation by digging a trench to 0.75 m depth, surrounding the cookie with stainless steel sheet metal, and then back-filling the trench with original soil. The depth of the trench was below the observed major rooting zone, and the sheet metal

prevented root in- our out-growth. The Untrenched treatment consisted of an adjacent (within 3 m), untrenched cookie of approximately the same neighboring vegetation, soil texture, and light environment (±50 μE m^{-2} s^{-1}). All Trenched and Untrenched pairs were located within 3 m of mature paper birch and Douglas-fir trees. Random selection of species pairs of mature trees along a transect within each site helped ensure that Trenched and Untrenched cookies were representative of the sites. Five pairs of Trenched and Untrenched cookies were established on each site, for a total of 30 treatment units. Each Trenched and Untrenched cookie was planted with 4 Douglas-fir seedlings in a 0.5 m x 0.5 m square (::) configuration.

Seedling establishment

One-year-old non-mycorrhizal Douglas-fir seedlings were shovel-planted into Trenched and Untrenched cookies in June, 1993. The seedlings were of the same seedlot as those planted for the field carbon translocation experiments (Chapter 5). Each cookie was carefully hand-weeded at the time of planting and monthly thereafter through the 1993 and 1994 growing seasons to minimize interspecific competition with neighboring understory vegetation. Severe deer browsing at Hidden Lake and Malakwa in the fall of 1993 necessitated re-planting in April, 1994. As a result, seedlings had been out-planted for 16 months at Adams Lake and only 6 months at Hidden Lake and Malakwa at the time of harvest. Blocks in the randomized block design therefore accounted for error associated with both site characteristics and seedling age. All seedlings were harvested for size (height, diameter, biomass) and mycorrhiza assessments in October, 1994.

Net photosynthetic rate, light availability and soil water content

Net photosynthetic rate and incident photosynthetically active radiation (PAR) of Douglas-fir seedlings in Trenched and Untrenched treatments were measured three times during the 1994 growing season. All measurements were conducted under sunny skies between 1100 and 1400 hours over 3-day periods (one site per day) in July, August, and September, 1994. One attached lateral shoot was randomly sampled from the top whorl of two seedlings per cookie. Leaf net photosynthetic rate and PAR were measured using a portable open CO_2 gas analyzer (LCA-2, Analytical Development Corp., Hoddeson, England). The leaves were harvested, pressed and dried flat, and leaf area (one side) later measured using a leaf area meter (Li-Cor 3100, Lincoln, Nebraska). Percent soil water content (15 cm depth) was measured gravimetrically in Trenched and Untrenched cookies at the same time as net photosynthetic rate and PAR were measured in August and September, 1994.

Mycorrhiza assessments

In October, 1994, one seedling per cookie was randomly harvested for ectomycorrhiza assessments. The seedlings were stored in a cooler for one month prior to assessment, and then the root systems washed in tap water and examined over a 2-week period. Ectomycorrhizal types were examined according to guidelines of Agerer (1987) and Ingleby *et al.* 1990. Root squashes and hand sections were examined microscopically to characterize fine details and determine the presence of a Hartig net. Distinguishing features were photographed on Fugichrome Tungsten film using a Zeiss compound microscope and a Zeiss dissecting microscope.

Abundance of each EM type was determined by placing the entire root system over a clear plastic grid with numbered (2.5 cm^2) squares (Smith *et al.* 1995). Root tips were sampled in randomly selected squares up to approximately 100 tips per seedling. The proportion of root tips colonized by each of the various EM types was calculated for each root system. After their mycorrhiza were identified and enumerated, each seedling was oven dried at 80°C for 2 days and then weighed.

Soil nutrients

Soil analyses were conducted at the British Columbia Ministry of Forests Research Laboratory, Victoria, B.C., using procedures adapted from Carter (1993). Mineral soil samples were collected in September, 1994, immediately following the final photosynthesis measurements. Four samples were collected to 15 cm depth from each cookie, and composited to form a single sample per cookie (total of 30 subsamples). The samples were air dried and sieved to 2 mm fraction. Total N and C were determined simultaneously on milled samples using a Leco CHN-600 elemental analyzer. Mineralizable N was analyzed colorimetrically on a Technicon Auto-analyzer following anaerobic incubation for 2 weeks at 30°C and KCl extraction. Exchangeable K, Ca, and Mg were analyzed on a Technicon Auto-analyzer following extraction with neutral ammonium acetate (Morgan's method). Exchangeable P was determined colorimetrically by UV/visible spectrophotometer following extraction with Bray-P1 extractant. Total extractable S was determined on an ARL 3560 ICP spectrometer following extraction with ammonium chloride Soil pH was measured in a 1:2 mixture of soil and water.

Statistical analysis

The effect of trenching treatments on ectomycorrhizal composition, physiology and growth of Douglas-fir seedlings, as well as micro-environment and soil characteristics, was compared between Trenched and Untrenched treatments using analysis of variance (ANOVA) in a

randomized block design. Blocks represented error associated both with site characteristics and seedling age, which were unavoidably confounded. Trenching treatments were also compared within sites using paired *t*-tests. Diversity was quantified using the following indices: Shannon-Weaver (Shannon and Weaver 1963), Simpson (Simpson 1949), McIntosh (McIntosh 1967), richness, redundancy, and evenness (Peet 1974). All analyses were performed using SAS procedures (SAS Institute Inc.).

Results

Twenty ectomycorrhizal types were identified on Douglas-fir seedlings over the three sites: 13 types at Adams Lake, eight at Hidden Lake, and eight at Malakwa (Table 16). Approximately twice as many ectomycorrhizal types occurred in the Untrenched (17 types) than Trenched (nine types) treatment. Morphological characteristics allowed for identification to the genus level for most ectomycorrhizal types and the species level for some. There were five types previously undescribed. All types are described in the Appendix.

Comparison of ectomycorrhizal colonization between trenching treatments

The Untrenched and Trenched treatments shared six ectomycorrhizal morphotypes in common: MRA, E-strain, *Rhizopogon vinicolor*, *Cenococcum geophilum*, *Thelephora terrestris*, and *Hebeloma* types (Table 16). Of the shared types, *Rhizopogon vinicolor* was 34% more frequent and almost 20 times more abundant in the Untrenched than Trenched treatment (mean abundance 11% versus <1%, p=0.002). Conversely, *Thelephora terrestris* was 20% more frequent and almost six times more abundant in the Trenched than Untrenched treatment (mean abundance >2% versus <1%, p=0.074). E-strain was 33% more frequent but less abundant (9% versus 19%) in the Untrenched than Trenched treatment (p=0.052).

An additional 11 morphotypes were unique to the Untrenched treatment (*Lactarius deliciosus*, *Tuber*, *Amphinema*, Chubby Bubby, *Rhizopogon*-like, Brown Zelig, *Laccaria*, *Cenococcum*-like, *Laccaria*-like type and Yellow-tip Grizzly types) and an additional three unique to the Trenched treatment (*Humaria*, Craigallechie, and Last Spike types). The dominant morphotypes in the Untrenched treatment were MRA (mean abundance 63%), *Rhizopogon vinicolor* (11%), E-strain (9%), *Amphinema* (7%), Chubby Bubby (2%), *Rhizopogon*-like (2%), *Lactarius deliciosus* (1%) and *Tuber* (1%) types. The dominant morphotypes in the Trenched treatment were MRA (mean abundance 70%), E-strain (19%), Craigallechie (4%), *Thelephora*

Table 16. Frequency and mean abundance of ectomycorrhiza types on Douglas-fir seedlings growing in Untrenched and Trenched treatments at Adams Lake, Hidden Lake and Malakwa.

1Ectomycorrhizal types in descending order of abundance.
[2]Frequency of colonized seedlings in percent.
[3]Not applicable because type absent from all control host seedlings.
[4]Mean abundance is the average percent of root tips colonized per seedling.
[5]Numbers in parentheses are standard errors.
[6]Values followed by the same letter are not significantly different at the 5% level.

Table 16.

Ectomycorrhiza type[1]	Treatment	No. of seedlings colonized (1 seedling plot^{-1})	No. colonized seedlings with <5% of tips	Mean abundance (%) of type[4]	p-value
MRA type	Untrenched Trenched	15/15 (100%)[2] 14/15 (93%)	0/15 (0%) 1/14 (7%)	63.4 (7.9)[5]a[6] 70. 4 (10.1)a	0.358
E-strain type	Untrenched Trenched	11/15 (73%) 6/15 (40%)	2/11 (18%) 1/6 (17%)	8.9 (2.1)b 18.8 (8.1)a	0.052*
Rhizopogon vinicolor	Untrenched Trenched	7/15 (47%) 2/15 (13%)	2/7 (29%) ½ (50%)	10.9 (4.8)a 0.6 (0.5)b	0.002***
Cenococcum type	Untrenched Trenched	4/15 (27%) 2/15 (13%)	1/4 (25%) 2/2 (100%)	0.5 (0.3)a 0.7 (0.5)a	0.680
Hebeloma type	Untrenched Trenched	2/15 (13%) 3/15 (20%)	½ (50%) 2/3 (67%)	0.4 (0.3)a 0.8 (0.5)a	0.472
Thelephora terrestris	Untrenched Trenched	2/15 (13%) 5/15 (33%)	2/2 (100%) 2/5 (40%)	0.4 (0.3)b 2.3 (1.0)a	0.071*
Lactarius deliciosus type	Untrenched Trenched	3/15 (20%) 0/15 (0%)	½ (33%) 0/0 (na)[3]	1.3 (0.8)a 0.0 (0.0)b	0.074*
Tuber type	Untrenched Trenched	3/15 (20%) 0/15 (0%)	2/3 (67%) 0/0 (na)	0.7 (0.4)a 0.0 (0.0)b	0.075*
Amphinema type	Untrenched Trenched	4/15 (27%) 0/15 (0%)	2/4 (50%) 0/0 (na)	6.7 (4.4)a 0.0 (0.0)b	0.093*
Chubby Bubby type (H30)	Untrenched Trenched	2/15 (13%) 0/15 (0%)	0/2 (0%) 0/0 (na)	2.4 (2.1)a 0.0 (0.0)a	0.244
Rhizopogon-like type	Untrenched Trenched	3/15 (20%) 0/15 (0%)	2/3 (67%) 0/0 (na)	2.1 (1.7)a 0.0 (0.0)a	0.188
Brown Zelig type (A20)	Untrenched Trenched	3/15 (20%) 0/15 (0%)	2/3 (67%) 0/0 (na)	1.2 (1.1)a 0.0 (0.0)a	0.258
Laccaria type	Untrenched Trenched	2/15 (13%) 0/15 (0%)	½ (50%) 0/0 (na)	0.8 (0.7)a 0.0 (0.0)a	0.228
Cenococcum-like type (H28)	Untrenched Trenched	1/15 (7%) 0/15 (0%)	1/1 (100%) 0/0 (na)	0.1 (0.1)a 0.0 (0.0)a	0.327
Laccaria-like type (H29)	Untrenched Trenched	1/15 (7%) 0/15 (0%)	1/1 (100%) 0/0 (na)	0.3 (0.3)a 0.0 (0.0)a	0.327
Yellow-tip Grizzly type (A21)	Untrenched Trenched	1/15 (7%) 0/15 (0%)	1/1 (100%) 0/0 (na)	0.0 (0.0)a 0.0 (0.0)a	0.327
Lactarius pubescens type	Untrenched Trenched	1/15 (7%) 0/15 (0%)	1/1 (100%) 0/0 (na)	0.1 (0.1)a 0.0 (0.0)a	0.327
Craigallechie type (M26)	Untrenched Trenched	0/15 (0%) 3/15 (20%)	0/0 (na) 2/3 (67%)	0.0 (0.0)a 4.3 (3.4)a	0.189
Humaria type	Untrenched Trenched	0/15 (0%) 1/15 (7%)	0/0 (na) 0/1 (0%)	0.0 (0.0)a 2.1 (2.1)a	0.327
Last Spike type (M27)	Untrenched Trenched	0/15 (0%) 1/15 (7%)	0/0 (na) 1/1 (100%)	0.0 (0.0)a 0.1 (0.1)a	0.327

terrestris (2%) and *Humaria* (2%) types. There was a significant difference between the Untrenched and Trenched treatments in the proportion of strand forming mycorrhizae. In the untrenched treatment, eight types colonizing an average of 23% of root tips were strand-formers, while only four strand-forming types colonized 4% of tips in the trenched treatment.

