GREEN-TREE-RETENTION HARVESTING AS A TOOL TO MAINTAIN SOIL MICROBIAL DIVERSITY AND FUNCTION IN HARVESTED FORESTS

by

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Abstract

Green-tree or variable-retention harvesting is increasingly being used in the Pacific Northwest due to the perceived benefits to aboveground biodiversity. Little research has been conducted on the value of this harvesting practice for soil organisms, though retained live trees on harvested sites are thought to benefit belowground biodiversity by acting as “hubs” of both species-specific and symbiotic microbial communities. Access to these communities may be necessary for seedling growth and forest regeneration after harvest. Live trees support microbial communities by maintaining a constant source of labile carbon through litter and root exudates. The aim of this thesis dissertation project was to trace the flow of carbon from live trees retained on clear-cut sites in different variable-retention harvesting regimes into the soil microbial community to determine which variable-harvesting regime best maintained pre-harvest soil microbial communities and soil microbial function. Two variable-retention strategies were compared: aggregate-retention, where intact forest patches are retained, and dispersed-retention, where individual trees are retained across the site. Both harvesting strategies decreased the fungal to bacterial ratio although the dispersed-retention harvesting treatment mitigated the effects of harvesting on soil nutrient availability. Aggregate-retention harvesting, even within 9 meters of the retention patch, did not appear to influence nutrient availability, but evidence suggested the microbial community within this area was supported by recent plant-carbon. Through analysis of stable-isotope natural abundance and application of a novel stable-isotope labeling stem-injection technique, I was able to discern that individual trees support the fungal community up to 20 meters into a clear-cut. However, the lack of recently-derived labile plant carbon in clear-cuts resulted in changes to soil carbon-cycling. The microbial community in clear-cut sites appeared to rely on tightly recycled labile microbial-derived carbon that was probably released during microbial turnover, rather than dissolved organic carbon. In the highly disturbed clear-cut areas, the microbial communities may have lost some of their ability to break down recalcitrant soil organic matter. Both variable-retention strategies investigated affected soil microbial community composition; though it appeared that dispersed-retention best maintained microbial community function on harvested sites.
Preface

Chapters 2 to 5 of this thesis were written as a series of manuscripts for publication in peer-reviewed journals. For Chapters 2 to 5 I designed the research questions, designed the studies and collected, processed and analyzed all soil samples. I also processed and analyzed all PLFAs, soil respiration, DOC, and SOM samples as well as calculated the $\delta^{13}\text{C}$ values of each. For all research Chapters I took the lead in writing the draft manuscripts, in addition to all the tables and figures. My co-author Per Bengtson carried out the PC analysis in Chapter 2, the geostatistical analysis in Chapter 3, and the PLFA extraction in Chapter 5, as well as provided assistance with data interpretation, and editing in Chapters 2 and 3. Cindy Prescott provided assistance with data interpretation, and editing of Chapter 2. The stem-injection technique described in Chapter 4 was co-designed with Andrew Weatherall. Maria J. Briones conducted the faunal collection and identification for Chapter 4, and provided assistance with editing. My supervisor, Sue Grayston, provided assistance with proposal development, experimental design, data interpretation and editing throughout the thesis.

A version of Chapter 2 has been submitted for publication: Churchland C, Bengtson P, Prescott C, Grayston SJ. 2013. Dispersed variable-retention harvesting mitigates N losses on harvested sites despite changes in soil microbial community structure.


A version of Chapter 5 will be submitted for publication as: Churchland C, Bengtson P, and Grayston SJ. In situ priming effects along a gradient of forest soil disturbance.
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Appendix B. This figure shows the major components of the C budget in forest ecosystems, and the partitioning of gross primary production into aboveground biomass (annual net primary production of wood/ foliage) aboveground respiration (wood, foliage) and total belowground C.
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List of Abbreviations, Acronyms and Symbols

‰  Per mil
δ  Delta
C  Carbon
CLPP  Community-level physiological-profiling
CWD  Coarse woody debris
DGGE  Denaturing gradient gel electrophoresis
DNA  Deoxyribose nucleic acid
DOC  Dissolved organic carbon
ECM  Ectomycorrhizae
FACE  Free-air CO₂ enrichment
GPP  Gross primary production
GTR  Green-tree-retention
N  Nitrogen
NPP  Net primary production
OTUs  Operational taxonomic units
P  Phosphorus
PCA  Principle component analysis
PCR  Polymerase chain reaction
PDB  PeeDee belemnite
PGPB  Plant-growth promoting bacteria
PLFA  Phospholipid fatty-acid
PRS  Plant-root simulator probes
PVC  Polyvinyl Chloride
rRNA  Ribosomal ribose nucleic acid
SOC  Soil organic carbon
SOM  Soil organic matter
STEMS  Silviculture treatments for ecosystems management in the Sayward
T-RFLP  Terminal restriction fragment length polymorphism
VPDB Vienna PeeDee belemnite
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To my parents,

Thank you for inspiring me
1 Introduction and objectives

Clear-cut logging was once the dominant harvesting practice in coastal British Columbia. However, due to the poor visual appearance of clear-cut sites and increased public knowledge of the impacts of clear-cut harvesting on aboveground biodiversity, green-tree-retention (GTR) harvesting (also known as variable-retention harvesting or just retention harvesting) has become the prevailing practice in the Pacific Northwest (Gustafsson et al. 2012). Green-tree-retention harvesting has been found to retain greater aboveground biodiversity compared to clear-cutting (Rosenvald and Lõhmus 2008), yet the ability of retained trees to maintain pre-harvest belowground communities remains unclear, and is considered a key knowledge gap in variable-retention forestry (Gustafsson et al. 2012). The purpose of this thesis was to explore the impact of two GTR practices, aggregated-retention (where patches of trees are retained) and dispersed-retention (single-tree-retention), on the spatial variability of belowground microbial and faunal community structure, ecosystem carbon (C) flow and nutrient availability.

The process of tree harvesting affects the soil microbial and faunal community through the reduction of organic matter inputs, the compaction and mixing of soil layers, and changes in ground vegetation and canopy cover. These factors, in turn, affect the distribution of soil organic matter (SOM), soil porosity, water content, temperature, pH and nitrogen (N) availability (Jones et al. 2003), which, consequently, can affect soil microbial biomass, microbial C to N ratios, microbial activity, and microbial community composition (Marshall 2000). Free-living soil microbes control an array of functional processes (Waring and Sclesinger 1985; Prescott and Vesterdal 2005; Paul 2007), but their major function is nutrient cycling, mineralizing nutrients through the breakdown of SOM (Prescott and Vesterdal 2005). Plants are able to take up mineralized SOM directly, but are more likely to obtain the nutrients through their symbiotic associations (Paul 2007). Mycorrhizal fungi and some bacteria form symbiotic relationships with the roots of plants, which may be necessary for acquisition of nutrients such as N and for successful growth of seedlings. Retained trees on clear-cut sites are thought to act as ‘life boats’ or ‘hubs’ for these symbiotic microbes, maintaining soil microbial diversity and acting as a source of inoculum for seedlings (Franklin et al. 1997). Retaining trees on harvested sites, thus
maintaining a source of species-specific symbiotic microbes in the soil, ensures seedlings have access to microbial communities necessary for growth and survival.

In order to determine the influence of retention trees left during the practice of GTR harvesting on belowground communities and soil processes, the flow of C from these trees into the surrounding soil microbial and faunal community must be determined. Trees supply C to the soil microbial community via litter, coarse woody debris and root exudates (Högberg et al. 2001; Harmon et al. 2004), but the spatial and temporal distribution of this C in the ecosystem is unclear. Successfully observing C flow from retained trees on clear-cut sites into the soil microbial community and characterizing which groups of microbes depend on recently photosynthesized C will allow us to determine if GTR is a useful tool to retain pre-harvest microbial communities on harvested forest sites.

1.1 Soil microbial communities and activity in forests

Soil can support a highly complex and diverse array of bacterial and fungal communities. A single gram of forest soil can contain up to 53 000 bacterial operational taxonomic units (OTUs), representing thousands of species over a broad range of phyla (Roesch et al. 2007). Many of these species are thought to be functionally redundant, and this redundancy may help ensure ecosystem resilience to disturbance (Torsvik et al. 1998). However, the diversity of the soil microbial community makes it very challenging to study, especially given that approximately 1% of soil bacteria can be cultured (Torsvik et al. 1998). Techniques for studying soil microbial diversity can be broadly categorized into biochemical and physiological-based methods and molecular-based methods. Biochemical and physiological methods include techniques such as community-level physiological-profiling (CLPP) where soil respiration is used as a measure of C substrate-use and phospholipid fatty acid (PLFA) analysis is used to characterize the microbial community at the phenotypic level (Leckie 2005; Table 1.1). Molecular, PCR-based methods include techniques that determine members of the microbial community to the species level, such as DNA microarrays, denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphisms (T-RFLP; Kirk et al. 2004; Table 1.1). Each technique has advantages and disadvantages; the most appropriate method will depend on the nature of the
study. Interestingly, in a study of grassland soils, Grayston et al. (2004) observed that the most popular techniques—CLPP, PCR-based methods and PLFA analysis—did not differ in their ability to differentiate between grasslands varying in management intensity. However, PLFA analysis, which assesses broad-scale microbial community structure, was found to be a more sensitive technique (Grayston et al. 2004), and is thought to be the most powerful approach for demonstrating changes in the microbial community structure (Ramsey et al. 2006). As PLFA analysis is not able to detect subtle changes in specific microbial populations, it is not always the most appropriate method of microbial community analysis. My study explored how two different variable-retention regimes, aggregated and dispersed, affect the microbial community structure through their supply of C sources to the community. Because I was interested in how the microbial community changes as a whole and to track how C flows from trees directly into the microbial community, PLFA analysis was the most appropriate and cost-effective method, and was used to measure the effects of the different harvesting regimes on microbial community structure.

Tree species have selective effects on mycorrhizae and free-living microorganisms in their rhizosphere (Shi et al. 2012; Pires et al. 2012). Depending on the tree species, numbers of ectomycorrhizal (ECM) associates can range from 50 ECM (e.g. alder; Pritsch et al. 1997) to 2,000 ECM (e.g. Douglas-fir, Molina et al. 1992). Trees are thought to recruit mycorrhizae through distinct chemical signals exuded from their roots that trigger gene activation, initiating a response of hyphal growth towards the plant root and subsequent development of symbiotic tissue such as short roots and the Hartig net (Martin et al. 2008; Podila et al. 2009). Like trees, many ECM have broad host range (e.g. Lactarius; Kennedy et al. 2003), whereas others will have only narrow host range (e.g. Suillus; Bruns et al. 2002). Trees support the ECM and soil rhizosphere microbial community through root exudates. The composition and total amount of root exudates varies among tree species (Martin et al. 2008; Tuason and Arocena 2009), and varies within a given tree species depending on which ECM colonize the tree roots (Sandnes et al. 2005; van Hees et al. 2005). Different ECM can increase organic-acid exudation (Sandnes et al. 2005; van Hees et al. 2003; 2005; Johansson 2009) and change organic-acid composition (van Hees et al. 2005; Klugh and Cumming 2003). Differences in root exudation among different tree species is due, in part, to differences in exudation rates from ECM hyphae, creating an area of
greater microbial biomass and activity, termed the ectomycorrhizosphere or hyphosphere (Jones et al. 2004; Frey-Klett et al. 2007; Finlay 2008; Tedersoo et al. 2009; Nazir et al. 2010; Figure 1.1). Frey-Klett et al. (2005) examined the mycorrhizosphere of Scots pine inoculated with \textit{L. bicolor} ECM fungi. The ECM formed a distinct habitat relative to the bulk soil that was host to different bacterial populations. Despite bacteria and archaea being ubiquitous in the rhizosphere and ectomycorrhizosphere, their roles in ecosystem processes are only beginning to be understood.

Plant growth-promoting bacteria (PGPB), which are found in the rhizosphere and mycorrhizosphere, are thought to benefit the plant by: creating biofilms that protect the root against pathogens (Akhtar and Siddiqui 2009), inducing systemic acquired resistance (preparing the plant for attack; Pieterse et al. 2003), enhancing plant growth, increasing plant nutrient uptake (Adesemoye et al. 2008), and improving abiotic stress tolerance (Yang et al. 2009).

Traditionally, it has been difficult to study rhizosphere microbial communities \textit{in situ}. Grayston and Campbell (1996) observed microbial communities in the rhizosphere using C utilization analyses (CLPP techniques) and found that hybrid larch and Sitka spruce had distinct microbial communities in their rhizosphere. Golinska and Dahm (2011) used 16S rRNA analysis to characterize microorganisms from the rhizosphere and bulk soil of mature stands of silver birch, Scots pine and black alder. They found the greatest number of eubacteria associated with alder (bulk soil, rhizosphere and rhizoplane), and the smallest number with pine; whereas actinomycetes were most numerous in the birch rhizosphere. Similarly, Grayston (2000) observed distinct C-utilization profiles in rhizoplane communities under larch, Sitka spruce and sycamore trees. Shi et al. (2011), using reverse-transcribed 16S rRNA genes, presented evidence that the structure of the active microbial community in the rhizosphere of radiata pine was affected by organic acids released by roots. Furthermore, Shi et al. (2012), using rRNA DGGE, found significant differences in the microbial communities (specifically alpha proteobacteria and pseudomonads) in the rhizosphere of genetically modified radiata pine vs. wild type radiata pine grown under controlled conditions in a biotron. These studies attributed tree species microbial community differences to differences in root exudates and hypothesized that the trees were ‘selecting’ the rhizosphere microbial communities. Evidence for root-exudate specificity is not limited to temperate tree species; it has been seen in other ecosystems such as mangrove forests.
(Gomes et al 2010; Pires et al 2012), grasslands (Grayston et al 1998, 2001) and arable systems (Neal et al 1973).

1.2 Soil faunal activity in relation to forest nutrient cycling

Soil faunal grazing on mycorrhizal fungi (Coleman 2004) in forests affects C flow into the mycorrhizosphere. Since fauna can preferentially feed on distinct mycorrhizae and organic matter fractions (Klironomos and Kendrick 1996; Schmidt et al. 2004), faunal impacts will likely vary depending on mycorrhizal species abundance. Soil faunal groups are often defined by size – micro, meso and macro – though their functional role in decomposition and nutrient cycling may be a more appropriate means of grouping. Microfauna consist of protozoa and nematodes, both of which are important for breaking down organic matter as well as mobilizing nutrients indirectly though controlling microbial populations (Paul 2007).

Soil mesofauna play an important role in nutrient cycling. Although the term mesofauna covers a broad range of taxa, including rotifers, tardigrada, pauripods, insects, and diplura, it is the numerically dominant enchytraeids and microarthropods, specifically mites and collembola, that have the greatest impact on soil C cycling (Coleman et al. 2004). There are three types of microarthropods, those that feed on fungi (microphytophages), those that feed on decomposed vegetable matter (macrophytophages) and those that feed on both (panphytophages). The presence of any or all of these microarthropods can have a significant impact on decomposition. Oribatid mites, the most numerically dominate mesofauna in forest soil (Travé et al. 1996), influence SOM decomposition and soil nutrient cycling by grazing on microbial populations or fragmented plant detritus. For example, mites and collembolan preferentially graze fungi growing on litter, but when offered only arbuscular mycorrhizae growing on maple they consumed the fine hyphae most distant from the root (Klironomos and Kendrick 1996). Collembola are usually highly specialized feeders on soil microbes and control the population dynamics of microorganisms in the soil (Bengtsson et al. 1994). Alternatively, some collembola have enzymes that enable them to utilize different constituents of organic matter in soil (Rusek 1998).
Soil enchytraeids, typically of a smaller size range than earthworms, are thought to be generalist feeders. However, previous studies have found that enchytraeids can exploit litter and SOM to a greater extent than rhizosphere C (Standen and Latter 1977; Briones and Ineson 2002; Schmidt et al. 2004; Schmidt and Baldwin 2009). However, it has been noted that enchytraeids vary widely in their ability to assimilate C from living plant roots (Bull et al. 2002). Enchytraeids ingest both mineral and organic particles in the soil (Didden 1990; Didden 1993), but will also eat plant materials coated with fungal hyphae, most likely because they lack gut enzymes that break down more recalcitrant organic matter.

Macrofauna (i.e. millipedes, earthworms, insect larvae, ants, etc) influence decomposition rates and nutrient cycling in soil by stimulating microbial activity and fragmenting organic matter. In coastal BC forests millipedes are the most dominant macro-fauna (Battigelli et al. 1994; Setälä et al. 1996). Macrofauna are also important in forming soil structure. They mix organic and mineral particles, redistribute organic matter, redistribute microorganisms, create biopores, promote humification and produce fecal pellets. Worms have been found to selectively feed on fungi degrading water-soluble sugars and cellulose (Moody et al. 1995), and worm casts and fecal pellets have been associated with flushes of microbial activity, which is thought to be due to increased moisture and increased access to labile C (Aira et al. 2003), though ultimately, soil microbial biomass is lower in worm-worked soil (Zhang et al. 2000). Millipede fecal pellets were also thought to be associated with flushes of microbial activity. However, recent work by Suzuki et al (2013) and Snyder et al. (2009) have shown that millipede feces and millipede-worked soil ultimately have lower soil respiration compared to ground feces and unworked feces-free control soil, indicating that millipedes play a role in soil C sequestration.

1.3 Green-tree-retention harvesting

The research conducted for this thesis formed part of the Silviculture Treatments for Ecosystems Management in the Sayward (STEMS) experiment (http://www.for.gov.bc.ca/hre/stems/intro.htm), a long-term, large-scale multidisciplinary field trial replicated at three locations within the Sayward Forest District, Vancouver Island. The three locations contain second-growth, 60-70-year-old, western hemlock and Douglas-fir mixed
stands and are located within a very dry variant (biogeoclimatic zone: CWHxm2) of the coastal western hemlock biogeoclimatic zone (Meidinger and Pojar 1991). The STEMS experiment was initiated to examine forest productivity, economics and public perceptions of seven different silvicultural systems. The research goals of this project include: creation of alternate harvesting regimes to be used by foresters/planners involved in ecosystem management, evaluation of the feasibility and cost of alternative harvesting practices, evaluation of the long-term effects of harvesting on timber production and other non-timber values, and evaluation of the reduction of visual impacts on forestry operations (de Montigny 2004). The aggregated- and dispersed-retention treatments of the third replicate of the STEMS project (STEMS 3) located at Gray Lake near Campbell River, B.C., were the focus of this study. The first two STEMS replicates (STEMS 1 and 2) have been used to study a broad array of topics including: tree growth, stand development, seedling regeneration, light availability, aesthetic quality of harvested sites, and public response (Evans et al. 2003; de Montigny 2004). Earlier studies examined the effects of different harvesting regimes, tree mortality, coarse woody debris recruitment, harvesting production, residual tree damage, and soil disturbance on wind-throw at the STEMS site (Scott 2005; Di Lucca et al. 2006). A meta-analysis of the effects of GTR harvesting on birds, mammals, lichens, mycorrhizae and other organisms determined this type of harvesting reduced the loss of populations or individual organisms in 41 of 57 studies relative to clear-cut harvesting (Rosenvald and Lõhmus 2008). Green-tree-retention harvesting almost always improved insect and bird habitat and was most successful at ‘life-boating’ for epiphytic lichens, small ground-dwelling animals, and ECM fungi. Effects of GTR harvesting on soil microbial community composition and function was studies at STEMS 2 in 2005 and at STEMS 1 in 2006 (Daradick 2007; Jones et al. 2008; Dewi 2009) and the results are discussed below.

Tree harvesting changes the quality and decreases the quantity of plant-C inputs into the surrounding soil (Marshall et al. 2000), and affects soil nutrient availability and climatic conditions (Covington 1981; Chen et al. 1995; Hassett and Zak 2005; Nave et al. 2010). However, the type of harvesting strategy employed can reduce the impact of harvesting on soil temperature, moisture, pH and available N. Immediately after clear-cut harvesting soil nutrient availability decreases, followed by a period of increased availability (Keenan and Kimmins 1993). This trend is particularly evident for soil nitrate (NO₃⁻) (Gordon and VanCleve 1983;
Mlandenoff 1987; Fisk and Fahey 1990; Denslow et al. 1998; Gravelle 2009; Jerabkova et al. 2011). Nitrate levels increase within a year of clear-cutting, and remain elevated for 3 to 5 years (Prescott et al. 2003), depending on site fertility (Vitousek et al. 1979) and the dominant tree species. For example in coniferous forest soil the nutrient response will be delayed, yet prolonged relative to deciduous forests, due to coniferous forest soils having greater buffer capacities (Futter et al. 2010). However, there are exceptions to this trend. Sometimes an increase in soil nitrate concentration after harvest is not observed (Bock and van Rees 2002; Jerabkova et al. 2006), instead, there may be an increase in ammonium ($\text{NH}_4^+$) concentrations (Carmosini et al. 2002; Theil and Perakis 2009). Soil N availability tends to resemble the adjacent forest for a distance of up to 11 meters from the edge of the forest into the clear-cut (Hope et al. 2003; Jerabkova et al. 2006; Theil and Perakis 2009).

Soil under variable-retention harvesting regimes has nutrient and microclimate conditions intermediate to uncut and clear-cut forests (Barg and Edmonds 1999; Lajzerowicz et al. 2004; Lapointe et al. 2005; MarenHoltz et al. 2010). Specifically, nitrate levels are higher relative to uncut forest sites when canopy gaps are as small as 0.07 to 1.7 hectares (Prescott 1992; Parsons et al. 1994; Bauhus and Bartel 1995; Prescott et al. 2002; Hope et al. 2003; Prescott et al. 2003). Single-tree-retention appears to mitigate the effects of harvesting on soil N availability, even when up to 60% of the trees are removed (Knight et al. 1991; Parsons et al. 1994; Prescott et al. 2003; Hope et al. 2003; Redding et al. 2003; Jerabkova et al. 2011). This is in part because residual trees reduce the extremes of temperature and moisture (Carlson and Groot 1997), and maintain inputs of C through litter and root exudates (Lee et al. 2002), both of which can affect microbial community structure and activity.

Few studies have explored the effects of harvesting on total microbial biomass, and even fewer have explored the effects on microbial diversity and function. The impact of harvesting on soil microorganisms is inconsistent (Siira-Pietkäinen et al. 2001; Hernesmaa et al. 2005). Microbial biomass in clear-cut sites can increase (Sundman and Hunta 1978; Entry et al. 1986), decrease (Bradley et al. 2001; Siira-Pietkäinen et al. 2001; Moore-Kucera and Dick 2008b; Mummey et al. 2010), or remain the same (Smolander et al. 1998; Barg and Edmonds 1999; Li et al. 2004). Severe SOM removal, soil compaction and tree removal tend to result in: an increase in
prokaryotic organisms such as *Arthrobacteria* and *Pseudomonas* species (Axelrood et al. 2002a; Axelrood et al. 2002b; Ponder and Tadros 2002; Busse et al. 2006; Schnurr-Putz et al. 2006; Fierer et al. 2007), an increase in organisms that thrive in oxygen-poor environments (Schnurr-Putz et al. 2006), a decrease in Gram-negative bacteria (Mummey et al. 2010), and a shift in the ammonia-oxidizing community (Bäckman et al. 2004). However, fungal biomarker PLFAs after clear-cutting are consistently reduced even 9 years after harvest (Mummey et al. 2010) and the fungal community composition (determined by various methods) is altered up to 15 years after harvest, particularly ratios of ECM to saprotrophic genera, with saprophytes increasing in dominance (Hagerman et al. 1999; Byrd et al. 2000; Jones et al. 2003; Busse et al. 2006; Hartmann et al. 2009; Hartmann et al. 2012). However, harvesting can also have no effect on microbial community structure defined by PLFA biomarkers (Ponder and Tardos 2002; Hannam et al. 2006; 4 and 5 years after harvest, respectively).

Fewer studies have examined the impact of variable-retention harvesting on the soil microbial community. Soil microbial biomass can be reduced (Lindo and Visser 2003), or remain similar to retention patches (Churchland et al. 2013, Chapter 3 this thesis). Chatterjee et al. (2008), using PLFA analyses, observed a reduction of fungi and Gram-negative bacteria 12 years after variable-retention harvest. Luoma et al. (2006), using molecular methods, found a decrease in ECM fungi with increasing distance from retention patches. Similarly, Jones et al. (2008), after morphotyping lateral roots tips, used rRNA sequencing and found that 2 years after harvest that ECM fungal communities in GTR aggregate-retention patches as small as 5 m and up to 40 m in diameter, and in clear-cut edge areas within 10 m of retention patches, did not significantly differ from those in uncut forests. In the 5-40 m diameter aggregated-retention patches at STEMS 2 Daradick (2007) found significant changes in the activities of soil enzymes involved in C, N and P cycling two years after harvest, and determined that GTR harvesting retained enzyme activity within 20 m of the aggregated-retention plots. At STEMS 1, 5 years after harvest, Dewi (2009), using PLFA analysis, found that GTR harvesting shifted the structure of the soil microbial community, but maintained activities of certain enzymes including β-glucosidase and NAGase. Dewi (2009) also found that dispersed-retention was better than aggregated-retention at maintaining soil enzyme activities across harvested sites. However, high levels of soil heterogeneity made it difficult to draw conclusions about the influence of retention trees on the
soil microbial community. Single-tree retention has been found to mitigate changes to the microbial community structure (molecular methods; Hagerman et al. 1999), but changes in the fungal community, particularly ECM, still occur (fungal community determined by various morphological and molecular methods: Kropp and Albee 1996; Buée et al. 2005; Teste et al. 2012; Bach et al. 2013).

Soil faunal responses to clear-cut harvesting are generally consistent, with most soil animals (including mites, spiders and earthworms) decreasing in abundance in the short-term (1 year) (Bird and Chaterpaul 1986; Addison 1996; Addison and Barber 1997; Marshall et al. 2000). Changes in mite assembly may persist for several years (Blair and Crossley 1988; Siepel 1996; Setälä and Marshall 1994). Eight years after clear-cut harvesting in the Appalachians, oribatid mite abundance was still lower relative to the uncut forest, and there was a shift in mite species composition (Abbott et al. 1980; Blair and Crossley 1988). Collembolans and enchytraeids, alternatively, have both been found to increase in abundance after clear-cutting, or not change (Addison 1996; Addison and Barber 1997; Marshall et al. 2000). Partial cutting and thinning reduces the impact of harvesting on soil faunal communities (Addison 1996; Lindo and Visser 2004).

In order to retain soil biota after harvest, it has been recommended by the British Columbia forest practices code that coarse woody debris be retained on site and that removal of SOM be minimized (http://www.for.gov.bc.ca/code/). Coarse woody debris will take years to decompose and in coastal western hemlock and Douglas-fir forests coarse woody debris has been found to lower soil pH, increase available aluminum and increase available iron (Spears and Lajtha 2004). In order to maintain consistent, labile C inputs and in turn maintain pre-harvest soil microbial community structure after harvest, small hubs of retained forest community structure should be included, like those associated with GTR harvesting. These hubs will also act as a source of inoculum, maintaining species-specific pre-harvest mycorrhizal communities. Teste and Simard (2008) explored seedling establishment on harvested sites with retained mature trees. They found that seedling height, shoot biomass, needle biomass and nutrient uptake peaked 2.5 to 5 m from retained trees. They attributed the increase in seedling growth to their direct access to the mycorrhizal network of retained trees.
1.4 Carbon cycling in forests

Soil organic matter is the largest C pool in terrestrial ecosystems (Falkowski et al. 2000; Fontaine et al. 2003). Formed primarily through decomposition of litter, SOM consists of a broad spectrum of plant-derived C compounds such as lignin, cellulose, glucose and plant lipids as well as microbial-derived byproducts and remnants of dead microorganisms. Soil organic matter accumulation depends on the balance between plant supply of litter and root exudates, and microbial activity, specifically decomposition, mineralization and transformation (Schmidt et al. 2011). Bulk SOM turns over every 50-100 years, with different components varying in turnover time from hours to centuries (see Appendix A) (Schmidt et al. 2011). Carbon enters the SOM pool via litter (leaves, coarse and fine roots), brash (branches and coarse woody debris) and root exudates. The amount of recently photosynthesized C allocated to leaves, storage, metabolism and root exudates varies depending on the environment, plant type, age of the plant and nutrient availability (Litton 2007, Epron 2012; Appendix B). The fate of these compounds is difficult to measure and varies depending on the age of the tree, tree species, spatial heterogeneity of belowground structures and environmental conditions (Subke et al. 2009; Kuzyakov and Gavrichkova 2010; Mencuccini and Holtta 2010; Warren et al. 2012). Consequently, there has been a recent focus on measuring the amount of C flow belowground as well as its spatial and temporal distribution (Högberg et al. 2001, 2008, 2010; Litton and Giardina 2008; Chapin et al. 2009; Warren et al. 2012).

Several studies have successfully measured C allocation belowground. Carbone et al. (2007) examined the fate of recently assimilated C in 31- to 36-year-old Sitka spruce forests and the associated understory by pulse-labeling with $^{14}$CO$_2$. The mean residence time of the labeled C was <1, 6 and 15 days in the understory, canopy and rhizosphere, respectively. After 30 days, 91 % of the assimilated label was respired (Carbone et al. 2007). Litton et al. (2007) conducted a meta-analysis of tree C budgets (the proportion of photosynthates allocated to different parts of the tree) and determined that usually between 25-63 % of primary production is allocated belowground. It is difficult to tease apart the relative contributions of symbiotic fungi, and free-living bacterial and fungal respiration in the rhizosphere, and root respiration, to total belowground respiration. As a result, C-allocation studies often combine the four, and generally
refer to all C partitioned belowground as total belowground C flux (root biomass + root respiration + mycorrhizal biomass + mycorrhizal respiration + rhizosphere respiration + exudates). However, a few studies have successfully separated apart C allocated to different sinks belowground. Pumpanen et al. (2009) demonstrated that roots and ECM growth accounts for 13-21 % of recently assimilated C, whereas 9-26 % of recently assimilated C is respired from the roots and rhizosphere. Nadelhoffer and Raich (1992) analyzed 59 separate studies and determined that approximately 33 % of all C allocated belowground is used for fine-root production, but 50-60 % of this C is derived from stored C (Gaudinski et al. 2009). Residence time and allocation of C to the roots varies between the fine roots and the root tips (Keel et al. 2012), and among seasons and species (Anderson et al. 2010; Epron et al. 2011). C allocated to the fine roots and root tips will be used for growth and respiration, and will also be exuded. Considering pulse-labeling, continuous labeling and C3 vs. C4 plant methodological studies, Kuzyakov et al. (2001) found that 1-30 % of total photosynthates are exuded by the roots, the amount and content of root-exuded C will varies depending on the age, species, season, nutrient availability and soil type. Recently photosynthesized C can be found within days in PLFAs (Högberg et al. 2008, 2010), especially fungi (Epron et al. 2011). Using a girdling experiment Högberg et al. (2001, 2008) determined that 37-54 % of recently assimilated carbohydrates are respired by roots and symbiotic microbes during the growing season.