Comparison of ectomycorrhizal colonization among sites

Thirteen ectomorphotypes were found at Adams Lake, and only eight each at Hidden Lake and Malakwa, perhaps reflecting differences in seedling age at the time of harvest. The three sites shared MRA and E-strain types in common (Table 17). Of those, MRA was more abundant at Hidden Lake and Malakwa (mean abundance 87%) than at Adams Lake (27%, p=0.000), and E-strain was more abundant at Adams Lake (31%) than Hidden Lake or Malakwa (5%, p=0.000).

At Adams Lake, 11 types occurred in the Untrenched and seven in the Trenched plots. Of the common types, *Rhizopogon vinicolor* was more abundant in the Untrenched (mean abundance 32%) than Trenched (2%) treatment (p=0.008), and E-strain more abundant in the Trenched (56%) than Untrenched (6%) treatment (p=0.006). *Cenococcum* (mean abundance 2%) and *Hebeloma* (2%) types were of similar abundance in the two trenching treatments (p>0.10). Unique to the Untrenched plots were *Lactarius deliciosus, Lactarius pubescens, Amphinema, Rhizopogon*-like, Brown Zelig, and Yellow-tip Grizzly types, and unique to the Trenched plots were *Thelephora terrestris* and *Humaria* types.

At Hidden Lake, all eight types occurred in the Untrenched plots and only two (MRA and *Thelephora terrestris*) in the Trenched plots (Table 17). Of the common types, MRA tended to be more abundant in the Trenched (mean abundance 97%) than Untrenched (79%) treatment (p=0.114), but *Thelephora terrestris* abundance (2%) was independent of treatment (p=0.428). Unique to the Untrenched plots were E-strain (mean abundance 11%), Chubby Bubby (7%), *Amphinema* (1%), *Laccaria*-like (1%), *Tuber* (<1%) and *Cenococcum*-like (<1%) types.

At Malakwa, five types occurred in each trenching treatment (Table 17). Of the common types, E-strain (p=0.060) was on average more abundant in the Untrenched than Trenched plots, but MRA abundance was unaffected by trenching treatment (p=0.920). Unique to the Untrenched plots were *Rhizopogon vinicolor* (mean abundance 1%), *Tuber* (2%), and *Laccaria* (2%) types, and unique to the Trenched plots were *Hebeloma* (<1%), Craigallechie (13%) and Last Spike (<1%) types.

Table 17. Frequency and abundance of ectomycorrhiza types on Douglas-fir seedlings in Untrenched and Trenched treatments at Adams Lake, Hidden Lake and Malakwa.

EM type[1]	Adams 16 month-old seedlings		Hidden 6 month-old seedlings		Malakwa 6-month-old seedlings		p-value[6]
Site	Untrench	Trenched	Untrench	Trenched	Untrench	Trenched	
MRA type	5 (100%)[2] 26.2[3] (2.5)[4]	4 (80%) 27.8 (16.1)	5 (100%) 78.8 (10.1)	5 (100%) 97.0 (2.0)	5 (100%) 85.2 (5.3)	5 (100%) 86.4 (10.2)	p=0.358
	p=0.924[5]		p=0.114		p=0.920		
E-strain type	3 (60%) 5.8 (4.0)	5 (100%) 56.2 (12.8)	5 (100%) 11.0 (3.0)	0 (0%) 0.0 (0.0)	3 (60%) 10.0 (4.5)	1 (20%) 0.2 (0.2)	p=0.052*
	p=0.006***		p=0.006***		p=0.060*		
Rhizopogon vinicolor	5 (100%) 32.0 (8.5)	2 (40%) 1.8 (1.6)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	2 (40%) 0.5 (0.4)	0 (0%) 0.0 (0.0)	p=0.002**
	p=0.0.008***		p=1.000		p=0.172		
Cenococcum type	4 (80%) 1.4 (0.7)	2 (40%) 2.0 (1.3)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.680
	p=0.687		p=1.000		p=1.000		
Hebeloma type	2 (40%) 1.2 (1.0)	2 (40%) 2.0 (1.3)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	1 (20%) 0.4 (0.4)	p=0.472
	p=0.629		p=0.347		p=0.347		
Thelephora terrestris	0 (0%) 0.0 (0.0)	3 (60%) 4.0 (2.2)	2 (40%) 1.2 (0.8)	2 (40%) 3.0 (2.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.071*
	p=0.100*		p=0.428		p=1.000		
Lactarius deliciosus type	3 (60%) 3.8 (2.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.074*
	p=0.099*		p=1.000		p=1.000		
Tuber type	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	1 (10%) 0.4 (0.4)	0 (0%) 0.0 (0.0)	2 (40%) 1.8 (1.1)	0 (0%) 0.0 (0.0)	p=0.075*
	p=1.000		p=0.145		p=0.145		
Amphinema type	3 (60%) 19.6 (11.9)	0 (0%) 0.0 (0.0)	1 (10%) 0.6 (0.6)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.093*
	p=0.138		p=0.347		p=1.000		
Chubby Bubby type (H30)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	2 (40%) 7.2 (6.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.244
	p=1.000		p=0.267		p=1.000		

[1]Ectomycorrhizal types in descending order of abundance.
[2]Frequency of plots with colonized seedlings in percent.
[3]Mean abundance is the average percent of root tips colonized.
[4]Numbers in parentheses are standard errors.
[5]p-values for paired t-test comparing Untrenched and Trenched treatments by individual site. ***p<0.01, **p<0.05, *p<0.10
[6]p-values for ANOVA of randomized block design comparing Untrenched and Trenched treatments ***p<0.01, **p<0.05, *p<0.10

Table 17, continued. Frequency and abundance of ectomycorrhiza types on Douglas-fir seedlings in Untrenched and Trenched treatments at Adams Lake, Hidden Lake and Malakwa.

EM type[1]	Adams		Hidden		Malakwa		p-value[6]
Site	Untrench	Trenched	Untrench	Trenched	Untrench	Trenched	
Rhizopogon-like type	3 (60%)[2] 6.4[3] (4.7)[4]	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.188
	p=0.212[5]		p=1.000		p=1.000		
Brown Zelig type (A20)	3 (60%) 3.6 (3.1)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.258
	p=0.280		p=1.000		p=1.000		
Laccaria type	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	2 (40%) 2.4 (1.9)	0 (0%) 0.0 (0.0)	p=0.228
	p=1.000		p=1.000		p=0.251		
Cenococcum-like type (H28)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	1 (10%) 0.2 (0.2)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.327
	p=1.000		p=0.999		p=1.000		
Laccaria-like type (H29)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	1 (10%) 1.0 (1.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.327
	p=1.000		p=0.999		p=1.000		
Yellow-tip Grizzly type (A21)	1 (10%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.327
	p=0.999		p=1.000		p=1.000		
Lactarius pubescens type	1 (10%) 0.2 (0.2)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.327
	p=0.347		p=1.000		p=1.000		
Craigallechie type (M26)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	3 (60%) 13.0 (9.6)	p=0.189
	p=1.000		p=1.000		p=0.267		
Humaria type	0 (0%) 0.0 (0.0)	1 (10%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	p=0.327
	p=0.347		p=1.000		p=1.000		
Last Spike type (M27)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	0 (0%) 0.0 (0.0)	1 (10%) 0.2 (0.2)	p=0.327
	p=1.000		p=1.000		p=0.347		

[1]Ectomycorrhizal types in descending order of abundance.
[2]Frequency of plots with colonized seedlings in percent.
[3]Mean abundance is the average percent of root tips colonized.
[4]Numbers in parentheses are standard errors.
[5]p-values for paired t-test comparing Untrenched and Trenched treatments by individual site. ***p<0.01, **p<0.05, *p<0.10
[6]p-values for ANOVA of randomized block design comparing Untrenched and Trenched treatments ***p<0.01, **p<0.05, *p<0.10

Comparison of richness and diversity between trenching treatments

Richness refers to the number of distinct ectomorphotypes that could be identified, which does not necessarily correspond to the number of species (*i.e.*, a single species could contain two or more ectomorphotypes). Over the three sites, about two times as many types were identified in the Untrenched (17 types) than Trenched (nine types) treatment (Table 16). Mean richness per seedling also was almost two times greater in the Untrenched (average of 4.3 types seedling^{-1}) than Trenched (2.5 types seedling^{-1}) treatment (p=0.002, Table 18, Figure 24).

Table 18. Trenching treatment comparison of mean richness, diversity, evenness and redundancy indices per seedling. Seedlings were harvested in October, 1994, 16 months following out-planting at Adams Lake and 6 months following out-planting at Hidden Lake and Malakwa.

Index	Untrenched	Trenched	p-values[2]
Ectomorphotype richness	4.33±0.36[1]	2.47±0.32	p=0.002***
Shannon-Weaver diversity index	0.67±0.13	0.27±0.09	p=0.027**
Simpson diversity index	0.40±0.11	0.20±0.04	p=0.049**
Simpson evenness index	0.60±0.09	0.27±0.09	p=0.043**
Simpson redundancy index	0.40±0.09	0.73±0.09	p=0.043**
McIntosh diversity measure	25.0±2.9	12.0±2.6	p=0.005***
McIntosh evenness measure	0.53±0.09	0.20±0.09	p=0.035**
McIntosh redundancy measure	0.47±0.09	0.80±0.09	p=0.035**

[1]mean ± standard error
[2]ANOVA of randomized complete block design. ***p<0.01, **p<0.05, *p<0.10

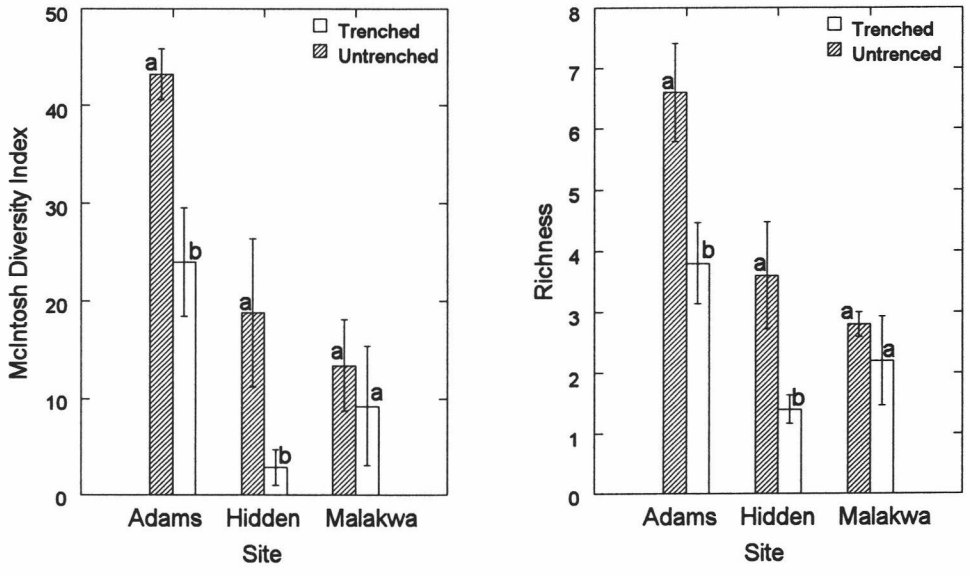

Figure 24. Comparison of (a) McIntosh Diversity Index and (b) morphotype richness between Untrenched and Trenched treatment at Adams, Hidden and Malakwa. Within a site, trenching means denoted by different letters differ significantly ($p<0.10$).

The diversity indices combine measures of richness and abundance using different approaches; the Shannon-Weaver Index is more sensitive to changes in proportion of rare species than the Simpson Index, and the McIntosh Index is more sensitive to changes in species abundance than either of the other two indices. Diversity of ectomorphotypes was approximately twice as great in the Untrenched than Trenched treatments using all three indices ($p<0.05$, Table 18). The evenness measures reflect how evenly the various morphotypes occur over the root system; a root system that has several, evenly proportioned types would have a higher evenness measure than a root system with one or two dominant types which occupy the majority of root tips. In our study, evenness was higher in the Untrenched than Trenched treatment ($p<0.05$, Table 18). The redundancy index is the antithesis of the evenness measure. It reflects the degree of repetition of types on a root system, and was greater in the Trenched than Untrenched treatment ($p<0.05$, Table 18). The richness, diversity, evenness and redundancy measures show that there were a greater number of ectomorphotypes that were more evenly portioned over the root systems in the Untrenched than Trenched treatment.

Richness, diversity and evenness indices were consistently greater at Adams Lake than either Hidden Lake or Malakwa, and redundancy indices were consistently greater at Hidden Lake and Malakwa than Adams Lake ($p<0.05$, Figure 24).

Comparison of seedling performance between trenching treatments

Net photosynthetic rate averaged almost 2 times greater in the Untrenched (0.64 mmol CO_2 s^{-1}) than Trenched (0.36 mmol s^{-1}) treatments in July ($p=0.031$) and August ($p=0.040$), 1994 (Table 19, Figure 25). By September, however, there was no difference in net photosynthetic rate between treatments (mean=0.29 mmol s^{-1}, $p=0.713$). At the time of harvest, there were no trenching treatment effects on seedling diameter, height or biomass ($p>0.10$), but height:diameter ratio was significantly greater in the Untrenched than Trenched treatment ($p=0.052$, Table 19). Seedling net photosynthetic rate in August and September, as well as seedling diameter and height:diameter ratio, were consistently greater at Adams Lake than either Hidden Lake or Malakwa ($p<0.05$, Table 19, site data not shown).

Comparison of PAR, soil water content and soil nutrient concentrations between trenching treatments

Photosynthetically active radiation (PAR) incident on seedlings at time of photosynthesis measurements averaged 114±14 μE m^{-2} s^{-1}, 79±9 μE m^{-2} s^{-1} and 54±5 μE m^{-2} s^{-1} in July, August and September, respectively. PAR did not differ between trenching treatments ($p>0.05$, Table 20),

Table 19. Comparison of net photosynthetic rate, seedling biomass and seedling size between trenching treatments. Seedlings were harvested in October, 1994, 16 months following out-planting at Adams Lake and 6 months following out-planting at Hidden Lake and Malakwa.

Seedling performance measure	Untrenched	Trenched	p-values[2]
Net photosynthetic rate (μmol m^{-2} s^{-1}) in July, 1994	0.59±0.09[1]	0.33±0.05	*T*=0.031**
Net photosynthetic rate (μmol m^{-2} s^{-1}) in August, 1994	0.68±0.06	0.40±0.07	*T*=0.040**
Net photosynthetic rate (μmol m^{-2} s^{-1}) in September, 1994	0.32±0.06	0.24±0.05	*T*=0.713
Biomass (g)	1.39±0.16	1.53±0.16	*T*=0.335
Height (cm)	17.25±1.29	14.84±1.37	*T*=0.135
Stem diameter (cm)	2.75±1.0	2.81±0.08	*T*=0.669
Height:diameter ratio	6.34±0.38	5.32±0.28	*T*=0.052*

[1]mean ± standard error
[2]ANOVA of randomized complete block design. ***p<0.01, **p<0.05, *p<0.10

was consistently greater at Adams Lake than either Malakwa or Hidden Lake at each measurement date (p<0.05, site data not shown).

Soil water content averaged 34±4% in August and did not differ between trenching treatments (p=0.992). Soil water in September, however, was lower in the Untrenched (24±2%) than Trenched (35±4%) treatment (p=0.046, Table 20). This difference may reflect soil water depletion by tree roots in the Untrenched plots, but not in the Trenched plots due to root exclusion. Soil water content was consistently greater at Malakwa than either Adams Lake or Hidden Lake on each measurement date (p<0.05, Figure 26).

Soil nutrient concentrations did not differ between trenching treatments when soils were sampled in September, 1994 (Table 20). Mean carbon concentration was 3.86±0.39%, C:N ratio

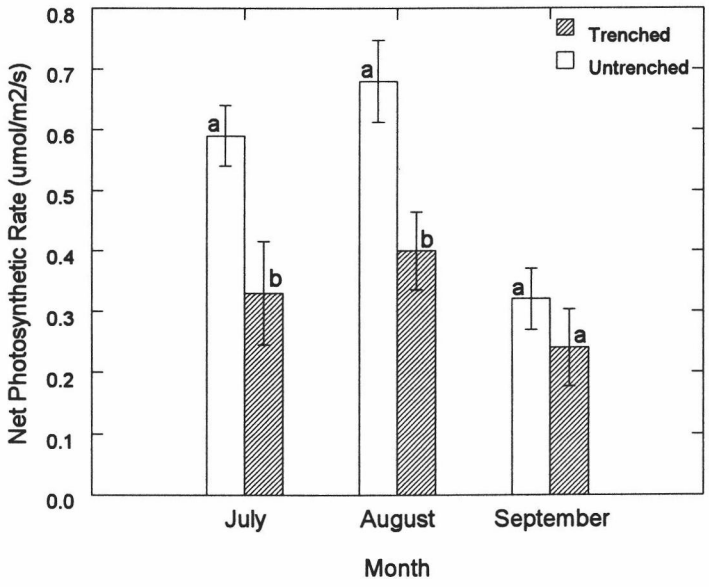

Figure 25. Comparison of net photosynthetic rate between Untrenched and Trenched treatments in July, August and September, 1994. Means denoted by different letters differ significantly ($p<0.05$).

Table 20. Comparison of PAR and soil water between trenching treatments.

Resource	Untrenched	Trenched	p-values[2]
PAR ($\mu E\ m^{-2}\ s^{-1}$) in July, 1994	120.0±18.9	108.3±17.6	*T*=0.678 *S*=0.000*** *TxS*=0.698
PAR ($\mu E\ m^{-2}\ s^{-1}$) in August, 1994	64.9±6.79	96.3±11.8	*T*=0.177 *S*=0.000*** *TxS*=0.535
PAR ($\mu E\ m^{-2}\ s^{-1}$) in September, 1994	59.5±6.2	48.3±3.05	*T*=0.377 *S*=0.000*** *TxS*=0.620
Soil water (%) in August, 1994	34.95±7.27	34.95±5.63	*T*=0.992 *S*=0.064 *TxS*=0.696
Soil water (%) in September, 1994	23.95±2.07	34.91±3.60	*T*=0.046** *S*=0.001*** *TxS*=0.313

[1]mean ± standard error
[2]ANOVA of randomized complete block design, where T=trenching treatment, S=site (block), and TxS=interaction. ***p<0.01, **p<0.05, *p<0.10

21.00±0.66, total nitrogen 0.19±0.02%, NH_4-N 7.75±0.63 ppm, anaerobically measured mineralizable nitrogen 47.90±5.16 ppm, exchangeable calcium 1563±148 ppm, exchangeable magnesium 118.4±12.6 ppm, exchangeable potassium 133.4±10.0 ppm, and pH 5.90±0.06. Total carbon, total nitrogen, mineralizable nitrogen, NH_4-N, exchangeable magnesium and exchangeable calcium concentrations were greater at Malakwa than Adams or Hidden Lakes, and pH was lower and C:N ratio higher at Hidden Lake than either Adams Lake or Malakwa ($p<0.05$, Table 21, Figure 27).

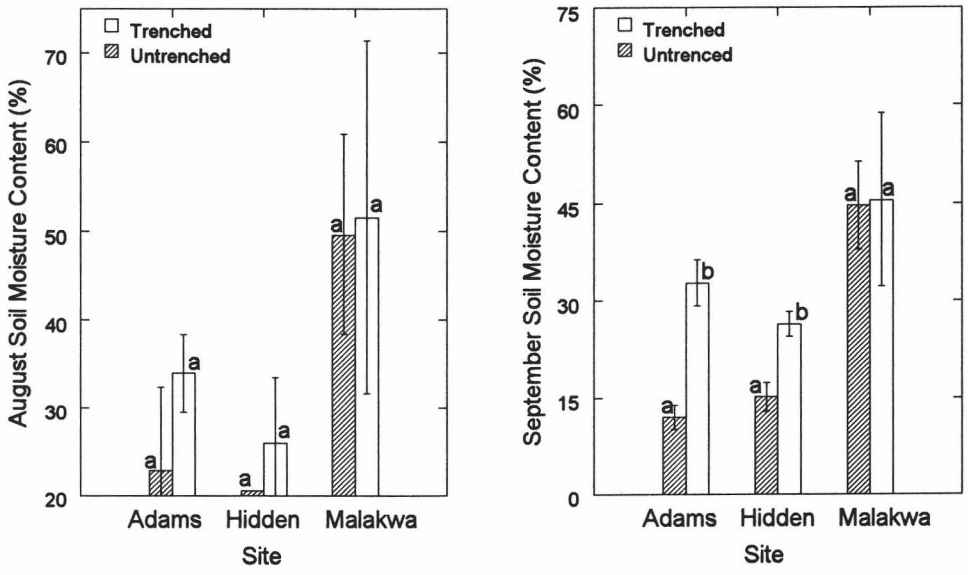

Figure 26. Comparison of soil water content in (a) August and (b) September between Untrenched and Trenched treatment at Adams, Hidden and Malakwa. Within a site, trenching means denoted by different letters differ significantly ($p<0.05$).

Table 21. Comparison of soil nutrients between trenching treatments.

Soil nutrient	Untrenched	Trenched	p-values[2]
Total carbon (%)	4.11±0.61	3.60±1.04	*T*=0.534 *S*=0.000*** *TxS*=0.765
C/N Ratio	21.0±1.18	20.9±1.70	*T*=0.932 *S*=0.000*** *TxS*=0.254
Total nitrogen (%)	0.21±0.03	0.17±0.04	*T*=0.392 *S*=0.000*** *TxS*=0.723
NH4-N	7.45±0.81	8.06±1.79	*T*=0.632 *S*=0.010** *TxS*=0.723
Mineralizable nitrogen	53.54±13.41	42.35±9.83	*T*=0.288 *S*=0.000*** *TxS*=0.192
Calcium (ppm)	1595±332	1532±302	*T*=0.835 *S*=0.000*** *TxS*=0.803
Magnesium (ppm)	124.6±27.4	112.3±24.0	*T*=0.630 *S*=0.002*** *TxS*=0.813
Potassium (ppm)	137.9±22.5	128.9±24.6	*T*=0.658 *S*=0.817 *TxS*=0.970
pH (water)	5.96±0.14	5.84±0.13	*T*=0.327 *S*=0.016** *TxS*=0.314

[1]mean ± standard error
[2]ANOVA of randomized complete block design, where T=trenching treatment, S=site (block), and TxS=interaction. ***p<0.01, **p<0.05, *p<0.10

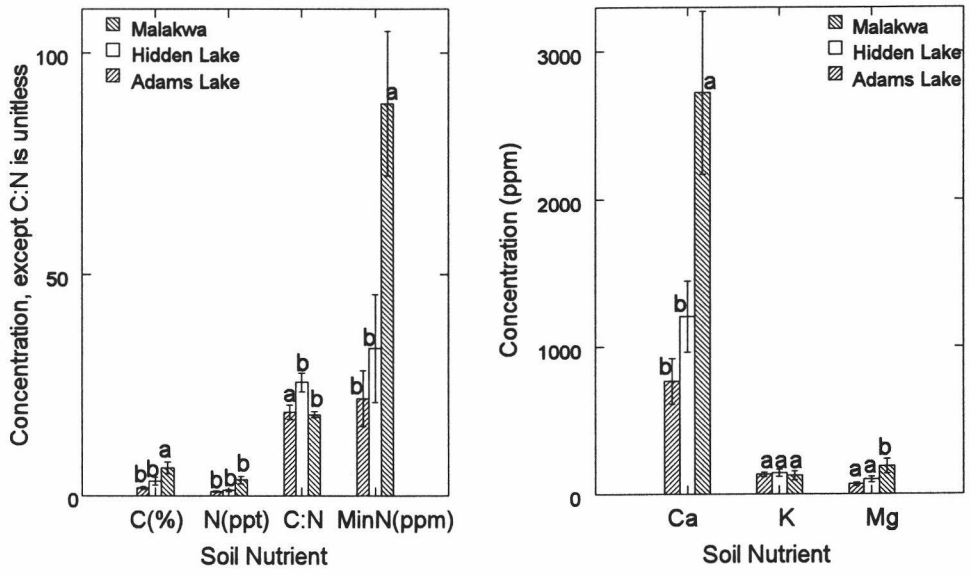

Figure 27. Comparisons of (a) total C (%), total N (ppt), C:N ratio, mineralizable N (ppm), and (b) exchangeable Ca (ppm), K (ppm) and Mg (ppm) among sites. Site means denoted by different letters differ significantly ($p<0.05$)

Discussion

Effects of trenching on occurrence of ectomycorrhizae

The field bioassay resulted in formation of ectomycorrhizae on Douglas-fir seedlings with 20 fungal types: 17 types occurred in the untrenched treatment (in order of abundance, underlined if present at ≥ 2 sites, and marked with * if characterized by strands: MRA, *Rhizopogon vinicolor**, E-strain*, *Amphinema**, Chubby Bubby, *Rhizopogon*-like*, *Lactarius deliciosus**, Brown Zelig, *Laccaria**, *Tuber*, *Cenococcum geophilum*, *Hebeloma**, *Thelephora terrestris**, *Laccaria*-like, *Lactarius pubescens**, *Cenococcum*-like, and Yellow-tip Grizzly types), and nine in the trenched treatment (MRA, E-strain*, Craigallechie, *Thelephora terrestris**, *Humaria*, *Hebeloma**, *Cenococcum geophilum*, *Rhizopogon vinicolor**, and Last Spike* types). The known genera of types found in our study have earlier been described as commonly associated with Douglas-fir in western North America (Trappe 1962 and 1977, Molina and Trappe 1982 and 1994, Molina *et al.* 1992, Smith *et al.* 1995, Jones 1995). The five unknown types (Chubby Bubby, Brown Zelig, Yellow-tip Grizzly, Craigallechie, and Last Spike) apparently have never previously been reported.