Plants release several types of root exudates including: mucilage that maintains a constant moisture environment, metal chelators that mobilize iron and zinc, and various forms of C, dominated by low-molecular-weight organic-acids, carbohydrates and amino acids that support the rhizosphere microbial community (Bais 2006; Appendix C). Evidence of the impact of roots and root exudates on soil C availability becomes apparent when measuring labile C content of the soil. In root-free soil there may be less than 50 µM of labile C (Monreal and McGill 1985; Jones 1998; van Hees et al. 2005). In rooted soil, labile C can increase to 100 µM and in the rhizosphere it may be upwards of 500 µM (Schneckenberger et al. 2008). This increase in C is partially responsible for the 10-100 times greater bacterial biomass observed in the rhizosphere relative to the bulk soil (Matilla et al. 2007). However, not all microbes in the rhizosphere benefit the plant. The spectrum of plant-microbe relationships in the rhizosphere can range from mutualistic (e.g. PGPB and mycorrhizae) to pathogenic.
Root exudation changes under different nutrient regimes and increases in the presence of microorganisms (Grayston, et al. 1997). For example, ECM trees allocate a third more C to their roots than non-ECM trees (Durall et al. 1994; Rygiewicz and Andersen 1994; Qu et al. 2004), probably because extramatrical hyphae are a large carbon sink (Rygiewicz and Anderson 1994; Cairney and Burke 1996; Cairney 2012). Lab studies have shown that up to 29 % of plant assimilated C is allocated to extramatrical hyphae (Rygiewicz and Andersen 1994; Ek 1997; Bidartondo et al. 2001). Plants are thought to allocate more C to their roots and mycorrhizal symbionts under nutrient-poor conditions (Zak et al. 1993, Franklin et al. 2012). In systems that are not N-limited, or in systems where N has been added, fungal biomass can decrease up to 45 % (Högberg et al. 2007). This decrease in fungal biomass was assumed to be the result of decreased C supply from the trees to the mycorrhizal fungi. The composition of microbial communities in the rhizosphere is determined in part by the differences in quality and quantity of C released in root exudates (Merbach et al. 1999). Variation in exudates produced by different tree species, as well as their mycorrhizal symbionts, can result in different rhizosphere microbial community structure (Prescott and Grayston 2013).

1.5 Priming effects of recent photosynthates on soil organic matter decomposition

Root exudates have been found to influence the soil microbial community and alter microbial utilization of SOM (Kuzyakov and Cheng 2001; Brant et al. 2006, Bird et al. 2011). The increase or extra decomposition of SOM that occurs when microbes are stimulated by the addition of labile C is called a ‘priming effect’ (Dalenberg and Jager 1989; Kuzyakov et al. 2000). Removal of trees from forest sites and subsequent removal of the soil microbial communities’ dominant carbon source (root exudates and litter) may cause long-term changes to microbial community composition, soil C cycling and soil nutrient availability. At present it is unclear how harvesting practices and disturbance affect the soil microbial community’s ability to respond to labile C inputs.

It is generally assumed that soil microorganisms are C-limited, and a large addition of labile C can cause a spike in microbial activity, microbial biomass and enzyme production (Fontaine et al. 2004). This spike in activity and enzyme production is predicted to enable decomposition of
older and more recalcitrant SOM. A priming effect occurs when a C addition results in an increase in CO$_2$ efflux greater than that of the C added. If the increase in CO$_2$ efflux is due to pool substitution (i.e. enhanced microbial growth and turnover), and not an increase in SOM decomposition, then it is termed an ‘apparent priming effect’. In this instance it is usually the previously dormant microorganisms that respond to the added substrate, resulting in an increase in microbial biomass (Kuzyakov et al. 2000, Kuzyakov 2010). However, if the size of the microbial community remains stable, and the community actively decomposes recalcitrant SOM, then it is termed a ‘positive’, or ‘real’, priming effect (Kuzyakov et al. 2000, Kuzyakov 2010).

In some instances the addition of labile C suppresses microbial activity or results in greater C storage. Those instances are termed ‘negative’ priming effects (Kuzyakov et al. 2000, Kuzyakov 2010).

Priming effects can be induced rapidly following changes in substrate availability, and can persist for many weeks to months in the soil (Kuzyakov et al. 2000; Dijkstra and Cheng 2007; Nottingham et al. 2009). The addition of plant residues, simple sugars and root exudates have all been found to stimulate priming effects (Fontaine et al. 2003; Dijkstra and Cheng 2007; Rasmussen et al. 2007; Nottingham et al. 2009). Depending on the type of C added to the soil, different microbial groups will respond more rapidly, initiating the priming effect. Typically bacteria, specifically r-strategist bacteria, are the first group to immobilize and metabolize labile C added to the soil (Paterson et al. 2007). This results in an increase in microbial activity and microbial biomass, initiating an apparent priming effect (Blagodatskaya et al. 2007; Nottingham et al. 2009). Garcia-Pausas and Paterson (2011) added $^{13}$C-enriched glucose to grassland soil and found that Gram-negative bacteria PLFA biomarkers are the first to take up the $^{13}$C, followed by Gram-positive bacteria and fungi. Waldorp and Firestone (2004) added four different $^{13}$C-labelled compounds (starch, xylose, vanillin and pine litter) to an oak forest soil and also found Gram-negative bacteria were the first microbial group to take up the $^{13}$C, despite them being the bacterial group of lowest abundance. Similarly, Nottingham et al. (2009) observed Gram-negative bacterial uptake of added C after addition of C4 plant-derived sugars (sucrose, maize leaf litter) to C3 soil, and attributed it specifically to the PLFA biomarkers 16:1ω5 and 16:1ω7. Lee et al. (2011) added $^{13}$C-glucose to soil in the form of a rice callus, then successfully traced the label into Arthrobacter, Pseudomonas, Actinobacteria, Bacilli, γ-Proteobacteria, Chloroflexi,
Sphingobacteria, Flavobacteria, Clostridia, Acidobacteria and cyanobacteria using DNA stable-isotope-probing. Over time the r-strategist bacteria that immobilize the added C will turn over, releasing bacterial lysates into the soil. Ultimately, the added C stimulates the K-strategist fungi and bacteria, increasing SOM decomposition. Thus the apparent priming effect transitions to a real priming effect (Kuzyakov et al. 2000). However, if the C added is in the form of cellulose then it is fungi that initiate the priming effect (Fontaine et al. 2011).

In situ studies have found that priming effects are most pronounced in nutrient-poor soils (Fontaine et al. 2003; Garcia-Pausas and Paterson 2011) and in near-neutral pH soils (Blagodatskaya and Kuzyakov 2008). In nutrient-poor soils, the low-quality C and low nutrient availability limit the amount of energy available for soil mineralization. Any C added will be quickly immobilized, though the amount of C added to the soil in relation to the size of the microbial biomass influences not only the strength of the priming effect, but also the type (Blagodatskaya and Kuzyakov 2008). If the amount of C added is greater than the microbial biomass, both microbial growth (apparent priming effect) and changes in community structure (potential real priming effect) can occur. If the amount of C added is less than or equal to the microbial biomass, then it stimulates microbial activity and turnover (real priming effect). If the amount of C added is much less than the microbial biomass, then there is not enough C to accelerate microbial turnover (Blagodatskaya and Kuzyakov 2008). Priming effects may also occur in response to N addition (Hoosbeek et al. 2006; Ramussen et al. 2007).

The majority of priming effect studies have been carried out in laboratory settings with agricultural soil; only a few have been conducted in situ (Kuzyakov 2010), and fewer still have been conducted in forest ecosystems. Kuzyakov (2010) stated that future studies need to be conducted in the field, as there have been issues in scaling-up laboratory studies to ecosystems. Kuzyakov (2010) also stressed the importance of examining which microbial groups immobilize the added C and how disturbance and land-use change will influence observed priming effects. Determining the impact of disturbance, and land use change on the potential priming effect will give insight into the ecological significance of priming in natural ecosystems.
**1.6 Stable isotopes**

Natural variations in the ratio of stable isotopes of $^{13}$C/$^{12}$C can be used to infer origin and flow of carbon in the environment (see Figure 1.2). Isotopic enrichment and depletion varies depending on isotopic fractionation. Isotopic fractionation is defined as “the isotopic effect in conversion from substrate to product: $\Delta = (\delta_{\text{substrate}} - \delta_{\text{product}})/(1 + \delta_{\text{product}}/1000)$” (Bowling et al. 2008). The delta value is the isotopic ratio of the sample, over the isotopic ratio of a standard, minus one, times 1000 (see example for C below).

$\Delta$ is the isotopic ratio of the sample, over the isotopic ratio of a standard, minus one, times 1000.

PDB refers to a Cretaceous Belemnite formation in PeeDee, South Carolina.

The relative proportion of $^{13}$C in trees differs from the amount of $^{13}$C in the atmosphere due in part to diffusion resistance across the stomata. Within the leaf there is further discrimination in reaction rates involving rubisco during carboxylation (Bowling et al. 2008). Many factors influence the $\delta^{13}$C values between plants, including: light photosynthetic capacity, nature of the photosynthetic pathway, water availability (Read and Farquhar 1991), and atmospheric humidity (Bowling et al. 2008). Furthermore, different compounds within the tree have different isotopic signatures and at an even finer scale, different products of metabolism have different $\delta^{13}$C values (Bowling et al. 2008; Figure 1.2). As a rule, during metabolism, catabolic reactions are less likely to involve molecules that contain $^{13}$C, due to the fact that the slightly lighter molecules containing less $^{13}$C will have slightly weaker bonds, therefore have lower activation energies (easier to break down), whereas those molecules containing more $^{13}$C are more likely to be involved in biomass production. This is because the heavier molecules have stronger bonds and require more energy to breakdown (and therefore are more likely to be used for other things). This leads to the prediction that $\delta^{13}$C values will be higher in microbial biomass and lower in the respired CO$_2$, relative to consumed material (Šantrůčková et al. 2000). The greater the activity of microorganisms the more $^{13}$C-enriched the microbial biomass C will be, since active cells will be breaking down more C compounds (Denies 1980).
Consumer’s tissues are 1‰ $^{13}$C-enriched relative to their food source, and bacteria are 1-2‰ $^{13}$C-enriched relative to their substrates. The $\delta^{13}$C value of soil fauna is a reflection of different feeding strategies and tissue turnover rates that are a result of variable metabolism and assimilation rates (Briones and Ineson 2002). Respired CO$_2$ is typically $^{13}$C-depleted by 1-2‰ relative to the food source. In addition many herbivorous food sources have different $^{13}$C content (e.g. leaves, wood, SOM; Schweizer et al. 1999; Weber et al. 2008). Therefore, $^{13}$C/$^{12}$C ratios can be used to help identify the consumers’ diet and ultimately trace the initial food source (Schweizer et al. 1999; Weber et al. 2008). However, natural variation in stable isotope ratios are often greater than the variation due to isotopic discrimination. Consequently, scientists tend to add labeled substrates, highly enriched in the heavy isotopes $^{13}$C or $^{14}$C in order to observe the flow and movement of nutrients.

1.7 Using stable isotopes to elucidate C-flow from trees to soil in situ

In situ $^{13}$C-pulse-labeling of plant C and subsequent tracing into soil organisms has been mostly limited to grasslands (Johnson et al. 2002; Leake et al. 2006; Ostle et al. 2007; Denef et al. 2009; Clayton et al. 2010; Lattanzi et al. 2012), small trees (Dyckmans and Flessa 2001; Esperschütz et al. 2009; Palacio et al. 2011) and crop species such as rice (Lu et al. 2004; Wu et al. 2009) and leek (Elfstrand et al. 2008; Table 1.3). The notable exceptions are Högberg et al.’s (2008; 2010) successful labeling of pine trees, the IsoFACE systems– developed by Talhelm et al. (2007) to label small trees, and Grams et al. (2011) to label tall trees (Table 1.3). Small plants and seedlings interact differently with their environment than established trees (Litton et al. 2007; Poorter et al. 2012). The latter have the potential to greatly influence above and belowground forest communities due to their developed root system, large canopy, and large amount of photosynthates that can be exuded through their roots.

Pulse-labeling of low-lying shrubs or grassland is typically conducted using chamber methods and has successfully allowed tracing of $^{13}$C into arbuscular mycorrhizal fungi (Johnson et al. 2002), soil PLFAs (Denef et al. 2009), rhizosphere microbial DNA (Clayton et al. 2010), and collembola (Ostle et al. 2007). In order to label isolated plant seedlings, the “bag method” has been developed. In this method seedlings are isolated from atmospheric CO$_2$ by a gas-tight
polyethylene/nylon bag sealed around the base of the stem and the bag is filled with either $^{13}$C- or $^{14}$C-enriched (or depleted) CO$_2$. The seedling incorporates this CO$_2$ during photosynthesis, producing $^{13}$C- or $^{14}$C-enriched (or depleted) photosynthates (Simard et al. 1997a, b, c; Teste et al. 2009). The disadvantages of both methods described above is that the size of the plant being labeled is limited, the bags/chambers will diffuse light (Högberg et al. 2008), and the injection of $^{13}$CO$_2$ into the bag or head space may cause CO$_2$ partial pressure changes, which will change the rate of photosynthesis. These devices are also expensive and could be impractical in the field as they need a power supply and equipment to control the conditions within the enclosure (Talhelm et al. 2007).