Richness and diversity of ectomycorrhizal types per seedling were approximately twice as great where Douglas-fir seedling roots had access to overstory trees (Untrenched treatment) than where seedlings were isolated (Trenched treatment). Our results support observations by Borchers and Perry (1990) and Deacon *et al.* (1983), who found that Douglas-fir or birch seedlings formed a greater diversity (richness and abundance) of ectomycorrhizal types when grown in close proximity rather than at distance from older hardwoods. They also corroborate results of Massicotte *et al.* (1994), who showed that richness of ectomycorrhizal types associated with Douglas-fir seedlings increased when grown in mixture with a primary host tree species rather than alone, which they suggest resulted from greater inoculum density and/or additional energy (e.g, carbohydrates) supplied by the well-colonized companion seedling.

On average, 4.3 types (maximum 9) were identified on a single Douglas-fir seedling from the Untrenched treatment, and only 2.5 types (maximum 4) per seedling in the Trenched treatment. Several species of ectomycorrhizal fungi have previously been isolated from single trees growing in undisturbed forest, and each fungus can have quite different effects on host physiology under different environmental conditions (Molina *et al.* 1992). The difference in richness observed between trenching treatments in our study may reflect differences in mycorrhizal inoculum potential (e.g., Deacon *et al.* 1983, Perry *et al.* 1987), competitive replacement among fungal species (Bruns 1995), and/or the magnitude of available niches in the rhizosphere (Bills *et al.* 1986). Seedlings which were not trenched, for example, potentially had access to a greater array of fungal inoculum (e.g., mycelium, strands or spores) from neighboring overstory trees, a supplementary carbon and nutrient source if trees became interconnected during seedling infection

(e.g, Deacon *et al.* 1983, Read *et al.* 1985), greater potential for co-infection and hence competitive replacement involving several fungal species, greater access to nutrient pools beyond the experimental unit (plot) boundary, and conversely more competition from overstory trees for nutrients and water.

Eight ectomycorrhizal types formed strands or rhizomorphs over an average of 23% of Douglas-fir root tips in the Untrenched treatment, whereas only four types formed strands over 4% of the root tips in the Trenched treatment. In addition, the main strand-forming type, *Rhizopogon vinicolor*, was on average 20 times more abundant in the Untrenched than Trenched treatment, forming in the latter only on 16 month-old seedlings at Adams Lake. Ectomycorrhizae with extensive strand networks (e.g., *Rhizopogon vinicolor*, *Lactarius* spp., and *Amphinema* sp. in this study) may impose a greater carbon drain on Douglas-fir seedlings than those with weak sheath and extramatrical hyphal development based on their fungal biomass (Deacon and Fleming 1992) and demonstrated stimulation of seedling photosynthesis (Parke *et al.* 1983, Dosskey *et al.* 1990). In our study, the combination of low seedling biomass, low light intensity and low net photosynthetic rates suggest that high carbon demands of the robust strand-forming mycorrhizae may have been difficult to meet in the forest environment.

The simplest explanation for greater abundance and frequency of strand forming ectomycorrhizae in the Untrenched than Trenched treatment is that ectomycorrhizal hyphae emanating from neighboring overstory trees contacted and colonized understory Douglas-fir seedlings. Carbohydrate supplied by the overstory trees may have allowed the seedlings to form ectomycorrhizae with a fungus that otherwise it energetically could not afford to support. Once the ectomycorrhizal association formed, the carbohydrate-deficient ectomycorrhiza would continue to directly benefit from carbon transfer from the connected overstory tree. The Douglas-fir seedlings also would indirectly benefit from the association through exploration of a soil volume beyond its root's normal capabilities and increased capacity for gathering water and nutrients (Amaranthus and Perry 1994). The seedlings may or may not directly benefit from carbon and nutrient gain through transfer from overstory trees; direct physiological support of understory seedlings supposedly is more costly to connected overstory trees than indirect support through the ectomycorrhizal association. However, results from our laboratory (Chapters 3 and 4) and field (Chapter 5) studies suggest potential for substantial interspecific transfer between established plants. There is considerable evidence that carbon is transferred between connected plants as the carbon skeleton of amino acids (e.g., Martin *et al.* 1988, Abuzinadah and Read 1989, Smith and Smith 1990, Arnebrant *et al.* 1993), and that transfer is driven more by interplant nitrogen than carbon concentration gradients, because fully developed receiver leaves are usually strong sinks for nitrogen and sources rather than sinks for carbon (Pearcy *et al.* 1987). Photosynthate gradients may play a greater role where receiver seedlings are growing in low light environments, however, as supported by other studies where carbon isotope transfer was greater among seedling growing

in full shade than full light (Francis and Read 1984, Read *et al.* 1985, Finlay and Read 1986, Chapter 5 this thesis).

These mechanisms require that hyphal links establish between overstory trees and seedlings, whether momentarily or for a prolonged period. Ectomycorrhizal connections and/or interspecific carbon transfer have been demonstrated previously between several tree species, including species in the families Pinaceae and Betulaceae (Read *et al.* 1985, Arnebrant *et al.* 1993, Chapters 3, 4 and 5 this thesis). In this study, hyphal or strand linkages between overstory trees and understory seedlings in Untrenched plots were never traced. Nor were ectomycorrhizal types that colonized overstory trees identified so that we could evaluate potential for linkage with seedlings. The most direct method of identification and enumeration of linkages would involve microscopic examination of extramatrical hyphae and strands in the field (Miller and Allen 1992). Alternatively, overstory trees could be injected with isotope and understory ectomycorrhizal seedlings later examined for its presence (e.g., Bjorkman 1960, Read *et al.* 1985). Finally, ectomycorrhizal species shared in common between overstory trees and understory seedlings could be identified (Deacon *et al.* 1983) and their genetic similarity examined using molecular genetic tools (Bruns 1995).

The pattern of colonization observed in our study is comparable to Fleming (1983), who assayed ectomycorrhizae on *Betula pendula* seedling growing in untrenched (untreated) and trenched (cored) positions around *Betula pubescens* parent trees. Fleming (1983, 1984) found that strand-forming mycorrhizae of *Lactarius* and *Leccinum* established most frequently and abundantly where seedling roots were in contact with parent tree roots, and suggested that colonization occurred by means of strands attached to the parent tree. The parent tree supplied the inoculum and the photosynthate necessary to meet high carbon demands of the ectomycorrhizal association (Mason *et al.* 1983, Fleming 1985). The strand-forming types were called "late stage" fungi, and are characterized by high carbon demands, establishment on older root systems or in older soil, and poor establishment from spores (Deacon and Fleming 1992). Once established, however, late stage fungi apparently are effective competitors (e.g., K-strategists). In contrast, when Fleming (1983) planted seedlings into cored positions they developed mycorrhizae only with "early stage" fungi (e.g., *Laccaria* and *Thelephora terrestris*), which are characterized by low carbon demands, ready establishment from spores or mycelium, and a ruderal behavior strategy (Deacon and Fleming 1992).

In our study, more ectomycorrhizal types with late stage characteristics occurred in the Untrenched than Trenched treatment (e.g., *Rhizopogon vinicolor*, *Lactarius deliciosus*, *Tuber*, *Amphinema* types). Conversely, most of the dominant types which formed in trenched plots had early stage characteristics (e.g., MRA, E-strain, *Thelephora terrestris*, *Humaria* types), although some with late stage characteristics were also present in low abundance (e.g., *Rhizopogon vinicolor*, Last Spike). *Rhizopogon* spp. behave like early stage fungi in that their spores germinate

well and serve as effective inoculum (Danielson 1982, Molina and Trappe 1994), but also have strong late stage characteristics due to common formation of abundant strands (Molina and Trappe 1994). Both classes of fungi were present and some types were of equal abundance (MRA, *Cenococcum* and *Hebeloma*) in both trenching treatments. The ubiquity of these types regardless of influence of parent trees is reminiscent of phenomenon documented by Molina *et al.* (1992) in which Douglas-fir formed ectomycorrhizae with *Rhizopogon* types regardless of host size, age or disturbance history. Apparently inoculum of both early and late stage classes of fungi exist in young and old-growth forests of western North America, presumably due to patterns of disturbance and succession.

That differences in ectomycorrhizal colonization patterns between Trenched and Untrenched treatments persisted for at least 16 months indicates that some fungi were unable to inoculate seedling roots in the Trenched treatment in spite of the assumed presence of spores or hyphae on excised roots in the plots. Within a trenched plot, ectomycorrhizal fungi could survive with an inadequate carbon source by entering a resting stage, by becoming saprophytic or by receiving sustained input of spores from surrounding areas (Amaranthus and Perry 1994). However, only some fungi have saprophytic capabilities for nutrient or carbon uptake (Dighton 1991), most hypogeous fungi disperse spores belowground (Molina *et al.* 1992), and spores alone often are inadequate for maintaining the inoculum potential of a microsite (Perry *et al.* 1989).

Effects of trenching on soil water and nutrients

One year after trenching, there were no differences in soil pH, total carbon concentration, C:N ratio, total, mineralizable and ammonium nitrogen concentrations, or exchangeable cations (Ca, K, Mg) between Untrenched and Trenched treatments. Soil water content also did not differ between trenching treatments in August, when seedlings were physiologically active, but was higher in Trenched than Untrenched plots in September, after seedlings had quiesced. As a result, we presume that the effect of trenching on patterns of ectomycorrhizal colonization of Douglas-fir seedlings were due primarily to isolation from neighboring trees rather than indirectly through changes in soil properties.

Trenching is desirable for this study because effects of neighboring inoculum sources on seedling ectomycorrhizal colonization can be examined in a natural setting with large trees; however, the act of trenching, inclusion of severed roots in the cookie, and the suspension of nutrient uptake by large trees potentially could affect rates of decay, associated nutrient pools (Silver and Vogt 1993) and water content, and perhaps, as a result, ectomycorrhizal colonization. That soil trenching did not affect measured soil properties or seedling growth in our study contrasts with some previous studies, which have shown trenching to sometimes increase soil water (Copeland 1953, Lorio and Hodges 1977) and nutrient availability (Grosz and Dyck 1979, Silver

and Vogt 1993), decrease subsurface water flow (Mirjat and Kanwar 1992), increase litter decomposition rates (Romell 1938, Silver and Vogt 1993), alter the structure of soil organism communities (Jenik *et al.* 1954, Wallis and Buckland 1955), and/or increase plant growth rates (Romell 1938, Mann 1950, Vaartaja 1950, Vogdt 1953, Surmac 1958). Romell (1938), for example, found that plant growth, health and seasonal longevity were enhanced one year following trenching treatments in an old-growth spruce-*Vaccinium* forest in Sweden, which he attributed to increased nitrogen availability as a result of increased root decomposition and decreased competition from surrounding trees.

Lack of increased nitrogen availability one year following trenching in our study, however, appears to corroborate slow root decomposition rates observed by Silver and Vogt (1993) and Ferrier and Alexander (1985). Silver and Vogt (1993) found one year after trenching in subtropical wet forests in Puerto Rico that root decay was slow, fine root concentrations of Ca, Mg, K, P and N relatively stable, and soil water content the same as soil outside the trenched plots. Ferrier and Alexander (1985) also observed persistence of fine roots and mycorrhizae of spruce seedlings nine months after trenching in mature Sitka spruce plantations. Slow decomposition rates observed in our study may be due either to high concentrations of secondary chemicals in fine roots (Bloomfield *et al.* 1993), high carbohydrate reserves in the excised root tissues (Ferrier and Alexander 1985), or low cold soil temperatures under the closed forest canopies.

We expect eventual changes in nutrient availability in our the trenched plots, however, and will continue monitoring soil nutrients for future interpretation of the field bioassays. For example, soil pH, Ca and Mg concentrations eventually declined in the trenched plots of Silver and Vogt (1993), which they attributed to coupled leaching with nitrate. They suggested that nitrate leaching resulted from lack of root uptake and increased mineralization rates. Grosz and Dyck (1979) also found that nitrate leaching was higher in unvegetated trenched than control plots in Douglas-fir stands in New Zealand. Nitrate leaching was not measured in the present study, but similar cation (Ca, K, Mg) concentrations between treatments suggest that leaching one year after trenching was insignificant.

Effect of trenching on seedling performance

Mean net photosynthetic rate of Douglas-fir seedlings was greater in Untrenched than Trenched treatments in July and August but not September, 1994. Alternative mechanisms which may be responsible for this response include greater colonization by ectomycorrhizal fungi (e.g., Dosskey *et al.* 1990), enhanced soil nutrient availability (e.g., Brix 1981) or enhanced soil water availability (e.g., Lopushinsky 1990) among seedlings in the Untrenched relative to Trenched treatment. However, trenching had no measurable effect on soil water content in July and August nor on soil nutrient concentrations at the end of the growing season (September), so we presume

soil water and nutrient availability were not responsible for differences in seedling physiology. Soil water content was higher in the Trenched than Untrenched plots in September, after mycorrhizae had formed and seedlings had quiesced, but did not differ between trenching treatments in August when seedling photosynthetic rates were significantly affected by treatment. Furthermore, soil water contents (Lopushinksy 1990) and nutrient concentrations (Russell 1973, Barber 1984, Stevenson 1986) did not appear to be in the limiting range for seedling growth when compared with other studies. Soil analyses provide only indirect estimates of plant nutrient and water availability, however; a better approach would have been direct measurement of foliar nutrient concentrations (Perry 1994) and seedling transpiration rates (Lopushinsky 1990).

The association between seedling photosynthesis and ectomycorrhizal composition suggests that colonization patterns may explain differences in seedling performance between Untrenched and Trenched treatments. Earlier studies have shown that presence of ectomycorrhizae on seedling root systems is correlated with higher rates of net photosynthesis rates (Parke *et al.* 1983, Reid *et al.* 1983, Nylund and Wallander 1989, Dosskey *et al.* 1990). Alternative mechanisms which may have been responsible for this effect include *(i)* increased photosynthate sink generated by fungal growth and metabolism (Reid *et al.* 1983, Dosskey *et al.* 1990), *(ii)* enhanced foliar photosynthate, water or nutrient status through transfer from neighboring trees (e.g., Read *et al.* 1985), *(iii)* improved water status resulting from mycorrhizal-enhanced water uptake and drought resistance (Parke *et al.* 1983, Dosskey *et al.* 1990), *(iv)* improved plant mineral nutrition resulting from mycorrhizal-enhanced nutrient uptake (Nylund 1988), or *(v)* changes in plant hormonal balance (Slankis 1973, Nylund and Wallander 1989). In our study, seedling photosynthetic rates were most likely limited first by light availability, because ambient light in each forest (PAR<200 $\mu E\ m^{-2}\ s^{-1}$) was consistently near Douglas-fir's light compensation point (Chapter 3 this thesis, Price *et al.* 1986). In that case, gains in photosynthate through transfer from neighboring trees may have accounted for enhanced photosynthetic rates. However, photosynthetic responses may also have resulted from nutrient and/or water transfer or enhanced uptake where slight water and nutrient deficiencies existed. Greater diversity of ectomycorrhizal fungi on seedling root systems may have played an important role in exploring a greater array of environmental niches and thereby improving uptake and overall seedling physiology, particularly under these marginal environmental conditions (Perry *et al.* 1987 and 1989, Molina *et al.* 1992, Bruns 1995).

The species of ectomycorrhizal fungal associate also has been shown to affect magnitude of photosynthetic response (Parke *et al.* 1983, Dosskey *et al.* 1990). Dosskey *et al.* (1990) found that ectomycorrhizae with extensive hyphal and/or rhizomorph growth (e.g., *Rhizopogon vinicolor*) increased net photosynthetic rates of Douglas-fir seedlings more than those with smooth mantles (e.g., *Laccaria laccata*), which they attributed to greater photosynthate demand for fungal growth. Similarly, Parke *et al.* (1983) showed that *Rhizopogon vinicolor* lessened the severity of drought on

photosynthesis of Douglas-fir seedlings more than other fungal associates, which they suggested was largely a result of efficient water uptake by *Rhizopogon*'s extensive rhizomorph network. In out study, estimates of water use efficiency (net photosynthetic rate/soil water content) were consistently higher in the Untrenched than Trenched treatment, which may have resulted from greater abundance and frequency of strand forming mycorrhizae such as *Rhizopogon vinicolor.* Other functions which have been suggested of rhizomorphs include enhanced nutrient uptake and transport (Read 1992), carbon, nutrient and water transfer between linked plants (e.g., Read *et al.* 1985, Duddridge *et al.* 1988), and linkage between the root system and diffuse mycelial front (Read 1992).

Trenching had no effect on average height, diameter or biomass of Douglas-fir seedlings, but did result in greater height:diameter ratio in the Untrenched than Trenched treatment in October, 1994. Inoculation of seedlings with mycorrhizal fungi has resulted in variable effects on seedling growth in other studies, possibly due to differences in fungal species used, degree of mycorrhizal development and hyphal growth, or condition of the growing environment (Nylund and Wallunder 1989, Villeneuve *et al.* 1991). For example, Bledsoe *et al.* (1982) found that biomass of Douglas-fir seedlings inoculated with *Laccaria laccata* or *Hebeloma crustuliniforme* was lower than that of non-inoculated seedlings two years after outplanting. Similarly, Nylund and Wallander (1989) found that relative growth rate of *Pinus sylvestris* seedlings decreased two to four months after ectomycorrhizal colonization. In contrast, inoculation of Douglas-fir seedlings with *Rhizopogon vinicolor* (Castellano and Trappe 1985) or *Laccaria bicolor* (Villeneuve *et al.* 1991) resulted in greater seedling biomass relative to uninoculated controls two years after outplanting. Differences between treatments were not significant in either case, however, after only one year. Villeneuve *et al.* (1991) suggests that seedling growth increases due to mycorrhizal colonization may not be measurable until two years post-planting, after seedlings have recovered from planting shock.

In our study, net photosynthetic rate was a more sensitive response measure than seedling size to differences between trenching treatments, and may be a precursor to measurable growth differences two or more years after out-planting. The treatment difference in height:diameter ratio also indicates differences in seedling morphology that may become more pronounced with age. Height:diameter ratio is an indicator of seedling carbon allocation patterns and morphology; seedlings with high ratios allocate more carbon to height than diameter growth, resulting in more spindly morphology, relative to seedlings with low ratios. High height:diameter ratio is a measure of etiolation and is considered a compensatory response to low light intensities.

Significance of seedling age to ectomycorrhizal colonization patterns

Greater diversity and richness of ectomycorrhizal fungi associated with Douglas-fir coincided with greater age of seedlings harvested at Adams Lake (16 months) than Hidden Lake or Malakwa (6 months). The age comparison is most valid between Adams Lake and Hidden Lake sites because they did not differ in soil water content or soil nutrient concentrations (total C, total N, available N, NH_4-N, K, Ca or Mg) other than C:N ratio. In contrast, Malakwa was both wetter and more nutrient rich than Adams Lake, thereby confounding effects of seedling age with site quality. Gradual increases in richness, diversity, and abundance of specific ectomycorrhizal associates with seedling age also have previously been observed among *Betula pubescens* seedlings in Great Britain (Fleming 1983, 1984, Last *et al.* 1987), and Douglas-fir seedlings in the Pacific Northwest (Smith 1995) and New Zealand (Chu-Chou and Grace 1981). Dighton (1991) suggests that ectomycorrhizal diversity tends to be low when nutrient supply of the soil exceeds demand of the tree, and increases as demand approaches and exceeds supply. Possible reasons that diversity and richness were greater among older seedlings at Adams Lake than the younger cohort at Hidden Lake include *(i)* greater nutrient demand due to higher net photosynthetic rates of Douglas-fir through the 1994 growing season, *(ii)* greater available carbohydrate to support mycorrhizae with robust as well as weak hyphal development, *(iii)* greater root egress out to a wider variety of substrates, *(iv)* longer opportunity for spore immigration and germination, and *(v)* higher inoculum potential of specific fungi. Differences in inoculum potential between sites are possible due to differences in forest tree composition or type and severity of previous disturbances (Perry *et al.* 1987). For example, *Thuja plicata* and *Tsuga heterophylla* comprised a greater proportion of the stand at Hidden Lake than Adams Lake, which may have diluted the inoculum potential of genera specific to Douglas-fir. *Thuja plicata* is strictly arbuscular mycorrhizal (J. Trappe, personal communication) and *Tsuga heterophylla* typically associates with a narrower assemblage of fungal species than Douglas-fir (Molina *et al.* 1992). With regard to disturbance history, both stands originated from wildfire, but differences in the severity of fires and their effects on inoculum potential are unknown. Differences in richness, diversity or abundance of specific ectomycorrhizal fungi associated with different aged Douglas-fir seedlings at Adams and Hidden Lakes corroborate trends reported in some other studies that have focused on sporocarp inventories in age sequences of single species stands. For example, increases in richness and abundance of sporocarps with age of *Betula pubescens* saplings, *Picea abies* trees and *Pinus sylvestris* have been observed by Mason *et al.* (1982), Dighton *et al.* (1986), Last *et al.* (1987), and Shaw and Lankey (1994). Greater richness and abundance of ectomycorrhizal sporocarps have also been found in spruce and Douglas-fir stands at crown closure than in younger age classes (Gaper 1994, Gaper and Lizon 1995, Vogt *et al.* 1983, Jansen and De Nie 1988, O'Dell *et al.* 1992). In contrast, Smith *et al.* (1995) found that the number of unique species was greater in young and old-growth

than rotation-aged Douglas-fir stands. Several problems are associated with drawing comparisons between these sporocarp studies and our field bioassay. Although productivity of sporocarps is assumed to reflect importance of ectomycorrhizal associations, studies show variable correlations in results between the two (Menge and Grand 1978, Jansen and De Nie 1988, Dalbergh and Stenlid 1995). In general, sporocarp inventories tend to underestimate total mycorrhizal diversity (Menge and Grande 1978), whereas seedling bioassays tend to select against types which are specifically associated with different host ages, sizes or habitats. Other problems are associated with differences in type, substrate, spatial scale, and longevity of the observational units in the two different approaches.

Abundance of *Rhizopogon vinicolor, Cenococcum, Hebeloma, Lactarius deliciosus,* and *Amphinema* types was significantly greater and MRA lower among older seedlings at Adams Lake than younger seedlings at Hidden Lake in the Untrenched treatment. Of those that occurred in greater abundance on older seedlings, most were robust, strand-forming ectomycorrhizal fungi. Greater proliferation of *Rhizopogon* among older than younger western hemlock seedlings also was observed by Smith (1993), and may have been associated with greater root carbohydrate storage with age. Similarly, Fleming *et al.* (1984) observed increased abundance of several robust late stage mycorrhizae as birch seedlings aged over a two year period following out-planting in afforested birchwoods in Great Britain (Deacon and Fleming 1992). Increasing dominance of late stage fungi with seedling age was attributed to fungal succession in first generation birchwoods (Fleming *et al.* 1984), but capabilities for growth amidst high accumulations of recalcitrant organic matter in second generation birchwoods (Last *et al.* 1987). We cannot speculate that differences in colonization patterns between Adams and Hidden Lake reflected fungal succession, because sequential colonization or species replacement was not observed within sites. Alternatively, the greater abundance of strand-forming mycorrhizae may have reflected a higher inoculum potential at Adams than Hidden Lake, either due to presence or greater abundance of propagules or due to greater seedling photosynthate available to support their high growth and metabolic demands.