Fewer studies have been conducted on trees taller than 2 m because of the large amount of isotope required to label whole trees. Carbone et al. (2007) labeled 4-m-tall black spruce trees with $^{14}$CO$_2$ using a dome-shaped portable polyethylene yurt. Högberg et al. (2008; 2010) pulse-labeled 4-m-tall pine trees using the free-air CO$_2$ enrichment (FACE) technique (Hanson et al. 2000). This technique successfully traced $^{13}$C into soil microbial cytoplasm, fungal PLFAs, some bacterial PLFAs and collembola (Högberg et al. 2010). Keel et al. (2012) similarly labeled a mature deciduous forest using the FACE method ($^{13}$CO$_2$ depletion) that allowed for observation of C flow above and belowground at a large scale. However, Subke et al. (2009) stated that interpretation of CO$_2$ tracer data in these studies is prone to error initially after the pulse, because the soil air is enriched in $^{13}$C due to the physical diffusion of the trace gas into soil air pockets. Also, adding enriched or depleted $^{13}$CO$_2$ increases the total concentration of CO$_2$ in the atmosphere, which has been found to increase tree net primary production (NPP), change C allocation within the tree and increase root exudation (Curtis and Wang 1998). This is predicted to influence soil microbial community structure and soil nutrient cycling (Paterson et al. 1997; Phillips et al. 2011a). More recently the crowns of larger trees have been successfully labeled with $^{13}$CO$_2$ (Plain et al. 2009; Dannoura et al. 2011; Epron et al. 2011). However, these methods required large canopy chambers covering 10-50 m$^2$, or chambers covering the entire crown of a large tree (Epron et al. 2012). Consequently, other “low-cost” approaches have been developed in recent years. Stem-injection techniques have been successfully used to label 8-year-old larch trees with $^{13}$C, via tubing from battery-operated pumps (Talhelm et al. 2007), and 20-meter-tall maritime pine trees with a multi-inlet system (Wingate et al. 2010). Direct injection of a solution
of Na$_{13}^2$CO$_3$ into the xylem was used to label a small northern white cedar tree (Powers and Marshall 2011). Similarly, large-tree stem-injection studies have successfully traced $^{15}$N in the labile organic matter within 1 m of injected 18-year-old sweet gum trees (Garten and Brice 2009) and $^{15}$N in grasses surrounding injected 16-year-old legume trees (Sierra and Daudin 2010). However, all these studies only assessed the partitioning of plant-assimilated C and N between autotrophic (both from foliage and roots) and heterotrophic respiration; none linked tree C allocation and the extent of its translocation into the main biotic components of the grassland or forest soil.

1.8 Thesis objectives and overview

The effect of harvesting on soil organisms is a critical knowledge gap that precludes certainty about the sustainability of current and proposed forestry practices (Gustafsson et al. 2012). This thesis aimed to elucidate C flow from live trees retained on harvested sites, into the soil microbial and faunal community to determine if green-tree-retention harvesting regimes better retain pre-harvest soil microbial community structure and function compared with clear-cut harvesting, and the best arrangement of retention trees to achieve this aim. The use of a novel stable-isotope stem-injection technique provided a unique opportunity to trace the flow of C directly from the phloem into the microbial biomass. This innovative technique was used, in addition to $^{12}$C/$^{13}$C natural abundance stable-isotope-ratio analysis of soils and soil organisms, to enable determination of which microbial and faunal groups are most dependent on recent photosynthate and to increase our understanding of C-flow in forest ecosystems. Spatial and temporal dynamics of C-flow within different forest silvicultural systems also play a role in determining which C sources are preferentially used by microbes and how best to maintain pre-harvest soil microbial and faunal communities, and were also investigated. The research was conducted at the STEMS long-term research trial, a second-growth forest on Vancouver Island, BC, Canada, which had been harvested 2 years prior to the studies. In addition, the stem-injection stable-isotope-labeling technique (Chapter 4) was conducted in a Sitka spruce stand, located in the UK Forestry Commission long-term tree species trial at Gisburn Forest, Lancashire, UK. The following questions were addressed:
i) Chapter 2 - Which of aggregated versus dispersed-retention treatments best maintains pre-harvest nutrient availability and pre-harvest microbial community composition?

The study described in Chapter 2 aimed to identify the soil microbes most sensitive to the harvesting of trees, and determine which variable-retention design best preserves pre-harvest microbial community composition and soil nutrient availability. The objectives were achieved using a principle component analysis to determine differences in the microbial community composition (PLFAs) and nutrient availability (measured using plant-root simulator, PRS, probes) between four variable retention treatments: a dispersed-retention area, an aggregate-retention area, a clear-cut area and a clear-cut edge (next to an aggregate-retention area). $^{13}$C natural abundance of PLFAs was analyzed to elucidate microbial community C sources. I hypothesized that fungal biomass would be greatest in the aggregated-retention patch, and would decrease in the dispersed-retention area and the clear-cut areas. Furthermore, I hypothesized that ammonium levels would be greatest in the clear-cut, clear-cut edge relative to the aggregated patch, with the dispersed-retention area intermediate to these.

ii) Chapter 3 - What is the range of influence of retained trees on soil microbial communities? Which microbial communities are dependent on recent photosynthates?

The study presented in Chapter 3 was designed with two objectives in mind. The first was to determine the degree to which living trees influence the relative abundance and activity of mycorrhizal fungi, saprotrophic fungi and bacteria, and to identify the major C sources used by these organisms for growth. The second was to identify the influence of trees on soil C-cycling and respiration. The objectives were achieved using a geostatistical approach where the spatial variation in soil microbial community composition (PLFAs) and C turnover ($\delta^{13}$C values of soil-respired CO$_2$, DOC, SOM and PLFAs) was studied in an evenly-distributed, 100-point, 27x18 m plot located within a second-growth forest along a transition from an area of retention trees into a two-year-old clear-cut area. I hypothesized that there would be a shift in the microbial community from a mycorrhizal dominated community to a bacterial and saprotrophic fungal
community in the forested area to the clear-cut area in conjunction with a shift in plant-derived labile-C availability.

iii)  Chapter 4 – What are the spatial and temporal patterns of C transfer from individual trees to the soil microbial community in situ?

The study presented in Chapter 4 involved application of a novel stem-injection stable-isotope-labeling and probing technique with the aim of tracing the spatial and temporal distribution of recent photosynthates from mature trees into soil-respired CO₂, dissolved organic C production, soil microbes and mesofauna over the course of 30 days. The study was conducted in the Gisburn Research forest in Lancashire, UK on 22-year-old Sitka spruce. Microbial community composition was assessed using PLFAs. Soil-respired CO₂, DOC, SOM, PLFA and fauna were measured, along with δ^{13}C values of each. I hypothesized that ^{13}C-enrichment of all measured parameters would be observed from the base of the trees up to the canopy edge, where root and mycorrhizal density is greatest.

iv)  Chapter 5 – How do soils under green-tree harvesting regimes respond to labile C additions (resembling those that occur through root exudation)? Can evidence of priming effects be observed in situ?

The objective of the study discussed in Chapter 5 was to determine the impact of harvesting and harvesting practices on the potential priming effect of forest soils in situ. Here I added ^{13}C-labeled glucose to forest soils and compared the short-term priming effect in an aggregated-retention patch in the STEMS 60-year-old forest, an adjacent clear-cut area, and a highly disturbed landing area where logs were temporarily stored before transport. In addition to an increasing level of disturbance, the three sites had decreasing concentration of roots, increasing soil temperature extremes, and increasing soil moisture extremes. In order to meet the objectives I measured community composition (PLFAs) as well as the δ^{13}C values of DOC, SOM, PLFAs, and soil respiration. From this I was able to calculate the priming effect. I hypothesized that the landing site would have a greater proportion of bacteria, and consequently would have a greater positive priming effect on SOM decomposition.
In Chapter 6, I discuss the context and application of my research relative to other work in the field thus far. I summarize the main findings of this thesis, pointing out limitations of the research and possible future research directions that could be used to address these issues, and finally, I discuss the implications of my research for forest management.
Figure 1.1 Schematic view of root-mycorrhizal zones of influence. Rhizoplane describes the area adjacent to the root where the soil particles adhere. The rhizosphere is the area of soil around the root that is influenced by root-exuded labile C. The hyphosphere is the area of soil around mycorrhizal hyphae that is influenced by hyphal-exuded labile C, and enzyme production. The mycorrhizosphere is the area of soil influenced by the root and mycorrhizal communities combined.
Figure 1.2 $\delta^{13}\text{C}$ values of C sources in forest ecosystems.
Table 1.1 Methods to measure soil microbial community structure (Kirk et al. 2004; Leckie 2005; Nakatsu 2007; Dewi 2009).

<table>
<thead>
<tr>
<th>Method</th>
<th>Overview</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical- and physiological-based</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate counts</td>
<td>Assessment of community structure by selective plating and direct viable counts</td>
<td>Fast, Inexpensive</td>
<td>Unculturable microorganisms not detected</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Bias towards fast-growing individuals</td>
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<td></td>
<td></td>
<td></td>
<td>Bias towards fungal species that produce large quantities of spores</td>
</tr>
<tr>
<td>Phospholipid Fatty Acid (PLFA) analysis</td>
<td>Assessment of community structure based on fatty acids assemblages</td>
<td>Direct extraction from the soil organisms, Targets the entire community</td>
<td>Results can be confounded by other microorganisms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>More variability associated with fungi than with bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Overlap among groups for marker PLFAs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No information about species composition</td>
</tr>
<tr>
<td>Enzyme activity analysis</td>
<td>Index of microbial functional diversity and nutrient cycling potential</td>
<td>Sensitive, fast, accurate, Inexpensive, Wide range of substrates</td>
<td>Only measures potential, not in situ activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Substrate specific, therefore not representative of activity in soil</td>
</tr>
<tr>
<td><strong>Community Level Physiological profiling (CLPP)</strong></td>
<td>Asses the sole source carbon utilization patterns to determine the metabolic diversity of soil community</td>
<td>Fast and reproducible, Inexpensive, Targets active populations</td>
<td>Only represents culturable, fast-growing microorganisms that can utilize the available carbon sources</td>
</tr>
<tr>
<td>1 – using SIR</td>
<td></td>
<td></td>
<td>Only shows metabolic diversity</td>
</tr>
<tr>
<td>2 – using Biolog® microplates</td>
<td></td>
<td></td>
<td>Sensitive to inoculum density</td>
</tr>
<tr>
<td><strong>Molecular-based</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine plus cytosine (G+C)</td>
<td>Assessment of bacterial diversity as microbial groups differ in their G+C content</td>
<td>No PCR bias, Includes rare members of the community, When diversity is low can be used to indicate overall community changes</td>
<td>Requires large quantities of DNA, Dependent on lysing and extraction efficiency, Overlap in among species in base composition</td>
</tr>
<tr>
<td>Nucleic acid reassociation and hybridization</td>
<td>Estimates diversity based on DNA reassociation as a measure of genetic complexity</td>
<td>No PCR bias, Includes all DNA extracts, Can be studied in situ</td>
<td>Time Consuming, Lack of sensitivity, Dependent on lysing and extraction efficiency</td>
</tr>
<tr>
<td>Denaturing and temperature gradient gel electrophoresis (TGGE/DGGE)</td>
<td>Asses microbial genetic diversity by separating DNA sequences based on melting point</td>
<td>PCR fragments can be used for sequencing, Sensitive, Fast and simple</td>
<td>PCR Bias, Laborious sample handling, Only detects dominant species, Dependent on lysing and extraction efficiency, Sample handling can influence the community</td>
</tr>
<tr>
<td>Method</td>
<td>Overview</td>
<td>Advantages</td>
<td>Disadvantages</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>-----------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Molecular-based continued</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single strand conformation polymorphism</td>
<td>Assess bacterial and fungal diversity and relies on electrophoresis separation based on differences in DNA</td>
<td>Same as DGGE (above)</td>
<td>PCR Bias Some DNA can form more than one stable conformation</td>
</tr>
<tr>
<td>Terminal restriction fragment length</td>
<td>Detects structural changes in microbial communities by using polymorphism of target genes</td>
<td>High replicable</td>
<td>PCR Bias Choice of restriction enzymes will influence community fingerprint</td>
</tr>
<tr>
<td>polymorphism</td>
<td></td>
<td>Can be automated</td>
<td></td>
</tr>
<tr>
<td>Ribosomal intergenic spacer analysis/</td>
<td>Assess microbial richness and evenness via ribosomal-based fingerprinting</td>
<td>Reproducible</td>
<td>Requires large quantities of DNA</td>
</tr>
<tr>
<td>Intergenic spacer analysis</td>
<td></td>
<td>Can be automated</td>
<td></td>
</tr>
<tr>
<td>Fluorescent in situ hybridization</td>
<td>Detects single cells by fluorescently labeling primers</td>
<td>No PCR bias</td>
<td>Poor sensitivity Cells with slow growth or low ribosome content may not be detected</td>
</tr>
<tr>
<td>RNA blot hybridization</td>
<td>Determine taxonomic composition of microbial communities and quantifies target microorganisms</td>
<td>Able to identify dominant community members</td>
<td>Requires large quantities of RNA Time-consuming</td>
</tr>
<tr>
<td>Microarrays</td>
<td>Uses target genes to provide functional diversity information or assess diversity</td>
<td>Rapid and sensitive Thousands of genes can be analyzed No PCR bias</td>
<td>Only detects numerically dominant species Only accurate in low-diversity systems</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td>Uses target genes to provide information on abundance and diversity</td>
<td>Rapid and sensitive Tens of thousands of sequences can be analyzed</td>
<td>PCR bias Volume of data</td>
</tr>
</tbody>
</table>
Table 1.2 Methods used in previous studies to determine distribution of C from trees belowground.

<table>
<thead>
<tr>
<th>Method</th>
<th>Tree species/age</th>
<th>% C distribution</th>
<th>Equation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girdling</td>
<td>Scots pine 45-55 yrs</td>
<td>46% autotrophic soil respiration</td>
<td>Autotrophic respiration = CO$_2$ efflux control - CO$_2$ efflux girdled plot</td>
<td>Högberg et al. 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54% heterotrophic soil respiration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{14}$C Pulse Labeling</td>
<td>Scots pine 3 months</td>
<td>43-75% shoots</td>
<td>Root respiration = (CO$_2$ soil with roots) - (CO$_2$ Control (without roots))</td>
<td>Pumpanen et al. 2009</td>
</tr>
<tr>
<td></td>
<td>Norway spruce, silver birch</td>
<td>9-26% roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 months</td>
<td>13-21% ECM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FACE</td>
<td>Various</td>
<td>55% autotrophic soil respiration</td>
<td>Root respiration = ($^{13}$CO$_2$ soil - $^{13}$CO$_2$SOM)/($^{13}$CO$_2$ root - $^{13}$CO$_2$SOM)</td>
<td>Hanson et al. 2000 review</td>
</tr>
<tr>
<td></td>
<td>45% heterotrophic soil respiration</td>
<td></td>
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</tr>
</tbody>
</table>
2 Dispersed variable-retention harvesting mitigates N losses on harvested sites despite changes in soil microbial community structure

2.1 Synopsis

As an alternative to clear-cutting, variable-retention harvesting is consistently being employed due to the benefits associated with maintaining mature forest species and forest structural diversity. Although there is some evidence that variable-retention harvesting, particularly single-tree-retention will mitigate the impacts of clear-cutting on soil microbial communities and nutrient cycling, findings have been inconsistent. In this study I examined microbial community structure (phospholipid-fatty acid), and nutrient availability (PRS™ probes) over four treatments: dispersed-retention, aggregated retention, clear-cut and clear-cut edges two years after harvest. I observed elevated nitrogen concentrations, specifically elevated ammonium in the harvested areas. Availability of N and other nutrients were similar between the dispersed-retention area and the aggregated-retention patch. The microbial community, however, was different in the clear-cut and dispersed-retention area, mostly due to significantly lower (p<0.001) fungal abundance, combined with an increase in bacteria, specifically Gram-negative bacteria. This shift in the microbial community composition in the dispersed-retention area did not appear to have a major impact on microbial functioning and nutrient availability, indicating that this harvesting practice has the potential to maintain generic microbial functions/processes. However, manganese (Mn) levels were twice as high in the retention patch, compared to the harvested sites, indicating that ‘narrow’ processes (those performed by a small number of specialized microorganisms), such as lignin degradation, catalyzed by Mn peroxidase, which concomitantly removes Mn from solution, or alternatively, Mn-reduction by Mn-reducing bacteria in the mycorrhizosphere, may be altered by harvesting.