Significance of site quality to ectomycorrhizal colonization patterns

Relationships between site quality on colonization patterns can only be drawn between Hidden Lake and Malakwa without confounding effects of site quality and seedling age, Diversity, richness and abundance of ectomycorrhizal associates did not differ among 6-month-old seedlings growing in Untrenched plots at Hidden Lake and Malakwa, in spite of site differences in soil water content and soil nutrient concentrations. This contrasts with trends in fertilization trials, where increased site fertility has resulted in lower abundance of ectomycorrhizal tips (Menge *et al.* 1977, Bledsoe 1992). The effect of trenching on mycorrhizal colonization did differ between sites, however; diversity and richness were reduced by trenching at Hidden Lake but not Malakwa.

Apparently access to host root systems was important to maintain inoculum potential at Hidden Lake, but made no difference at Malakwa. We can speculate that ready availability of nutrients at Malakwa negated advantages conferred by a diverse assemblage of ectomycorrhizal associates, so that access to carbon supply and inoculum from parent trees was irrelevant to ectomycorrhizal colonization patterns. Conversely, low fertility at the Hidden Lake site suggests that a diverse array of ectomycorrhizal associates could affect seedling physiology by providing access to a wider diversity of nutrient sources. In that case, access to supplemental carbohydrate and inoculum from neighboring parent trees may be critical.

Summary

In our study, diversity and richness of ectomycorrhizae as well as seedling photosynthetic rates were reduced by isolating seedlings from root systems of overstory trees. The association between ectomycorrhiza diversity and seedling performance in our study supports the hypothesis of Perry *et al.* (1987) that diversity contributes to seedling success. The abundance of *Rhizopogon vinicolor* also was dramatically reduced, which has been shown in other studies to affect drought stress and net photosynthetic rates of Douglas-fir seedlings (Parke *et al.* 1983, Dosskey *et al.* 1991). Further research is needed to determine the benefits that other ectomycorrhizal types and different mixtures confer on regenerating seedlings in highly variable field conditions. Since trenching had no effect on soil water content, soil nutrient concentrations, or light availability, we assume that influence of overstory trees through ectomycorrhizal colonization patterns was the main factor affecting seedling performance. Other factors also operated to create some differences in colonization patterns among sites, including age of seedlings at harvest and site quality.

Although questions remain regarding the mechanism of ectomycorrhizal-mediated influence of overstory trees on performance of understory seedlings, the study provides useful insights to the value of older trees in maintaining ectomycorrhizal inoculum potential of a site. Low inoculum potential, due to inadequate levels of either fungal propagules or fixed carbon input, can restrict performance of seedlings, particularly in physically rigorous environments such as deep shade or where the growing season is short. Trees that survive or stumps that sprout following disturbance such as harvest or wildfire can serve as legacies of mycorrhizal inoculum and energy for the regenerating forest, and probably influence success of different plant species, community dynamics and the successional trajectory (Perry 1994). The significant influence of inoculum potential of overstory trees on regenerating seedlings indicates that ectomycorrhizal linkages between old and young trees must be considered, along with resource competition, in predictions of the outcome of management practices on forest regeneration patterns.

Species-specific influences of overstory trees on regenerating Douglas-fir cannot be determined from our study because the forests were comprised of intimate mixtures of paper birch, Douglas-fir and other conifers. Because paper birch and Douglas-fir share several ectomycorrhizal types in common, and previous studies (Chapters 4 and 5) indicate that carbon is readily exchanged between them, we expect that both species contributed to inoculum potential of the sites. Further research is necessary to better understand the relative roles of residual tree species in providing legacies that influence the recovery of other species in forest communities following different management disturbances, such as clearcutting, partial cutting, site preparation, and stand tending.

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Appendix

Ectomycorrhizal types common to Untrenched and Trenched plots

MRA type

MRA, *Mycelium radicis atrovirens* Melin, was the most frequent and abundant ectomycorrhizal type on Douglas-fir in the Untrenched and Trenched treatments, colonizing 97% of the seedlings over 67% of their root tips (mean abundance comparison between trenching treatments, p=0.358, Table 16). MRA was present in Trenched and Untrenched treatments on all 3 sites (Table 17). The ectomycorrhizae were cylindrical, unbranched, smooth, dark brown to black, with a loose mantle. The seedling root tip was usually uncolonized because it grew through the mantle. The mantle surface was an irregular locking synenchyma, or fine jigsaw pattern (Smith 1993), with large (10 µm wide), pillowy hyphal cells. Extramatrical hyphae were 2-4 µm wide, light brown, septate, without clamps. Strands were absent.

E-strain type

E-strain colonized fewer seedlings (40% versus 73% of seedlings) but was more abundant (19±9%) in the Trenched (19±9%) than Untrenched (9±2%) treatment (p=0.052, Table 16). E-strain occurred on all three sites (Table 17). The ectomycorrhizae were branched, smooth, pale to reddish brown with a white tip. The mantle was discontinuous, thick (4-12 µm wide), irregular synenchyma lock, with large (2-8 µm wide), thick-walled hyphal cells. Extramatrical hyphae were rare, light brown, 3-10 µm wide, branched, without clamp connections. Loose, reddish brown strands were rare.

Rhizopogon vinicolor

Rhizopogon vinicolor section Villosuli occurred on 47% of Douglas-fir seedlings in the Untrenched treatment where it occupied on average 11±5% of the root system, and on only 13% of seedlings in the Trenched treatment over <1% of the root system (p=0.002, Table 16). It occurred in both trenching treatments at Adams Lake (16-month-old seedlings), and only the Untrenched treatment at Malakwa (6-month-old), and was absent from Hidden Lake (6-month-old) (Table 17). Ectomycorrhizae were single to pinnately-branched to pinnately-tuberculate-clumped, tomentose, dark brown to light brown to white. The mantle was a rough, crusty, felt prosenchyma with abundant external hyphae. Emanating hyphae were hyaline to grayish-brown, 2-3 µm wide, smooth, with infrequent branching and without clamp connections. Cystidia were up to 10 µm wide and 3 mm long, and frequently bent. Grayish-brown strands were abundant, forming mats.

Cenococcum geophilum

Cenococcum geophilum occurred only at Adams Lake (16-month-old seedlings), where it colonized 60% of the seedlings over <2% of their root systems in the Untrenched and Trenched treatments (p=0.687, Tables 16 and 17). The ectomycorrhizae were cylindrical, unbranched, up to 5 mm long, black, with a smooth texture under abundant emanating hyphae. The mantle was a compact, black, net synenchyma, with a radiating isocentric pattern (Trappe 1971). Emanating hyphae were bristly, fragile, black, smooth, 3-5 µm wide, septate, without clamp connections. Strands were absent.

Hebeloma type

Hebeloma type was found in both trenching treatments at Adams Lake only (16-month-old seedlings), where it occurred on 40% of the seedlings over <2% of their root systems (Table 17). Ectomycorrhizae were single to pinnately branched, light brown, smooth textured, with adhering soil. The compact mantle was a felt prosenchyma, with abundant emanating hyphae up to 8 µm wide. Emanating hyphae were hyaline to light brown, smooth to verrucose, infrequently branched, with abundant clamp connections. The abundant strands were loose, white and brown, and 7-13 µm wide.

Thelephora terrestris

Thelephora terrestris type occurred on 23% of Douglas-fir seedlings, and was more abundant in the Trenched (2.3±1%) than Untrenched (0.4±0.3%) treatments (p=0.071, Table 16). *Thelephora terrestris* occurred only at Hidden Lake and Adams Lake, at the latter only in the Trenched treatment (Table 17). The ectomycorrhizae were pinnately branched, light to medium brown. The compact mantle was smooth to rough textured, with infrequent emanating hyphae. The emanating hyphae were smooth, hyaline, 4 µm wide, infrequently branched, with clamp connections. Cystidia were hyaline, 4 µm wide and 100 µm long, with basal clamp connections. Light brown strands were occasionally present.

Ectomycorrhizal types unique to Untrenched plots

Lactarius deliciosus type

Lactarius deliciosus type occurred only at Adams Lake in the Untrenched treatment, where it occurred on 60% of the seedlings and occupied 4±2% of their root systems (p=0.071, Table 17).

Ectomycorrhizae were single to pinnately branched, smooth, swollen, blue-brown to dark brown with a bluish tip. The compact, smooth mantle was a net prosenchyma with bluish lactiferous hyphae up to 10 µm wide. Emanating hyphae were moderately abundant, 2 µm wide, hyaline-whitish-yellowish-brown, smooth, unbranched, with abundant clamp connections. Strands were loose, hyaline, approximately 2 µm thick.

Tuber type

Tuber type was present on 20% of Douglas-fir seedlings in the Untrenched treatment, and occupied on average 0.7±0.4% of the root systems (p=0.075, Table 16). *Tuber* type was found at Hidden Lake and Malakwa (Table 17). The ectomycorrhizae were single to infrequently branched, stout, smooth, creamy buff colored, with abundant, needle-like, tapering setae bristling out from the mantle surface. The setae were 50-150 µm long and 3-4 µm wide. The mantle was a smooth, compact, irregular interlocking synenchyma, 10-20 µm wide. Emanating hyphae were rare, hyaline, smooth, rarely branched, 2-5 µm wide, septate, without clamp connections. Strands were absent.

Amphinema type

Amphinema type occurred on 27% of the Untrenched Douglas-fir seedlings and occupied on average 7±4% of their root tips (p=0.093, Table 16). It was found in the Untrenched treatment at Adams and Hidden Lakes (Table 17). The ectomycorrhizae were yellowish-brown, rough textured, unbranched, approximately 1 cm long. The mantle was a felt prosenchyma with abundant external hyphae. Emanating hyphae were hyaline-yellow, smooth, 2 µm wide, frequently branched with abundant, spherical clamp connections. The abundant strands were white, variable thickness up to 1 mm, with a compact core but abundant emanating hyphae.

Chubby Bubby type (H30), previously undescribed

Chubby Bubby type (H30) occurred on 40% of the Douglas-fir seedlings and occupied 7±6% of their root tips in the Untrenched treatment at Hidden Lake (Table 17). The ectomycorrhizae were dark brown with a whitish crust, rough textured, stout, up to 8 mm long and 3 mm wide. The mantle was a loose net prosenchyma with cells up to 5 µm wide. The abundant emanating hyphae were reddish brown, smooth, 5 µm wide, infrequently branched, and with abundant, flattish clamp connections. Strands were absent.

Rhizopogon-like type

Rhizopogon-like type was found only at Adams Lake in the Untrenched treatment, where it occurred on 60% of the seedlings and occupied 6±5% of the root tips (Table 17). Ectomycorrhizae were single to pinnately-branched, brown and yellowish-green. The mantle was a rough, compact, net synenchyma with moderately abundant external hyphae. Emanating hyphae were hyaline-yellowish, 1-2 µm wide, smooth, unbranched and with abundant clamp connections. Compact, reddish brown strands were abundant, 25 µm wide.

Brown Zelig type (A20), previously undescribed

Brown Zelig type (A20) type occurred on 60% of the Douglas-fir seedlings and occupied 4±3% of their root tips in the Untrenched treatment at Adams Lake (Table 17). The ectomycorrhizae were cylindrical, unbranched, brown, rough, with a loose, felt prosenchyma mantle. Emanating hyphae were abundant and either fat or thin. The fat hyphae were 5 µm wide, smooth, branched, brown with abundant spherical clamp connections. The thin hyphae were 2 µm wide, smooth, branched, black, without clamp connections. Strands were absent.

Laccaria type

Laccaria type occurred on 40% of the Douglas-fir seedlings and occupied 2±2% of their root tips in the Untrenched treatment at Malakwa (Table 17). The ectomycorrhizae were single, 8-10 mm long and 3-4 mm wide, whitish to dark brown, cottony textured. The loose, cottony mantle was a net prosenchyma of variable density. Abundant emanating hyphae were hyaline to light brown, 3-4 µm wide, moderately branched, tortuous, verrucose, with abundant clamp connections 2-4 µm wide. Loose cottony strands were present.

Cenococcum-like type (H28), previously undescribed

Cenococcum-like type (H28) was found on one seedling only in the Untrenched treatment at Hidden Lake; it occupied <1% of the seedling's root tips (Table 17). The ectomycorrhizae were cylindrical, unbranched, up to 2 mm long, black, with a rough texture under abundant emanating hyphae. The mantle was compact, black, irregular non-locking net synenchyma, with a stellate-layered pattern. Emanating hyphae were black-brown-pinkish, verrucose, 5 µm wide, septate, with moderately frequent oblique branching, and without clamp connections. Strands were absent.

Laccaria-like type (H29), previously undescribed

Laccaria-like type (H29) was found on one seedling only in the Untrenched treatment at Hidden Lake; it occupied 1% of the seedling's root tips (Table 17). The ectomycorrhizae were single, 2 mm long and <1 mm wide, light brown to purple-pinkish with a rough-like texture. The outer mantle was felt prosenchyma and the inner mantle a net synenchyma. Abundant emanating hyphae were hyaline, smooth, 2-3 µm wide, moderately frequently and obliquely branched, with abundant round clamp connections 2 µm wide. Strands were absent.

Yellow-tip Grizzly type (A21), previously undescribed

Yellow-tip Grizzly type (A21) was found only at Adams Lake where it occurred on one seedling in the Untrenched treatment; it occupied <1% of the root system (Table 17). The ectomycorrhizae were unbranched, smooth, brown with a distinctive yellow tip. The compact mantle was a felt prosenchyma with moderately abundant external hyphae. Emanating hyphae were hyaline, smooth, unbranched, 2-3 µm wide, with frequent, round clamp connections. Strands were absent.

Lactarius pubescens type

Lactarius pubescens type was found only at Adams Lake where it occurred on one seedling in the Untrenched treatment; it occupied <1% of the root system (Table 17). Ectomycorrhizae were light brown to yellowish, smooth, swollen, long and tortuous, with frequent, irregularly spaced branches. The compact mantle was a net synenchyma with lactiferous hyphae up to 10 µm wide. Emanating hyphae were abundant, 2 µm wide, hyaline, smooth, unbranched, with rare, flattish clamp connections. Strands were white-yellow with a compact organization.

Ectomycorrhizal types unique to Trenched plots

Craigallechie type (M26), previously undescribed

Craigallechie type (M26) occurred on 60% of the Douglas-fir seedlings and occupied 13±9% of their root tips in the Trenched treatment at Malakwa (Table 17). The ectomycorrhizae were smooth, pinnately branched, light to dark brown with a thin, compact, felt prosenchyma mantle. Emanating hyphae were moderately abundant, hyaline, smooth, 2 µm wide, with frequently, oblique branching and moderately abundant spherical clamp connections. Strands were absent.

Humaria type

Humaria type was found on one seedling only in the Trenched treatment at Adams Lake, where it occupied <1% of the seedlings root tips (Table 17). The *Humaria* type is referred to as E-strain by Ingleby *et al.* (1990). The ectomycorrhizae were single, rough, dark brown with a pale tip. The mantle was discontinuous, compact, irregular synenchyma lock, with large (up to 8 µm wide) hyphal cells. Emanating hyphae were few, hyaline, 4-5 µm wide, minimally branched, without clamp connections. Strands were absent.

Last Spike type (M27), previously undescribed

Last Spike type (M27) was found on one seedling only in the Trenched treatment at Malakwa, where it occupied <1% of the seedling's root tips (Table 17). The ectomycorrhizae were smooth, unbranched, dark brown with a loose, up to 25 µm thick mantle that was irregular synenchyma non-locking at the tip and irregular synenchyma locking behind. Emanating hyphae were abundant, dark brown to hyaline, smooth, unbranched, 3 µm wide, with abundant hemispherical, flattish clamp connections. Strands were loose, dark tawny, 10-15 µm thick.

Chapter 7

Summary and Conclusions

This study investigated influences of ectomycorrhizal (EM) associations and interspecific carbon transfer on tree seedling performance, with the overall goal of advancing out ability to assess effects of species manipulations on productivity of mixed forests. The general objectives of the study were: *(i)* to determine the potential for EM fungi to form interspecific hyphal links between paper birch and Douglas-fir, *(ii)* to quantify gross and net carbon transfer between paper birch and Douglas-fir seedlings in the laboratory and under a range of environmental conditions in the field, and *(iii)* to evaluate the importance of the transfer phenomenon to seedling performance in species mixtures.

Issues addressed

The study addressed several controversial issues regarding interplant carbon transfer that recently have been debated in the literature (e.g., Newman 1988, Miller and Allen 1992). First, we found large overlap in EM morphotypes associated with paper birch and Douglas-fir, providing some evidence to support the hypothesis of Newman (1988) and Molina *et al.* (1992) that mycorrhizal linkages among plants are common. Second, we found that carbon transfer between paper birch and Douglas-fir was bi-directional, supporting Newman's (1988) assertion that results of one-way labeling studies do not necessarily prove net movement. Third, we measured net transfer of carbon isotope from paper birch to Douglas-fir in both the laboratory and the field, representing the first reports of net transfer between plants. Fourth, the amount of carbon transferred was substantially larger than that measured in previous studies, but was comparable to the amount of nitrogen transferred from alder to pine in the laboratory study of Arnebrant *et al.* (1993). This may be explained by the steep photosynthetic and foliar nutrient gradients that existed between paper birch and Douglas-fir. The extent and direction of carbon transfer may have been influenced by both photosynthate and nitrogen source/sink relationships between paper birch and Douglas-fir, providing some support to the hypothesis that carbon is transferred in amino acids primarily along a nitrogen concentration gradient (e.g., Smith and Smith 1990, Arnebrant *et al.* 1992). Fifth, substantially more isotope (>20% of fixed isotope) was translocated into shoots of receiver plants than has been observed in previous studies (<10% of fixed isotope, Newman 1988), which may significantly supplement carbon in photosynthate, and/or foliar nitrogen if carbon is transferred in amino acids. Similar amounts of carbon isotope transferred from parent plants into shoots of connected ramets plants have previously been suggested to increase survivorship and growth (Bradbury and Hutchings 1986, Alpert *et al.* 1991). Sixth, the amount of carbon isotope

transferred was compared to the amount fixed by photosynthesis by both paper birch and Douglas-fir, representing the first attempt at evaluating potential importance of interspecific carbon transfer to plant performance (Newman 1988). Finally, we found that mycorrhizal inoculation of Douglas-fir seedlings by overstory trees was important to seedling performance, suggesting that hyphal linkages play a significant role in plant community dynamics. Greater detail on results of individual chapters follow.

Chapter synthesis

The ability of paper birch and Douglas-fir to share compatible EM fungi, and thereby potentially form hyphal linkages, was investigated in a soil bioassay described in Chapter 2. Occurrence of EM fungi on paper birch and Douglas-fir seedlings was compared between monoculture and dual culture treatments to evaluate the influence of neighboring seedlings on EM development. Eleven EM morphotypes were recognized, seven of which paper birch and Douglas-fir shared in common over 90% of their root tips, suggesting high potential for interspecific hyphal connections. In addition, the number of common morphotypes and percent of root tips colonized by common morphotypes were slightly greater in dual culture than in monoculture, indicating that companion seedlings influenced connection potential. That interspecific genetic compatibility of common morphotypes was not tested using molecular tools, however, leaves in question the ability for the two tree species to form functional hyphal links. In addition, less than 5% of the root tips of both paper birch and Douglas-fir were colonized by EM that formed strands, which are viewed as important conduits for carbon and nutrient transfer (e.g., Duddridge *et al.* 1988, Cairney 1992).

Quantifying carbon transfer between paper birch and Douglas-fir in the field required that we develop a flexible pulse-labeling procedure in the laboratory that resulted in rapid and substantial enrichment of isotope in fine roots of both species. In Chapter 3, we modified the labeling procedures of Svejcar *et al.* (1990), and tested a range of $^{13}CO_{2(gas)}$ pulse and chase treatments on one-year-old seedlings to identify the appropriate pulse-chase regime for examining intra- and interspecific carbon distribution patterns. We found that the 100-mL $^{13}CO_{2(gas)}$ pulse and 6-d chase were appropriate for these purposes, resulting in ^{13}C root/shoot ratios of 0.00 immediately following the pulse and 0.87 six days later. After 6 d, paper birch had allocated on average 49% and Douglas-fir 41% of fixed ^{13}C to roots, of which over 55% occurred in fine roots in both species. We then tested the procedure for its utility in examining interspecific carbon transfer between paper birch and Douglas-fir growing in laboratory rootboxes. We found that 4.7% of excess ^{13}C fixed by paper birch was transferred to neighboring Douglas-fir, which distributed the isotope evenly between roots and shoots. Since roots of paper birch and Douglas-fir were intimately mingled in this experiment, amount transferred via different belowground pathways (e.g., root grafts, mycorrhizal linkages, or soil pool) was not possible to distinguish. In addition, since we

labeled only paper birch, we were able to assess only one-way transfer. We could not determine whether some isotope was also transferred in the reverse direction, from Douglas-fir to paper birch, and thus could not evaluate whether net transfer occurred.

In Chapter 4, we addressed the net transfer issue in a laboratory rootbox study. We used the labeling procedure to pulse-label pairs of six-month-old EM paper birch and Douglas-fir seedlings growing in individual, root-restrictive pouches filled with field soil. Interspecific carbon transfer was examined by labeling one seedling with $^{13}CO_{2(gas)}$ and its neighbor with $^{14}CO_{2(gas)}$, and then examining the amount of each isotope in each seedling after 6 d. The amount of carbon transferred directly through EM connections versus indirectly through the soil pool was examined by comparing rootboxes where interconnecting hyphae were left intact versus where they were severed immediately prior to labeling. We found that transfer between paper birch and Douglas-fir was bi-directional, but there was on average a net carbon gain by Douglas-fir ($p=0.10$). Net carbon transfer to Douglas-fir represented on average 4% of the total isotope assimilated by the rootbox system, 10% of the isotope assimilated by Douglas-fir alone, and 7% of that assimilated by paper birch alone. These values exceeded one-way mycorrhizal-mediated ^{14}C transfer measured in previous studies (e.g., Francis and Read 1984), but were comparable to one-way ^{14}C transfer between connected ramets of clonal plants (Alpert *et al.* 1991) and to one-way EM-mediated ^{15}N transfer from alder to pine (Arnebrant *et al.* 1993).

Mean values for net transfer were approximately three times greater where hyphae were intact than severed ($p=0.28$). Whether carbon isotope was translocated directly through EM fungi or indirectly through the soil pool remained equivocal because the treatment differences were not significant and because the functional status of hyphal connections between seedlings in unsevered rootboxes was not rigorously tested using autoradiography. Unidirectional gross transfer to Douglas-fir was 50% greater in unsevered than severed rootboxes ($p=0.15$), however, indicating an 85% probability that hyphal connections facilitated transfer from paper birch to Douglas-fir. In contrast, unidirectional gross transfer to paper birch was independent of severing treatments. These results suggest that Douglas-fir received isotope from paper birch directly through interconnecting fungi as well as the soil pool, whereas paper birch received all of its isotope from Douglas-fir simply through the soil pool.

In Chapter 5, we tested whether net transfer measured in our rootbox study reflected the magnitude of carbon transfer that occurs in the field. We applied the isotope labeling procedures in the field in 1993 and 1994 to quantify *(l)* carbon isotope translocation among paper birch, Douglas-fir and western redcedar seedlings, and (b) the effect of shading (5%, 50% and 100% of ambient sunlight) on amount of carbon isotope transferred. We found that out-planted western redcedar formed only AM and paper birch and Douglas-fir formed only EM, so western redcedar was effective in signaling isotope transferred indirectly between seedlings via the soil pool. As in the rootbox study (Chapter 4), transfer between paper birch and Douglas-fir was bi-directional, but

there was a net carbon gain by Douglas-fir in the 100% sun treatment (but 0 net transfer in the 5% and 50% sun treatments) in 1993, and in all three shading treatments in 1994. Gross transfer and net transfer represented 4% (averaged over all shading treatments) and 2% (full sun only), respectively, of the total isotope assimilated by both species in 1993, and increased to 7% and 6% (both averaged over all shading treatments), respectively, in 1994. In comparison, gross and net transfer in the rootbox study represented 29% and 4% of total isotope assimilated by both species. Greater gross transfer in the rootbox than field may have been due to closer proximity and entrainment of roots, as well as containment of all isotopes within the narrow confines of the rootbox. Transfer to western redcedar represented on average <1% of gross transfer between paper birch and Douglas-fir in 1993, and 18% in 1994, suggesting that most isotope transferred between paper birch and Douglas-fir occurred via EM fungi.