2.2 Introduction

Variable-retention harvesting, as opposed to clear-cut harvesting, is being increasingly employed in forest management because of the perceived ecological benefits. The goals of variable-retention harvesting (also known as green-tree-retention or retention harvesting) are to maintain
forest structural diversity, preserve species associated with mature forests, and support faster post-harvest recovery of biodiversity (Franklin 1997). The benefits of retaining aboveground tree and shrub species for the preservation of plant and animal diversity are well documented in the short-term (Work et al. 2003; Atwell et al. 2008; Aubry et al. 2009), particularly for ground-dwelling animals and epiphytic lichens (Rosenvald and Lõhmus 2008). Although not as well documented, there is reason to believe that variable-retention harvesting will impart similar benefits to belowground biodiversity and function. The trees retained on clear-cut sites are thought to act as ‘lifeboats’ for microbes, maintaining belowground plant-soil interactions and ensuring seedlings will have access to host-specific mycorrhizae and microbes, which could aid seedling success (Franklin 1997). Belowground plant–soil interactions have been increasingly recognized as necessary to ecosystem functioning (Bever et al. 1997; Wardle et al. 2004; Bardgett et al. 2005), and are considered a key knowledge gap and a major research challenge for retention forestry (Gustafsson et al. 2012).

Harvesting changes the quality and decreases the quantity of labile plant C inputs into the soil (Marshall et al. 2000), and affects soil temperature, moisture, pH and available nitrogen (Covington 1981; Chen et al. 1995; Hassett and Zak 2005; Nave et al. 2010). Generally, clear-cut harvesting is followed by a period of increased availability of soil nutrients (Keenan and Kimmins 1993), particularly nitrate (NO$_3^-$) (Gordon and Van Cleve 1983; Mladenoff 1987; Fisk and Fahey 1990; Denslow et al. 1998; Gravelle 2009; Jerabkova et al. 2011). Nitrate levels typically increase within a year of clear-cut harvesting, and remain elevated for 3 to 5 years (Prescott et al. 2003). However, this depends on site fertility (Vitousek et al. 1979) and the forest type, as the response in coniferous forests tends to be delayed, but prolonged, relative to deciduous forests (Futter et al. 2010; Jerabkova et al. 2011). Other studies have found that nitrate levels remain low after harvesting (Barg and Edmonds 1999; Bock and Van Rees 2002; Jerabkova et al. 2006; Jerabkova and Prescott 2007); instead, ammonium levels increase relative to uncut forests (Carmosini et al. 2002; Titus et al. 2006; Bradley and Parsons 2007; Theil and Perakis 2009). At the edges of clear-cuts (within 11 m of the forest edge), nitrogen availability tends to resemble that in the adjacent forest (Hope et al. 2003; Jerabkova et al. 2006; Theil and Perakis 2009).
Variable-retention harvested areas have been shown to have nutrient and microclimate conditions intermediate to uncut and clear-cut forests (Barg and Edmonds 1999; Lajzerowicz et al. 2004; Lapointe et al. 2005; MarenHoltz et al. 2010), with elevated inorganic nitrogen levels (relative to uncut forest sites) found in harvested areas as small as 0.07 to 1.7 hectares (Prescott 1992; Parsons et al. 1994; Bauhus and Bartsch 1995; Prescott et al. 2002; Hope et al. 2003; Prescott et al. 2003). However, dispersed-retention harvesting does not result in increase nitrogen availability, even when up to 60 % of trees are removed (Knight et al. 1991; Parsons et al. 1994; Hope et al. 2003; Prescott et al. 2003; Redding et al. 2003; Jerabkova et al. 2011). This muted effect can be attributed to the residual trees and associated microbial community continuing to immobilize soil N, minimizing temperature, pH and moisture extremes (Carlson and Groot 1997; Franklin 1997; Jerabkova et al. 2011), and maintaining inputs of C through litter and root exudates (Lee et al. 2002).

The impact of forest harvesting on soil microbes is less clear (Siira-Pietkäinen et al. 2001; Hernesmaa et al. 2005). Soil microbial biomass can increase (Sundman et al. 1978; Entry et al. 1986), decrease (Bradley et al. 2001; Siira-Pietkäinen et al. 2001; Moore-Kucera and Dick 2008b; Mummey et al. 2010), or remain similar to preharvest biomass (Smolander et al. 1998; Barg and Edmonds 1999; Li et al. 2004) following clear-cut harvesting. Similarly, the impact of clear-cut harvesting on microbial community composition is inconsistent. Gram-negative bacteria (Moore-Kucera and Dick 2008b, Mummey et al. 2010) and Actinobacteria (Hartmann et al. 2009) may be reduced; but more often fungal biomass is reduced (Mummey et al. 2010) and the ratio of ectomycorrhizal and saprotrophic genera is altered (Jones et al. 2003; Hartmann et al. 2009; Hartmann et al. 2012). Fewer studies have examined the impact of variable-retention harvesting on the microbial community composition, and the results are inconsistent; soil microbial biomass may be reduced (Lindo and Visser 2003) or unaffected in openings relative to retention patches (Churchland et al. 2013). Chatterjee et al. (2008) observed a reduction of fungi and Gram-negative bacteria in harvested areas. Similarly, Luoma et al. (2006) reported a decrease in ectomycorrhizal richness with increasing distance from retention patches. Single-tree removal has been found to mitigate changes to the microbial community structure (Kranabetter and Wylie 1998; Hagerman et al. 1999), although changes in the fungal community, particularly
ectomycorrhizae, still occur (Kropp and Albee 1996; Buée et al. 2005; Teste et al. 2012; Bach et al. 2013).

Retaining live trees on harvested sites provides soil organisms with a continually replenished source of labile carbon (C) from root exudates (Högberg et al. 2001; Zak et al. 2003), as well as above- and below-ground litter materials. Recently photosynthesized labile plant C exuded by roots is typically $^{13}$C-depleted relative to older C stored in coarse woody debris and soil organic matter (Kuzyakov 2006; Werth and Kuzyakov 2010). Natural variation in these carbon sources can be used to infer the flow of C in the environment (Schweizer et al. 1999). By comparing the ratio $^{13}$C/$^{12}$C within microbial signature phospholipid fatty acids to that of other C pools, including recently photosynthesized C, it is possible to determine which microbial groups rely the most on recently photosynthesized C from living trees for growth.

The objective of this study was to determine whether aggregated-retention harvesting (where living trees are retained in aggregated patches) or dispersed variable-retention harvesting (where single dispersed individuals are retained) best maintains pre-harvest soil microbial community structure and soil nutrient availability. In addition, through analysis of $\delta^{13}$C$_{PDB}$ values in the microbial community I could deduce which microorganisms are dependent on plant photosynthate for growth. In order to meet these objectives I used principle component analysis (PCA) to examine nutrient availability and $^{13}$C natural abundance stable-isotope ratios of soil microbial phospholipid fatty acids (PLFA) in a second-growth forest along (a) a transition from an area of retention trees into a clear-cut area and (b) in a dispersed-retention area, two years following harvesting. Soil nutrient availability at each point was estimated using plant root simulator (PRS) probes.

2.3 Methods

2.3.1 Study site

Samples were collected in the third replicate of the existing Silviculture Treatments for Ecosystem Management in the Sayward (STEMS) long-term research installation set up by the British Columbia Ministry of Forests and Range, close to Gray Lake on Vancouver Island, B.C.
(50°03’43.02”N, 125°35’24.35”W) in early May, 2009. The stand is in the submontane very dry maritime Coastal Western Hemlock biogeoclimatic zone (CWHxm2) (de Montigny 2004), receives mean annual precipitation of 1529.5 mm and has a mean annual temperature of 8.4 °C (Upper Campbell Lake climate station, National Climate Data and Information Archive, 1971-2000). Soils are mostly Orthic Humo-Ferric Podzols with moder humus form and sandy-loam structure (de Montigny 2004).

In February 2007 the existing stand of 60-70-year-old western hemlock (*Tsuga heterophylla*) and Douglas-fir (*Pseudotsuga menziesii*) trees was harvested using variable-retention, retaining trees in both aggregated and dispersed formats. Aggregate-retention patches, within which all trees were retained, were approximately 0.2 ha in size, with clear-cut spaces ranging from 4.4 to 11.5 ha, in a total area of 25.5 ha. In the dispersed-retention area, approximately 40 trees per ha were retained within an 18.6-ha area. Douglas-fir and western red cedar (*Thuja plicata*) seedlings were planted in 2008. The ground vegetation consisted mainly of salal (*Gaultheria shallon*) and sword fern (*Polystichum munitum*). The soils were a mix of mineral and organic layers in the harvested areas due to soil disturbance during harvesting; those areas that were not mixed had a forest floor 6-8 cm deep. The soil pH ranged from 3.5-5.0.

2.3.2 Soil sampling

Two equally distributed 10 x 10 point grids with sampling points 3 m apart in the north-south direction and 2 m apart in the east-west direction were laid out, one within the dispersed-retention area and the other positioned on the northern edge of an aggregate-retention patch such that it extended 12 m south into the patch, and 15 m north into the clear-cut area (Figure 2.1). Due to subtle changes in topography, the grid was laid out in a rectangle to maximize the area sampled. At each sampling point, three soil cores were collected using a 5-cm-diameter stainless-steel corer to a depth of 10 cm. The three cores were combined and stored at 4°C within 6 hours. The soil was sieved to < 2 mm prior to PLFA analysis.

Samples from the study area (dispersed-retention and, aggregated-retention) were separated into four treatments for comparison. Samples from the five southernmost rows in the aggregated-retention area, located at the edge and extending 12 m into the patch of retained trees, were
considered part of the retention patch (RP, 50 samples). Samples along the next two rows, just north of the retention patch, located 3 and 6 m into the clear-cut, were considered clear-cut edge (CCE, 20 samples). The three northernmost rows of samples at the aggregate-retention area, between 9 and 15 m from the retention patch, were denominated clear-cut (CC, 30 samples). The division into CCE and CC was based on prior knowledge from this particular site, and from other studies that have demonstrated that the influence of trees on belowground communities and processes is greatest within 10 m of the trees (Saetre 1999; Saetre and Bååth 2000; Bengtson et al. 2006; Churchland et al. 2013; Chapter 3 this thesis). Samples collected from the dispersed-retention site (DR) were not separated (100 samples).

2.3.3 Soil physical, biological and chemical analysis

Phospholipid fatty acids were extracted according to the Frostegård et al. (1991) method, based upon the Bligh and Dyer (1959) procedure, and further modified by White et al. (1979). Briefly, soil samples (1.2 g of freeze-dried soil) were vortex-extracted in a 0.8:1:2 (v/v/v) solution of citrate buffer, chloroform and methanol. The extracted lipids were then fractionated into neutral lipids, glycolipids and phospholipids on Accubond II Solid Phase Extraction silica columns (Agilent Technologies Inc., Santa Clara, CA) by elution with chloroform, acetone and methanol. A known amount of methyl nonadecanoate (19:0) was added to the fraction containing the phospholipids to act as an internal standard. Lipids were then transmethylated to their fatty-acid methyl esters using mild alkaline methanolysis. Following this, fatty-acid residues were flash-evaporated under N₂ gas and stored at -20 °C until analysis.

PLFA peaks were identified by means of a combination of mass spectra and retention times relative to the internal standard 19:0, and an external bacterial acid methyl-ester standard (BAME; Sigma-Aldric., 47080-U, Oakville, ON, Canada). PLFA \(^{13}C/^{12}C\) ratios were analyzed with a 6890N gas cromatograph (Agilent Technologies, Santa Clara, CA, USA) interfaced with a GC5-MK1 combustion furnace (GV Instrument, Cheadle Hulme, UK), in turn interfaced to an Isoprime ratio mass spectrometer (GV Instruments) at the Belowground Ecosystem Group Stable Isotope Facility in the Faculty of Forestry at the University of British Columbia. Sample batches were two-point calibrated to C20:0 isotopic standards that bracketed the expected range of δ\(^{13}\)C\(_{PDB}\) values (C20’s equaled -30.68 ‰, standard deviation 0.02 ‰ versus VPDB, and -6.91 ‰,
standard deviation 0.04 ‰ versus VPDB; isocanoic acid methyl ester, certified reference material, Indiana University). The following fatty acids were chosen to represent bacterial PLFAs: i15:0, a15:0, 15:0, i16:0, 16:1ω7c, i17:1ω8c, 10Me17:0, i17:0, a17:0, 18:1ω7c, 18:1ω5c, cy19:0 (Frostegård et al. 1993; Kroppenstedt 1985; Zogg et al. 1997). The branched PLFAs i15:0, a15:0, i16:0, i17:0, and a17:0 were considered to be indicative of Gram-positive bacteria, and 16:1ω7c, 18:1ω7c and cy19:0 of Gram-negative bacteria. There was one fungal biomarker PLFA, 18:2ω6,9. Abundance of identified fatty acids is expressed as nmols per gram of freeze-dried soil (nmols g⁻¹). Total microbial biomass was calculated as the sum of all PLFAs indicated above.

Three sets of anion and cation Plant Root Simulator (PRS)™ available-nutrient probes (Western Ag Innovations, Saskatoon, Canada) were incubated in the upper 10 cm of the soil 2-5 cm apart, at each of the sampling locations within the retention areas for 90 days. After removal the probes were washed with distilled and deionized water and scrubbed with a stiff brush to remove any residual soil before being shipped to the Western Ag Innovations laboratory in SK, Canada for chemical analysis (Hangs et al. 2004). The following cations and anions were analyzed: NO₃⁻, NH₄⁺, Ca²⁺, Mg²⁺, K⁺, H₂PO₄⁻, Fe³⁺, Mn²⁺, Cu²⁺, Zn²⁺, B³⁺, SO₄²⁻, Pb²⁺, Al³⁺. The three (PRS)™ probes at each sampling location were analyzed together, making one composite sample (200 samples in total). Values express the nutrient absorbed per surface area of the ion exchange membrane (µg of ion / 10 cm²; Qian and Schoenau 2002; Hangs et al. 2004) over 90 days.

2.3.4 Statistical analysis

Due to a lack of ‘true’ replication in the harvested sites, variations in microbial community composition and nutrient availability between sites and among harvesting treatments were assessed by a principal component analysis (PCA) of the relative abundance (mol %) of the different PLFAs and the concentrations of nutrients in the PRS probes, respectively. The factor scores for the samples in the different treatments were then used in a Kruskall-Wallis ANOVA by ranks followed by two-tailed multiple comparison of means, in order to determine if microbial community composition and nutrient availability were significantly affected by the treatments. The same tests (Kruskall-Wallis ANOVA by ranks followed by a two-tailed multiple comparison
of means) were used to assess treatment differences in the other measured variables. All statistical analyses were performed in STATISTICA, version 11 (StatSoft, Inc. Tulsa, OK, USA).

2.4 Results

2.4.1 Microbial biomass and community composition

Total microbial biomass (as indicated by PLFAs) did not differ between the aggregated-retention and dispersed-retention (DR) areas, and within the aggregated-retention area there were no significant differences between samples taken within the retention patch (RP), samples taken along the clear-cut edge (CCE), and samples taken within the clear-cut (CC) (Fig 2.2, p=0.66). However, PCA of the PLFA data indicated a clear difference in microbial community composition between the two areas and at different distances from the retention patch within the aggregated-retention area (Fig. 2.3A, p<0.001). The first principal component, which explained 44% of the variation, separated samples taken in the aggregated-retention area (including samples taken from the clear-cut edge and clear-cut) from samples taken in the dispersed-retention area (Fig 2.3A). The second principal component, which explained 17% of the variation, grouped together samples from within the retention patch and samples from the dispersed-retention area and also grouped together samples along the clear-cut edge and in the clear-cut. Thus, the major factor influencing microbial community composition, as indicated by the PCA, was the sampling area (dispersed-retention versus aggregated-retention areas). Within the aggregated-retention area, fungal PLFA abundance was significantly reduced in the clear-cut and clear-cut edge compared to within the retention patch (p<0.001).

The differences in microbial community composition between the two areas were mostly the result of the lower abundance of the fungal PLFA biomarker 18:2ω6,9 in the dispersed-retention area compared to the aggregated-retention area (p<0.001, Fig. 2.3B, Table 2.1). Within the aggregated-retention area the abundance of 18:2ω6,9 was highest in the retention patch and lowest in the clear-cut, with the clear-cut edge being intermediate (p<0.001,Table 2.1). In the dispersed-retention area the decrease in fungal abundance co-occurred with an increase in the relative abundance of the Gram-negative PLFA biomarker cy19:0 (p<0.05, Table 2.1). In fact, the
reduced fungal abundance due to tree harvesting seemed to generally be associated with increased bacterial abundance (p<0.001, Fig 2.4).

2.4.2 δ\(^{13}\)C\(_{PDB}\) values of microbial PLFAs

In the aggregated-retention area the weighted average δ\(^{13}\)C\(_{PDB}\) values of PLFA biomarkers were significantly lower in the retention patch relative to the clear-cut (Fig. 2.5, p<0.001), with the clear-cut edge being intermediate to these. This trend was also observed in the δ\(^{13}\)C\(_{PDB}\) values of fungal, as well as Gram-negative and Gram-positive biomarker PLFAs (Fig 2.5). The weighted-average δ\(^{13}\)C\(_{PDB}\) values of PLFA biomarkers in samples from the dispersed-retention area were generally similar to samples from the retention patch. There were no significant differences in δ\(^{13}\)C\(_{PDB}\) values of fungal, Gram-positive or Gram-negative biomarker PLFAs between the dispersed-retention area and within the aggregated-retention patch (p>0.05, Fig. 2.5).

2.4.3 Nutrient availability

The availability of soil nutrients, as indicated by the PRS-probes, was similar in the dispersed-retention area and within the aggregated-retention patch (Fig. 2.6A). Only two nutrients differed in availability: K and Mn (Table 2.2). Mn concentrations were almost twice as high within and at the clear-cut edge of the retention patch compared to the dispersed-retention area, whereas K concentrations were slightly lower within the aggregated-retention patch compared to the dispersed-retention area.

In contrast, the availability of nutrients in the clear-cut edge and clear-cut were significantly different than in the retention patch (Fig 2.6A, p<0.001). Most notably NH\(_4^+\) availability was highest in the clear-cut edge and clear-cut area (Fig 2.6B, Table 2.2). There were no differences in concentrations of NO\(_3^-\) between any locations. Higher concentrations of K\(^+\) and H\(_2\)PO\(_4^-\) in the clear-cut edge and clear-cut compared to the aggregated-retention patch were also observed, and the effect was most pronounced in the clear-cut (Fig 2.6B, Table 2.2). Concentrations of Ca\(^{2+}\) and Mg\(^{2+}\) were higher at the dispersed-retention site than in the clear-cut (p<0.05), and comparisons between the clear-cut edge, clear-cut and the aggregated-retention patch revealed a similar trend (Table 2.2). Manganese concentration in the clear-cut was similar to that at the dispersed-
retention area; however, it was almost twice as high within the aggregated-retention patch (Table 2.2). In contrast, there was no significant difference in Mn concentration between the aggregated-retention patch and the clear-cut edge.

2.5 Discussion

The aim of variable-retention harvesting is to maintain the stand structural diversity and biodiversity associated with mature forests, which includes soil microbial diversity (Franklin 1997). In this study, retention of living trees on harvested sites was not successful in preserving the structure of the soil microbial community and activity. Although total microbial biomass was unaffected by harvesting, there was a clear shift in microbial community composition due to clear-cutting in the aggregated-retention area, even within 9 m of the retention patch. The differences between the aggregated and the dispersed-retention areas were even more pronounced. The variation in the aggregated-retention area was mostly due to a lower abundance of the fungal PLFA biomarker 18:2ω6,9 in the clear-cut and clear-cut edge, indicating there was a change in the bacterial to fungal ratio. The same reduction in the fungal PLFA biomarker was found in response to dispersed-retention harvesting, but in the dispersed-retention area there was also a slight shift towards Gram-negative bacteria.

The observed decrease in 18:2ω6,9 in the clear-cut, clear-cut edge and dispersed-retention areas probably indicates reduced abundance of mycorrhizal fungi. Mycorrhizae are dependent on recent tree photosynthates for carbon and so are particularly susceptible to tree harvesting (Hagerman et al. 1999; Byrd et al. 2000; Busse et al. 2006; Hartmann et al. 2009). Girdling studies have shown that removal of recent photosynthates belowground reduces the fungal, and to a lesser extent bacterial communities within weeks (Högberg et al. 2008). Accordingly, Mummey et al. (2010) found that fungal biomass was significantly reduced in grand fir, lodgepole pine and Douglas-fir forest soils following clear-cutting. Similarly, Luoma et al. (2004) found a decrease in ectomycorrhizal (ECM) sporocarp production in sites with lower stem retention in both aggregated and dispersed harvesting regimes. However, other studies have shown that the presence of aggregated tree clumps can support mycorrhizal communities up to 10 m (Jones et al. 2008) and even 20 m into a clear-cut area through root-exuded C (Churchland et al. 2012).
The low $\delta^{13}\text{C}_{\text{PDB}}$ value of PLFAs in both the aggregated-retention patch and the dispersed-retention site, compared to the clear-cut areas, concurs with the fact that recently photosynthesized labile plant C exuded by roots is typically $^{13}\text{C}$-depleted relative to older C stored in coarse woody debris and soil organic matter (Kuzyakov 2006; Werth and Kuzyakov 2010). This suggests that the microbial community in the clear-cut and clear-cut edge is more reliant on C sources that are older and more recalcitrant, whereas recently photosynthesized plant C is a major C source in the retention patch and in the dispersed-retention area. Even if the average $\delta^{13}\text{C}_{\text{PDB}}$ value of microorganisms was similar in the retention patch and in the dispersed-retention area, differences in microbial community composition between the two suggest that the recently photosynthesized labile plant C was utilized by different microorganisms. Mycorrhizal fungi absorb recently photosynthesized C directly from the tree root and tend to be $^{13}\text{C}$-depleted (Bowling et al. 2008; Churchland et al. 2013; Chapter 3 this thesis). The dispersed-retention site had significantly lower abundance of the fungal PLFA biomarker 18:2ω6,9 than the retention patch. Instead, a greater proportion of plant C seemed to be directly utilized by bacteria, particularly Gram-negative bacteria, as suggested by the increased abundance of the Gram-negative biomarker PLFA cy19:0 in the dispersed-retention area relative to the retention patch. Accordingly, Gram-negative bacteria are the dominant bacteria associated with the rhizosphere (Paterson et al. 2007). The decline in mycorrhizal fungal abundance may have allowed the dominant Gram-negative bacteria to access more of the recently photosynthesized C, as also indicated by the lower $\delta^{13}\text{C}_{\text{PDB}}$ value of the Gram-negative PLFA’s, resulting in the observed increase in their abundance. Högberg et al. (2010) recently demonstrated through FACE studies that fungi and Gram-negative bacteria are the main utilizers of tree root exudate carbon.

Although microbial community composition was significantly altered by dispersed-retention harvesting, nutrient concentrations remained remarkably similar to those in the uncut patch of retained trees. In contrast, shifts in the microbial community composition in the clear-cut and the clear-cut edge co-occurred with altered nutrient availability. This was most clearly manifested as an increase in available $\text{NH}_4^+$ concentrations in the clear-cut and clear-cut edge. The different nutrient availability in the dispersed-retention area and the clear-cut and clear-cut edge cannot be explained by changes in the microbial biomass, as there was no significant difference in total microbial biomass among these treatments. However, microbial biomass has been shown to
respond slowly to environmental change, even when there is up to ten-fold variation in microbial activity (Bloem et al. 1992; Rousk and Bååth 2011). The isotopic data suggest that the soil microbes in the clear-cut and clear-cut edge may have had less access to labile C in the form of root exudates, compared to the retention patch and the dispersed-retention area, which would reduce microbial activity. Since the quantity and quality of C often limits microbial growth and activity (Bengtson et al. 2005; Demoling et al. 2007), the greater availability of recent photosynthates would result in more vigorous microbial growth, which in turn would influence the availability and turnover of nutrients (Bengtsson et al. 2003). There are also indications that when the availability of labile plant-C is low, gross N mineralization exceeds gross N immobilization, resulting in net mineralization of N, while net immobilization (by both microbes and tree roots) occurs at high availability of labile plant-C (Bengtson et al. 2012). This may explain the low NH$_4^+$ concentrations in the retention patch and in the dispersed-retention area.

The similarity in concentrations of nutrients in the dispersed-retention area and within the aggregated-retention patch suggests that dispersed-retention harvesting is effective for avoiding elevated concentrations and potential leaching losses of nutrients such as N and K. Most studies agree that single-tree retention mitigates N losses (Hope et al. 2003; Prescott et al. 2003; Redding et al. 2003; Jerabkova et al. 2011). Our results suggest that dispersed-retention harvesting that retains as few as 40 trees ha$^{-1}$, is sufficient to maintain pre-harvest N availability. In contrast, clear-cutting resulted in elevated NH$_4^+$ availability. Proximity to the aggregated-retention patch did not affect N availability in our study, unlike previous studies (Theil and Perakis 2009).

Taken together, our results suggest that as long as the microbial community has access to recent plant C, as was the case in the dispersed-retention treatment, the functions which the microbial community provides in terms of nutrient cycling remain largely unchanged after harvest. This indicates that these particular functions are generic —i.e., as long as the total microbial biomass and activity remains the same, decomposition and nutrient cycling will not be greatly affected by shifts in microbial community composition. There was, however, one major exception. The Mn$^{2+}$ concentration, as determined by PRS-probes, was almost twice as high in the retention patch (and in the clear-cut edge) compared to the dispersed-retention area and the clear-cut area. This may be related to the low abundance of ectomycorrhizal fungi in the dispersed-retention area and in
the clear-cut area. Manganese is only soluble in its reduced state (Schwab and Lindsay 1983). It has been demonstrated that the abundance of Mn-reducing bacteria is several times higher in the rhizosphere of plants colonized by arbuscular mycorrhizae compared to non-mycorrhizal plants, resulting in higher concentrations of soluble Mn (Nogueira et al. 2007). To our knowledge there have been no studies designed to test if ectomycorrhizae have the same stimulatory effect on Mn-reducing bacteria, but our results suggest that this may be the case. Manganese reduction is only performed by a few specialized microorganisms. Therefore, even though dispersed-retention harvesting methods are successful in preserving generic soil functions, specialist functions are more responsive to changes in microbial community composition. An alternative explanation to the reduced Mn concentrations in the dispersed-retention site and the clear-cut area is that the fungal communities in these two areas were dominated by saprotrophic fungi growing on woody debris and partly decomposed litter. Manganese peroxidase (MnP) is the most common lignin-modifying enzyme produced by white-rot fungi and other wood and litter decomposing basidiomycetes (Hofrichter 2002). Manganese peroxidase catalyzes the oxidation of soluble Mn$^{2+}$ to highly reactive Mn$^{3+}$, which effectively removes the Mn from the soil solution.

In conclusion, my results demonstrate that harvesting of trees influences the soil microbial community composition, alters the microbial communities’ access to labile C sources, and affects soil nutrient availability. The presence of a retention patch in the aggregated-retention site did not appear to lessen the impact of harvesting on soil microbial community structure or nutrient availability in the clear-cut, even within 6 meters. In contrast, dispersed-retention harvesting seemed to mitigate the effects of harvesting on nutrient cycling, as demonstrated by the remarkably similar concentration of nutrients in this treatment relative to the aggregated-retention patch. Dispersed-retention appears to be the most effective variable-retention harvesting system to maintain microbial functioning and nutrient cycling. Shifts in the microbial community composition in response to harvesting in the dispersed-retention area did not have a major impact on microbial functioning and nutrient availability. However, this may only be true for generic functions/processes; other ‘narrow’ processes (those performed by a small number of specialized microorganisms) such as lignin degradation, may be more sensitive.
Figure 2.1 Diagram of the aggregated-retention area showing the split into clear-cut, clear-cut edge and retention-patch treatments. Each square represents a sampling site, 2 m x 3 m.
Figure 2.2 Total microbial biomass in the dispersed-retention (DR) area, the retention patch (RP), in the clear-cut area (CC, 9 m or more from the retention patch) and in the clear-cut edge area (CCE, within 9 m of the retention patch). Vertical errors bars represent standard error.
Figure 2.3 Principal component analysis ordination showing a) soil microbial communities based on PLFA abundance (mol%) in the dispersed-retention (DR) area, the retention patch (RP), in the clear-cut area (CC, 9 m or more from the retention patch) and in the clear-cut edge area (CCE, within 9 m of the retention patch). b) The relative contributions of individual PLFAs to the community structure. Horizontal and vertical error bars represent standard error.
Figure 2.4. Bacterial abundance (expressed as mol% bacterial PLFAs) in the dispersed-retention (DR) area, the retention patch (RP), in the clear-cut area (CC, 9 m or more from the retention patch) and in the clear-cut edge area (CCE, within 9 m of the retention patch). Vertical errors bars represent standard error.
Figure 2.5 Weighted average $\delta^{13}$C$_{PDB}$ values of all analyzed PLFAs (square), the fungal biomarker PLFA 18:2w6,9 (triangle), and bacterial Gram-negative (diamond) and Gram-positive (square black) biomarker PLFAs in the dispersed-retention (DR) area, the retention patch (RP), in the clear-cut area (CC, 9 m or more from the retention patch) and in the clear-cut edge area (CCE, within 9 m of the retention patch). Vertical errors bars represent standard error.
Figure 2.6 Principal component analysis showing a) soil nutrient availability based on the concentration of anions and cations adsorbed onto PRS probes in the dispersed-retention (DR) area, the retention patch (RP), in the clear-cut area (CC, 9 m or more from the retention patch) and in the clear-cut edge area (CCE, within 9 m of the retention patch). b) Relative contributions of individual ions to differences in nutrient availability. Horizontal and vertical error bars represent standard error.
Table 2.1 Abundance (mol%) of PLFAs in the clear-cut area, the clear-cut edge area within 9 meters of the retention patch, the retention patch and the dispersed-retention area. SE represents standard error of the means.