Net transfer to Douglas-fir occurred only in full sun in the field experiment of 1993, but in all three shading treatments in 1994. In 1994, net isotope transfer to Douglas-fir was approximately two times greater in 5% sun than the 50% or 100% sun treatments. Similarly, shading had no effect on gross transfer in 1993, but was two times greater in 5% sun than the 50% or 100% sun treatments in 1994. This was due to increased one-way transfer from paper birch to Douglas-fir, but not in the opposite direction, suggesting that transfer was affected by changes in sink strength of Douglas-fir. Factors which may have been important to the change in shading effect on extent and direction of net transfer between 1993 and 1994 included greater extent of root development, improved seedling physiological vigor, later seasonal timing of the experiment, and more favorable micro climatic conditions for photosynthesis and translocation in 1994 than 1993.

In combination, results of the rootbox and field studies provided support for a source/sink mechanism for carbon transfer between paper birch and Douglas-fir. Others have suggested that direction and extent of interplant transfer is influenced by source/sink relationships, such as those established by differences in net photosynthetic rate, nutrient status, or capability of fixing atmospheric N_2 (e.g., Read *et al.* 1985, Ritz and Newman 1986, Arnebrant *et al.* 1993). In our studies, net transfer from paper birch to Douglas-fir coincided with whole seedling net photosynthetic rates which were up to 11 times greater and foliar nitrogen concentrations which were approximately two times greater for paper birch than Douglas-fir. The greater foliar nitrogen content may have resulted from greater associative nitrogen fixation rates of paper birch than Douglas-fir (Simard and Li, unpublished data).

In the field experiments, decreased net photosynthetic rates of Douglas-fir under full shade may have resulted in reduced assimilate supply to its roots and thus increased the assimilate concentration gradient between paper birch and Douglas-fir. Because foliar nitrogen concentration is positively correlated with net photosynthetic rate (e.g., Brix 1981, Wang *et al.* 1995) due to enzyme (especially RUBISCO) demands for nitrogen during photosynthesis, lowered photosynthetic rates may also have operated to steepen the interspecific foliar nitrogen gradient.

The link between carbon and nitrogen in driving extent and direction of transfer becomes clearer in view of the hypothesis of Smith and Smith (1990) that carbon is combined with nitrogen (and other nutrients) and transferred between connected plants through mycorrhizal fungi as amino acids. In fact, labeled amino acids have been shown to pass directly from mycorrhizal fungi into the xylem sap of host plants (Abuzinadah and Read 1989), whereas simple sugars have not (Smith and Smith 1990). In the rootbox and field studies, the *(i)* photosynthate and foliar nitrogen gradient between paper birch and Douglas-fir, *(ii)* similar proportion of fixed carbon transferred compared to fixed nitrogen transferred in the alder-pine system of Arnebrant *et al.* (1993), and *(iii)* translocation of substantial carbon into receiver foliage, together suggest that assimilated carbon was incorporated into amino acids and translocated between plants along a carbon-nitrogen gradient from paper birch to Douglas-fir. That fully developed leaves are usually strong sinks for nitrogen, and are sources rather than sinks for carbon (Pearcy *et al.* 1987), suggests that interplant carbon transfer may be driven more by a nitrogen rather than a carbon concentration gradient.

Nitrogen and other foliar nutrients did not appear limiting to paper birch, and some may have been in the luxury range, suggesting that organic nutrient transfer to neighboring Douglas-fir should not adversely affect performance of paper birch. In contrast, slight nutrient deficiencies in Douglas-fir may signal a potentially important benefit of transfer from paper birch, particularly during periods of nutrient stress. Based on the Mitscherlich curve of diminishing returns, transfer of organic nutrients via mycorrhizal fungi from nutrient-rich paper birch would result in greater gain by nutrient-poor Douglas-fir neighbors than the relative magnitude of birch's own requisite loss (c.f. Perry *et al.* 1992).

In Chapter 6, we examined the ability of overstory trees to influence EM inoculation patterns and performance of Douglas-fir seedlings growing in the understory. Others have suggested than companion hardwoods may influence formation of mycorrhizal associations of neighboring seedlings through contact between their emanating hyphae and seedling roots, and subsequent induction of colonization (e.g., Fleming 1983, Borchers and Perry 1990). Douglas-fir seedlings were grown for six to 16 months in untrenched and trenched treatments in three 90-120 year-old mixed forests of paper birch and Douglas-fir. Mean richness, diversity, and evenness of EM associates per seedling were approximately twice as great in the untrenched than trenched treatment. Of types that formed strands or rhizomorphs, eight occurred in the untrenched treatment where they occupied on average 23% of root tips, and only four occurred in the trenched treatment over 4% of the root tips. In addition, net photosynthetic rate of Douglas-fir seedlings was greater in the untrenched than trenched treatment in July and August, 1994. The effect of trenching on seedling performance was attributed mainly to differences in EM colonization patterns, because trenching had no effect on soil water content, soil nutrient concentrations, or light availability. These results suggested that influence of overstory trees and pattern of EM formation were important to seedling performance in the deeply shaded forest environments. We speculated

that fungi compatible and mycorrhizal with overstory Douglas-fir, paper birch, and other neighboring EM trees may have contacted, colonized and physiologically supported the stressed understory Douglas-fir seedlings, possibly through carbon or nutrient transfer.

Suggestions for improvement

Each of the five studies had room for improvement. In Chapter 2, the potential for hyphal connections between paper birch and Douglas-fir could have more conclusively been demonstrated by comparing DNA profiles of the shared morphotypes. In addition, the effect of resource competition on EM colonization patterns may have been clearer if paper birch and Douglas-fir had been grown in a replacement series design (i.e., two paper birch seedlings per tube, two Douglas-fir seedlings per tube, one paper birch seedling plus one Douglas-fir seedling per tube) rather than an additive design (Harper 1977). In Chapter 3, the effect of pulse on carbon allocation patterns may have been better understood if we'd monitored ambient CO_2 levels in the labeling chambers during the pulse period, as had been done in previous ^{13}C pulse-labeling studies (e.g., Kouchi and Yoneyama 1984, Svejcar *et al.* 1990). Several improvements could have been made to the interspecific ^{13}C transfer experiment of Chapter 3 (e.g., separation of interconnecting hyphae from other transfer pathways, dual-labeling of both paper birch and Douglas-fir to assess net transfer), which were later applied in Chapter 4.

In Chapter 4, the pathway of carbon transfer between paper birch and Douglas-fir in the rootboxes may have been clearer with *(i)* greater replication of severed and unsevered treatments, *(ii)* repeated severing of hyphal links through the entire chase period, and *(iii)* examination of hyphal interconnections using autoradiography. In addition, net transfer estimates may have been more precise if we had used similar amounts of ^{13}C and ^{14}C with which to pulse-label seedlings. The field study (Chapter 5) was an adventure, which by definition involved unpredicted twists and required continual trouble shooting. First, the source/sink gradient between paper birch and Douglas-fir was affected by poor vigor and deer browsing of paper birch seedlings. This could have been avoided by planting seedlings earlier in 1992, when soil water was closer to field capacity, and by protecting seedlings from browsers and curious bears. The source/sink gradient also could have been steepened by more prolonged shading of Douglas-fir prior to labeling. Second, differences in behavior of the stable and radioactive isotopes would have been avoided by simply using a single isotope applied to different species in paired seedling groups (e.g., Jakobsen 1991). The disadvantage to this approach, however, is added error associated with physiological differences between paired groups. Alternatively, results of reciprocal labeling with ^{13}C and ^{14}C may have been improved by using similar quantities of the two isotopes (as in the rootbox study). Third, the labeling experiment of 1994 should have included reciprocal labeling treatments, as was done in 1993. Application of the 1993 multipliers to the 1994 data was difficult due to the change in relative

labeling efficiencies of ^{13}C and ^{14}C. Finally, as with the rootbox study, hyphal connections as a transfer pathway could have more definitively been identified either by tracing hyphal strands through the soil (c.f. Miller and Allen 1992) or by applying autoradiography techniques in the field (c.f. Vose 1980).

Considerably more information may have been derived from Chapter 6 with a few changes. First, overstory trees could have more clearly been identified as sources of inoculum for understory seedlings if their EM associates also had been identified. Second, the trenching treatments could have been applied in pure stands of paper birch and Douglas-fir, rather than in mixtures, to determine the effect of overstory species on understory seedling colonization patterns. Third, Douglas-fir seedlings should have been protected from browsing critters to avoid the additional error associated with seedling age differences between Adams Lake and the other two sites. Fourth, the effects of soil trenching on nutrient and water availability would have been more convincing if, in addition to soil nutrient concentrations and water content, we'd measured foliar nutrient concentrations and seedling transpiration rates. Additional replicate seedlings are still growing in the trenching treatments, and the second harvest will include as many of these additional measurements as possible.

Further research

This thesis represents but a tiny contribution toward our overall understanding of interactions among host tree species, mycorrhizal fungi, and the environment. The main contribution of the work, that net transfer of carbon occurs between plants in substantial quantities, needs to be expanded to determine its role in plant community dynamics and the ecosystem as a whole. We see six interrelated directions as priorities for further research.

(1) Long-term effects of carbon transfer on plant fitness need to be studied in native plant communities in the field, which are characterized by considerably more spatial and temporal complexity than is encountered in the laboratory or typical experimental field plots. Because most native plant communities are comprised of species mixtures, this necessarily will involve carbon transfer effects on interspecific interactions and mixed community development. The well-known hypothesis, that interspecific carbon transfer may decrease interference and enhance species diversity (e.g., Newman 1988, Perry *et al.* 1989, Perry *et al.* 1992), needs to be tested in the field.

(2) Our field study indicated that carbon transfer is a variable process that is affected by plant age, root development and season (plant phenology). Although we measured net transfer from paper birch to Douglas-fir in mid-summer, the direction may reverse in early spring and late fall when paper birch is not foliated. In addition, the importance of transfer to plant fitness may change with over time and under different environmental conditions. Further research is needed to assess the importance of interplant connections on plant fitness under varying circumstances (e.g.,

different seasons, species, successional stages, site qualities (water, light, nutrients), disturbance regimes).

(3) The importance of hyphal links and carbon transfer to ecosystem recovery following disturbance must be better understood to facilitate efficacy of conservation programs. For example, understanding importance of hyphal connections on persistence of species planted into different successional stages may help us stabilize ecosystems against unprecedented changes in the future.

(4) More knowledge is needed on the mechanisms which underlie carbon and nutrient transfer so that reforestation techniques can be modified to strengthen rather than weaken the pathways. Research is needed on role of hyphal links versus the soil pool as pathways for transfer; on different fungal species or functional groups most involved in transfer; on the interrelated roles of carbon, nitrogen and other nutrients in driving transfer; and on the role of associative nitrogen fixers and other rhizosphere organisms in mediating material transfer.

(5) Finally, we must better understand the impacts of severing the interspecific links on resilience of ecosystems. For example, does removal of paper birch from Douglas-fir plantations through planting and weeding programs reduce vigor of Douglas-fir by reducing interspecific hyphal linkages or mycorrhizal/microbial inoculation potential? Does this then impact the resistance of the community as a whole against generalist pathogens such as *Armillaria* and *Phellinus* root diseases?

Conclusions

The main conclusions drawn from this study were:

(1) There is considerable overlap in EM morphotypes between paper birch and Douglas-fir, indicating high potential for hyphal interconnection.

(2) Net transfer occurs from paper birch to Douglas-fir along photosynthate and nitrogen concentration gradients, when both species are fully foliated in mid-summer.

(3) Gross and net carbon transfer represented 7% and 6% of total isotope fixed by paper birch and Douglas-fir in the second field season, representing substantial interspecific belowground exchange. The magnitude of carbon transferred indicates that interspecific interactions between paper birch and Douglas-fir involve more dimensions than resource competition alone.

(4) There is considerable evidence from the rootbox and field experiments combined for a mycorrhizal-mediated carbon transfer pathway; however, further research is necessary to unfold the full complexity of the pathway.

(5) Overstory trees strongly influenced EM inoculation patterns and physiology of understory Douglas-fir seedlings, suggesting an important role of residual trees (legacies) in plant community dynamics.

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