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<td>10.4 0.4</td>
<td>12.3 0.4</td>
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</table>
Table 2.2 pH (H$_2$O) and soil nutrient availability (PRS-probes, top 10 cm) in the clear-cut area, the clear-cut edge area within 9 meters of the retention patch, the retention patch and the dispersed-retention area. SE represents standard error of the means. Separate for each nutrient, similar letters indicate the individual treatments are not significantly different (Kruskall-Wallis ANOVA by ranks followed by a two-tailed multiple comparison of means, $\alpha>0.05$). n/a = not available.

<table>
<thead>
<tr>
<th></th>
<th>Clear-cut</th>
<th>Clear-cut Edge</th>
<th>Retention Patch</th>
<th>Dispersed-retention</th>
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<td></td>
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<td>SE(n=30)</td>
<td>Mean</td>
<td>SE(n=20)</td>
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<tr>
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3 Spatial variability of soil fungal and bacterial abundance: Consequences for carbon turnover along a transition from a forested to clear-cut site

3.1 Synopsis

Live trees retained on harvested sites are thought to act as hubs of pre-harvest microbial communities as a result of the continuous supply of C through their root exudates. In order to investigate to what degree living trees influence the abundance and activity of mycorrhizal fungi, saprotrophic fungi, and bacteria, a geostatistical approach was used to examine natural abundance stable-isotope ratios of soil microbial PLFAs, respired CO₂, and different SOM pools in a 100-point grid, extending from an area of retention trees into a clear-cut area. Labile C from trees was the major source of C for the fungal communities and influenced the composition of the microbial community and soil respiration rates up to ten meters into the clear-cut. When the input of labile plant C decreased, it appeared that microorganisms became increasingly dependent on recycled C released during microbial turn-over, resulting in a decrease in soil respiration. The findings demonstrate that by influencing the structure and function of soil microbial communities, plants act as important regulators of belowground C flux, soil C sequestration and, ultimately, soil C stocks.

3.2 Introduction

Shifts in public perception have resulted in clear-cutting becoming socially unacceptable due to environmental and aesthetic losses (Bliss 2000). Consequently, alternative silviculture practices, including those that retain live trees on the site, also known as variable-retention harvesting, have replaced clear-cutting in North America and Europe (Elfving and Jakobsson 2006; Work et al.)

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Live trees retained on clear-cuts sites are thought to minimize habitat loss, minimize above and belowground disturbance caused by logging equipment, retain above and belowground biodiversity, and more closely emulate natural disturbance events in forest ecosystems (Kimmins, 2004). Retained trees will ameliorate microclimate stress through reduction of transmitted light, air temperature, soil temperatures, and soil moisture levels (Heithecker and Halpern, 2006). Retained trees will also act as a seed source, ensuring seedlings are the correct ecotype (Franklin et al., 1997), and will maintain habitat connectivity (Heithecker and Halpern, 2006; Franklin et al., 1997).

The effect of retained trees on maintenance of soil quality following harvesting, through their effect on belowground microbial communities, is less established. Trees provide a continual source of carbon (C) to the microbial and faunal community through leaf, branch and root litter, as well as through root exudation of recent photosynthates (Franklin et al., 1997). The combination of access to labile C and maintenance of a suitable microclimate may result in retention trees on clear-cut sites acting as ‘life-boats’ or ‘hub trees’ for microbes, maintaining pre-harvest soil microbial community structure and function, and providing species-specific mycorrhizal inoculum for future seedlings (Franklin et al., 1997). Ectomycorrhizal (ECM) community diversity is maintained by retention patches as small as 5-40 meters in diameter, but are reduced at distances greater than 10 meters from the patch (Jones et al., 2008). Luoma et al. (2004) observed ECM sporocarp and truffle production in both aggregated and dispersed-retention at four levels of tree retention (100, 75, 40 and 15%). Treatments that reduced canopy cover in either a dispersed or aggregated-retention resulted in decreased sporocarp production; however, this effect varied among sporocarp types and among seasons in each treatment. Truffle sporocarps observations were more equally distributed in spring and fall, and more prevalent in lower levels of aggregate and dispersed-retention compared to ECM mushrooms. Daradick (2007) and Dewi (2009) determined that green-tree-retention (GTR) retained enzyme activities within 20 meters of aggregated-retention patches, four months and five years after GTR harvest, respectively. Daradick (2007) suggested that the supply of labile C in the form of root exudates and litter from the retained trees maintained the pre-harvest microbial community in this 20 meter zone of influence, but neither Daradick or Dewi explored how this C influences the microbial community structure and how it contributes to C cycling.
To understand how forest ecosystems respond to a changing environment, it is necessary to address the fate of plant-C allocated belowground and determine how plants influence the abundance and flow of C to different groups of soil microorganisms, and in turn, determine how retained trees influence soil C-cycling. Plants allocate approximately one third of recently photosynthesized C belowground, though it could increase to two thirds depending on the species and climatic conditions (Litton et al. 2007). The C allocated belowground will support the growth and activity of roots, mycorrhizae and rhizosphere microbial communities (Read 1991; Curtis et al. 2002; Read and Perez-Moreno 2003). However, how this C is allocated between root biomass, root respiration, mycorrhizal biomass and mycorrhizal respiration, and how much is exuded, varies (Goodale et al. 2002; Lipson et al. 2005; Kandeler et al. 2008; Castro et al. 2010), and has been historically difficult to assess. Nadelhoffer and Raich (1992) analyzed 59 studies that measured fine-root production in a variety of forest ecosystems using replicate soil cores over the course of at least one year. They determined that approximately 33 % of all C allocated belowground is used for fine-root production. By cutting off the flow of C belowground using a tree-girdling experiment Högberg et al. (2001) determined that 37-54 % of soil efflux is derived from roots and symbiotic microbes metabolizing recently assimilated carbohydrates. Using a variety of stable-isotope pulse-labeling, continuous-labeling and C3 vs. C4 plant methodological techniques, Kuzyakov and Domanski (2000) and Smith (1986) have found 1 to 30 % of total photosynthate can be exuded from tree roots. Allocation of plant-C to root exudates has also been found to stimulate soil organic matter (SOM) decomposition, often referred to as the ‘priming effect’ (Dijkstra and Cheng 2007; Phillips et al. 2009). However, it is generally assumed that C allocated to structures with long turnover times (weeks to years), e.g. roots and fungal hyphae, will contribute to long-term C storage (Langley et al. 2006; Cheng 2009). Concurrently, the rate-limiting step of heterotrophic soil respiration in the bulk soil appears to be decomposition of SOM into dissolved organic C (DOC) (Guggenberger and Zech 1993; Huang et al. 1998; Bengtson and Bengtsson 2007). Furthermore, evidence is emerging that fungal versus bacterial dominance in the soil could have profound effects on the belowground C-cycle (Jastrow et al. 2007; Cheng 2009; Strickland and Rousk 2010; Drake et al. 2011; Zak et al. 2011), and it has been suggested that compositional shifts in soil saprotrophs are the drivers of ecological response to environmental disturbance (Zak et al. 2011).
Stable-isotope-probing provides the means to tease out ecosystem C-flow \textit{in situ}. Natural variation in the ratio of $^{13}\text{C}/^{12}\text{C}$ is used to infer the origin and flow of C in the environment (Schweizer et al. 1999). Isotopic enrichment and depletion varies depending on fractionation, a process where lighter elements are preferentially used in catabolic reactions, and heavier elements are typically incorporated into biomass (Bowling et al. 2008). For example, the isotopic composition of microorganisms typically reflects the isotopic composition of their C sources (Abraham et al. 1998; Cifuentes and Salata 2001; Wick et al. 2003). Consumers’ tissues are $^{13}\text{C}$-enriched by 1 %o relative to their food source, and bacteria are $^{13}\text{C}$-enriched 1-2 %o relative to their substrates. Respired CO$_2$ is typically $^{13}\text{C}$-depleted by 1-2 %o relative to the food source. In addition, many herbivorous food sources have different $^{13}\text{C}$ content (e.g. leaves, wood, soil organic matter; Schweizer et al. 1999, Weber et al. 2008). Thus, by comparing the ratio of $^{13}\text{C}/^{12}\text{C}$ within microbial signature phospholipid fatty acids (PLFAs) to that of different C pools, including recently photosynthesized C, I was able to determine which microbial groups are relying on recently photosynthesized C for growth and determine their relative contribution to soil C-cycling.

Plants create and maintain a mosaic of environmental conditions through differences in nutrient demands, transpiration, and above- and belowground C inputs. This will translate into spatial variation in $\delta^{13}\text{C}$ values, due to different C inputs, age of plants and consumption of the material by soil microorganisms and fauna. Little is known about how proximity to plants, specifically large trees, translates into corresponding spatial variations in the activity and abundance of different groups of microorganisms. I, therefore, designed a study with two objectives. The first was to determine the degree to which living trees influence the relative abundance and activity of mycorrhizal fungi, saprotrophic fungi and bacteria, and identify the major C sources used by these organisms for growth. The second was to identify the influence of trees on soil C-cycling and respiration. The objectives were achieved using a geostatistical approach where the spatial variation in soil microbial community composition and C turnover was studied in an evenly distributed 100-point 27- by 18-meter plot located within a second-growth forest along a transition from an area of retention trees into a two-year-old clear-cut area. At each sampling point I measured quantitative values of soil-respired CO$_2$ (mg C m$^{-2}$ h$^{-1}$) and PLFAs (nmol PLFA g$^{-1}$ soil dry weight), $\delta^{13}\text{C}$ values of soil-respired CO$_2$, DOC, SOM and phospholipid fatty acids
(PLFAs), and compared relative δ^{13}C values of soil-respired CO₂, DOC, SOM and phospholipid fatty acids (PLFAs).

### 3.3 Methods

#### 3.3.1 Site description

Samples were collected on May 5^{th}-6^{th}, 2009 in the third replicate of the existing Silviculture Treatments for Ecosystem Management in the Sayward (STEMS) long-term research installation set up by the British Columbia Ministry of Forests and Range, close to Gray Lake, near Campbell River, on Vancouver Island, B.C. (50°03’43.02’’N, 125°35’24.35’’W). The site is a second-growth forest, composed of 60 to 70-year-old western hemlock (*Tsuga heterophylla*) and Douglas-fir (*Pseudotsuga menziesii*) trees, in the submontane very dry maritime Coastal Western Hemlock biogeoclimatic zone (CWHxm2) (de Montigny 2004). In 2008 the site was harvested by variable-retention practices, leaving aggregated tree patches approximately 0.2 ha in size, with clear-cut spaces ranging from 4.4 to 11.5 ha, in a total area of 25.5 ha. The undergrowth consisted mainly of salal (*Gaultheria shallon*), and sword fern (*Polystichum munitum*). The soils were podzolized, with a mix of mineral and organic layers in the clear-cut area due to harvesting machinery.

#### 3.3.2 Soil and gas sampling

An equally distributed 10x10 point grid with sampling points 3 m apart in the north-south direction (27 m) and 2 m apart in the east-west direction (18 m) was positioned such that half the sampling area was located within the aggregated-retention site, while the other extended due north into the clear-cut area. At each sampling point three soil cores were collected using a 5-cm-diameter stainless steel corer to a depth of 10 cm. The cores were composited on site, stored on ice within 2 hours of sampling and stored at 4°C within 6 hours. The soil was later sieved to 2 mm and split into subsamples for analysis of soil organic matter (SOM), dissolved organic carbon (DOC) and phospholipid fatty-acids (PLFA).
Soil CO₂ emissions were measured at each sampling point using a closed non-steady state incubation apparatus (Basiliko et al. 2009). A 10-cm-diameter, 7-cm-tall piece of Polyvinyl Chloride (PVC) piping was installed into the forest floor or soil at each sampling point, such that 3.5 cm of the pipe (collar) was exposed. Twenty-four hours after installation the collars were fitted with 20-cm-tall PVC pipe chambers, fitted with a rubber suba-seal. Gas sub-samples (14 ml) were collected after 0, 20, 40 and 60 minutes of incubation and stored in vacuumed exetainers (Labco) prior to analysis at the Belowground Ecosystem Group Stable Isotope Facility in the Faculty of Forestry at the University of British Columbia. Exetainers were over-pressured with sample to avoid atmospheric contamination, and the presence of atmospheric CO₂ in the sampling chambers was corrected for using Keeling plot procedures (Keeling 1958; Petaki et al. 2003).

3.3.3. Soil physical, biological and chemical analysis

Soil organic C was measured by acid-fumigating oven-dried soil to remove carbonates, which tend to skew isotopic results in mineral soil (Harris et al. 2001). The δ¹³C values of DOC was measured according to the technique in Bengtson and Bengtsson (2007). Briefly, 0.5 g of field moist soil was placed in 0.2-µl Z-spin filter units (Pall, Nanosep MF, Mexico). These were then centrifuged at 16000 × g for 30 minutes (DiaMed, Mirko 20, Canada). The resulting soil water in the collection tube was transferred into 8-mm tin cups, evaporated at 35°C and stored at room temperature before isotopic analysis. There was no difference in soil moisture between the forested and clear-cut area (data not shown).

Phospholipid fatty acids (PLFA) were extracted from 1.2 g of freeze-dried soil according to the Frostegård et al. (1991) method, based upon the Bligh and Dyer (1959) procedure, and further modified by White et al. (1979). Briefly, soil samples were vortex-extracted in a 0.8:1:2 (v/v/v) solution of citrate buffer, chloroform and methanol. The extracted lipids were fractionated into neutral lipids, glycolipids and phospholipids on Accubond II Solid Phase Extraction silica columns (Agilent Technologies Inc., Santa Clara, CA) by elution with chloroform, acetone and methanol, respectively. A known amount of methyl nonadecanoate (19:0) was added to the fraction containing the phospholipids and the lipids were then transmethylated to their fatty acid
methyl esters using mild alkaline methanolysis. Following alkaline methanolysis, fatty-acid residues were flash-evaporated under N2-gas and stored in 200 μl hexane at -20 °C until analysis. PLFA peaks were identified by means of a combination of mass spectra and retention times relative to the internal standard 19:0, and an external bacterial acid methyl ester standard (BAME; Sigma-Aldrich co., 47080-U, Oakville, ON, Canada). Each sample, including the external standard BAME, was anchored relative to an internal standard (C19:0). Sample batches were two-point calibrated to C20:0 isotopic standards that bracketed the expected range of δ13C values (C20’s equaled -30.68 ‰, standard deviation 0.02 ‰ versus VPDB, and -6.91 ‰, standard deviation 0.04 ‰ versus VPDB; Isocanoic acid methyl ester, certified reference material, Indiana University).

The following fatty-acids were chosen to represent bacterial PLFAs: i15:0, a15:0, 15:0, i16:0, i16:1ω7c, i17:1ω8c, 10Me17:0, i17:0, a17:0, 18:1ω7c, 18:1ω5c, cy19:0 (Frostegård et al. 1993; Kroppenstedt 1985; Zogg et al. 1997). Fungal biomarkers are limited to 18:2ω6,9 (See Table 3.1. for a full list of PLFA’s). Abundance of identified fatty-acids are expressed as nmols per gram of freeze-dried soil (nmols g⁻¹) and were calculated based on the chromatograph peak areas relative to the peak area of the internal standard 19:0. PLFA nomenclature identifies the number of C atoms in the fatty-acid chain (e.g. 18 in 18:1ω7), the number of double bonds in the chain (e.g. 1) and the position of the first double-bonded C from the methyl end of the fatty-acid molecule (e.g. ω7).

3.3.4. Isotope analysis

Gas and PLFA 13C/12C ratios were analyzed with Agilent Technologies Gas Chromatograph 6890N (Santa Clara, CA, USA), interfaced with a GV Instruments GC5 MK1 combustion furnace interfaced with a GV Instruments Isoprime ratio mass spectrometer (both Cheadle Hulme, UK) at the Belowground Ecosystem Group Stable Isotope Facility in the Faculty of Forestry at the University of British Columbia. An elemental analyzer (Elementar Vario EL Cube: in C, N mode; Hanau, Germany) interfaced with an isotope ratio mass spectrometer (GV Instruments Isoprime, with reference gas box; Cheadle Hulme, UK) via continuous flow, also at the University of British Columbia, was used to measure the 13C and 12C content of DOC.
3.3.5. Geostatistics

Variogram model fitting and kriging were performed with the geostatistical software GS\textsuperscript{+TM} version 9 (Gamma Design Software, Plainwell, Michigan, USA). Depending on the range of spatial autocorrelation, the active lag (the range over which the semivariance was calculated) was set to 18.1-27.1 m and the semivariance determined for uniform lag classes of 2.1 or 3.1 meter intervals. Exponential or spherical model variograms were fitted to the calculated semivariances using a least squares technique. The software’s inbuilt kriging function was used for interpolation of values between sampling points, and the interpolated values were calculated from a maximum of 16 neighbors. This resulted in low and relatively uniform standard deviations across the interpolated area. The interpolation estimates were placed on a uniformly spaced grid with 0.25 m intervals and mapped in STATISTICA version 9.1 (StatSoft, Inc. Tulsa, OK, USA).

3.4 Results

3.4.1. Soil respiration and $\delta^{13}$C$\text{PDB}$ values of CO$_2$, DOC and SOM

Soil respiration and $\delta^{13}$C values of CO$_2$, DOC and SOM were all spatially autocorrelated at scales varying from 7 m to >27 m (Table 3.2). Soil respiration rates varied from a maximum of 80 mg C m$^{-2}$ h$^{-1}$ to a minimum of 40 mg C m$^{-2}$ h$^{-1}$ and were autocorrelated at a scale of approximately 7 m (Fig. 3.1a). The respiration rate was highest in (and in proximity to) the patch of retention trees, and decreased with increasing distance from the retained trees. The $\delta^{13}$C values of the respired CO$_2$ were spatially autocorrelated at a similar scale (7.4 m) and had an average $\delta^{13}$C value of -28 to -30‰ in the forested area (Fig. 3.1b). The $\delta^{13}$C values of CO$_2$ gradually increased to between -26 to -27‰ in the clear-cut area.

The isotopic composition of DOC and SOM ($\delta^{13}$C values of -27 to -28‰) was less variable than CO$_2$ and did not seem to be related to the presence of trees (Figs 3.1c and 3.1d). However, when the relative $^{13}$C enrichment of CO$_2$ was compared with DOC and SOM, a clear pattern emerged. The CO$_2$ emitted from soil was $^{13}$C-depleted by 1-2‰ compared to DOC and SOM in the forested area, extending a few meters into the clear-cut area. In contrast, the CO$_2$ emitted from the clear-cut area was slightly $^{13}$C-enriched relative to DOC and SOM (Figs 3.1c and 3.1d).
3.4.2. Microbial biomass and the relative abundance of fungi and bacteria

The concentration of PLFAs, a proxy for microbial biomass, varied between 100 nmol PLFA g\(^{-1}\) soil to 180 nmol PLFA g\(^{-1}\) soil and was spatially autocorrelated at a scale of approximately 20 meters (6 m for bacteria; >27 m for fungi) (Table 3.2, Fig 3.2a). Although the highest biomass was found in the area with retained trees and the lowest in the clear-cut area, there was no obvious trend of decreasing biomass with increased distance to the wooded area. However, the relative abundance of fungi and bacteria was clearly dependent on the distance from the retained trees (Fig. 3.2b). The fungal biomarker PLFA 18:2\(\omega6,9\) comprised 20-25 % of the total PLFAs in the forested area. A similar abundance of the fungal PLFA biomarker was found up to 10 meters into the clear-cut area and then it sharply declined, such that 18:2\(\omega6,9\) only made up about 10 % of the total PLFAs in samples taken more than 10 meters from the trees. The \(\delta^{13}C\) values of 18:2\(\omega6,9\) exhibited a similar pattern, with \(\delta^{13}C\) values in the range of -29 ‰ in the wooded area extending a few meters into the clear-cut area, where it then gradually increased to approximately -26 ‰ (Fig. 3.2c). The weighted average \(\delta^{13}C\) value of bacterial PLFAs exhibited a similar pattern and varied between -24.8 to -26.8 ‰, with a range of spatial autocorrelation of 6.4 m (Table 3.2, Fig. 3.2d). The bacterial PLFAs were clearly \(^{13}C\)-enriched relative to DOC and SOM (Figs 3.3a and 3.3b). In the forested area, bacterial PLFA \(^{13}C\) enrichment was about 0.5-1 ‰, which gradually increased to almost 3 ‰ in the clear-cut area. Potential variations in the relative contribution of saprotrophic fungi and ectomycorrhizal fungi to the 18:2\(\omega6,9\) biomarker made it difficult to compare \(\delta^{13}C\) values of saprotrophic fungi relative to DOC and SOM, and therefore, this analysis was not attempted.

3.5. Discussion

My results demonstrate that trees influence the composition of the microbial community up to ten meters into the clear-cut, and that fungi are proportionally more abundant (approximately 2.5 times greater) in, and near, the forested area. The most likely explanation for the decrease in fungal biomass with increasing distance from the wooded area is that mycorrhizal fungi are less abundant in the clear-cut area. This conclusion is supported by an isotopic shift in the fungal biomarker 18:2\(\omega6,9\) in the clear-cut compared to the forested area. Studies of pure cultures of
fungi growing on single substrates demonstrate that δ^{13}C values of the fungal PLFA biomarker, 18:2ω6,9, follow the general pattern of being ^{13}C-enriched by ~1-2 % compared to the substrate (Abraham and Hesse 2003). Saprotrophic fungi are, on average, ^{13}C-enriched by 3 % relative to mycorrhizal fungi, as mycorrhizae obtain their carbon directly from recently photosynthetically-fixed C, which is ^{13}C-depleted relative to SOM (~26 % vs. -29 % respectively) (Bowling et al. 2008). The δ^{13}C value of 18:2ω6,9 in the forested area was intermediate to these (~ -27 to -29.3 %). Since 18:2ω6,9 is generally slightly ^{13}C-enriched compared to bulk biomass (Abraham and Hesse 2003), our results suggest that mycorrhiza made up at least half of the fungal community in the forested area. On the other hand, the δ^{13}C value of that same biomarker in the clear-cut area gradually increased (~ -26 to -27 %) 5-10 meters from the retention area, implying that the fungal community transitions to one that is dominated by saprotrophic fungi in the absence of trees. Luoma et al. (2004) observed a decrease in ectomycorrhizal (ECM) sporocarp production in forest sites with lower stem retention under both aggregated and dispersed harvesting regimes. Other studies have found a significant reduction in ECM species richness at a distance of 10 meters from retention trees (Jones et al. 2008). The finding that the δ^{13}C values of the bacterial PLFAs were more ^{13}C-enriched than fungi suggests bacteria are less reliant on recently photosynthesized plant C for growth than fungi. This conclusion is supported by the findings of the research outlined in Chapter 4 of this thesis, where I have shown, using stable-isotope-labeling of photosynthates of mature trees followed by stable-isotope-probing of microbial PLFAs, that it is the fungal community that utilizes labile C released from tree roots (Churchland et al. 2012; Chapter 4, this thesis).

The shift in microbial community composition, from mycorrhizal to saprophytic fungi and increased bacterial abundance, 5-10 m from the forested area is reflected in the soil CO₂ efflux as well as the isotopic ratio of soil-respired CO₂. This shows that the trees are not only influencing the composition of microbial communities, but also influencing microbial activity. Soil respiration was up to twice as high in the forested area compared to the clear-cut area. These findings mirror what has been found in girdling experiments, where 30 to 65 % of forest soil efflux is attributed to mycorrhizal root respiration (Singh et al. 2003; Högberg et al. 2001; Scott-Denton et al. 2006). However, the lower respiration rate in the clear-cut area cannot be explained by diminishing mycorrhizal and root respiration alone as the shift begins 3-5 meters from the
edge of the retention patch. The exudation of labile C from tree roots may also contribute to higher soil respiration in the forested area (Phillips and Fahey 2006; Dijkstra and Cheng 2007). Addition of glucose and other labile C sources has been found to increase SOM decomposition (through priming effects), resulting in an increase of up to 380% in heterotrophic respiration (Cheng 2009; Kuzyakov 2010). This topic is explored further in Chapter 5 of this thesis.

Heterotrophic soil microorganisms can only take up dissolved compounds in the soil solution, making dissolved organic C (DOC) their sole source of C. Microbial biomass is typically $^{13}$C-enriched compared to their C source, usually by approximately 1-2‰. The isotopic composition of bacterial PLFAs not only varies due to isotopic fractionation during synthesis, but also varies depending on C sources and growth conditions (Abraham et al. 1998; Cifuentes and Salata 2001; Wick et al. 2003). There is some concern that PLFA $\delta^{13}$C values might not reflect the $\delta^{13}$C values of microbial biomass. However, there is evidence that the $\delta^{13}$C values of bacterial PLFAs are, on average, very close to that of the substrate when the bacteria are grown heterotrophically on substances with more than one C source (Cifuentes and Salata 2001; Wick et al. 2003). Abraham et al. (1998) confirmed that pure cultures of different bacteria and fungi grown on a variety of $^{13}$C-enriched sources have many PLFAs (e.g. 16:0, 16:1ω7, 18:0, 18:1ω7), as well as total microbial biomass, with $\delta^{13}$C values similar to, or slightly $^{13}$C-enriched, compared to the substrates.

The $^{13}$C enrichment of microbial biomass during assimilation necessitates a corresponding $^{13}$C depletion in the fraction of the C source that is released as CO$_2$ (Schweizer et al. 1999; Weber et al. 2008). The expected microbial $^{13}$C enrichment compared to the C substrate corresponds well to what was found in the forested area, but in the clear-cut area the microbial PLFAs were $^{13}$C-enriched by up to 3‰ relative to DOC. Curiously, the same was true for the respired CO$_2$, which was $^{13}$C-enriched by up to 1.5-2‰ compared to DOC in the same area. This is by no means an unusual finding; Boström et al. (2007), Comstedt et al. (2006), Werth and Kuzyakov (2010), among others, have found microbial biomass, as well as respired CO$_2$, to be more $^{13}$C-enriched than SOM and DOC. Such findings conflict with isotopic mass balancing, which stipulates $^{13}$C enrichment in one compound must be accompanied by $^{13}$C depletion in another (Park and Epstein, 1961). It has been suggested that preferential utilization of labile $^{13}$C-enriched substrates
may explain these observations (Boström et al. 2007; Werth and Kuzyakov 2010), but the source of such substrates is not obvious. Plant-derived C is usually $^{13}$C-depleted relative to DOC, and there is no evidence that $^{13}$C-enriched labile C is preferentially released from SOM. On the contrary, microbially-processed SOM found at greater depths in the soil profile tends to be $^{13}$C-enriched (Ågren et al. 1996). Here I propose that the $^{13}$C enrichment of microbial C, as well as soil-respired CO$_2$, in the clear-cut area is caused by a microbial community that in large part is tightly recycling C released during microbial turn-over.

Carbon enters the DOC pool via three major routes: 1 - decomposition of SOM; 2 - active exudation and turnover of microbial cells; 3 - input of plant C, through root and mycorrhizal turnover and root exudation. Dissolved organic C produced by route 1 has approximately the same $\delta^{13}$C value as SOM. Dissolved organic C produced by route 2 is $^{13}$C-enriched compared to SOM and plant C, as C incorporated into microbial biomass will tend to be $^{13}$C-enriched relative to its C source. Dissolved organic C produced by route 3 will tend to be $^{13}$C-depleted, as plant C tends to be $^{13}$C-depleted relative to SOM. If the input of plant C decreases then the pool of DOC would gradually become $^{13}$C-enriched because microbial-derived DOC would constitute a greater portion of the DOC pool (see Figure 3.4). Bacteria have been shown to turn over every 1 to 54 days (Bååth 1998; Uhlírova and Šantrúčková 2003), and fungi every 130 to 150 days (Rousk and Bååth 2007). In the two years since the site was clear-cut the microbial community may have turned over several times, enriching both the labile DOC pool and succeeding microbial biomass with every cycle. This conclusion is supported by the increased $\delta^{13}$C value of the fungal and bacterial community within the clear-cut area. DOC and SOM have been found to gradually become $^{13}$C-enriched in the absence of plant C inputs (Ågren et al. 1996; Bengtson et al. 2005). Furthermore, a constant concentration and turnover rate of the DOC pool can only be maintained if 25-30 % of the influx of DOC originates from root exudation and root-associated degradation (Bengtson et al. 2005). Accordingly, respiration in this study decreased from 80 mg C m$^{-2}$ h$^{-1}$ in the forested area to a minimum of 40 mg C m$^{-2}$ h$^{-1}$ in the clear-cut area, suggesting a slower turnover rate of the DOC pool in the clear-cut area. This is well in-line with the proposed hypothesis that the microbial community in the clear-cut area is primarily growing on recycled-C released during microbial turn-over. This supposition is also supported by previous studies showing that approximately 50 % of soil respiration can be attributed to root-associated
respiration (Högberg et al. 2001). Of this root-associated respiration, 50-60 % is respired by heterotrophic growth supported by root exudates and other rhizodeposits (Kuzyakov 2002). However, no increase in the isotopic composition of DOC in the clear-cut area was observed. One explanation is that labile DOC sources have extremely fast turnover (half-life of ~ 20-50 minutes) and only constitute a minor part of the DOC pool, while more recalcitrant DOC forms have mean residence times on a scale of centuries (Boddy et al. 2007; Boddy et al. 2008; Kalbitz et al. 2003). Thus, two years after harvesting might be too short a time period to detect isotopic shifts in DOC and SOM, especially since residual roots and mycorrhizal hyphae will likely remain a major C source for some time.

In conclusion, these results demonstrate that decreased belowground C inputs from plants on harvested forest sites result in major shifts in the relative abundance of different soil microbial groups. Furthermore, when the input of labile plant C decreases, the microorganisms facilitating decomposition of SOM become increasingly dependent on recycled C released during microbial turnover for growth. Since DOC of microbial origin is less labile compared to the DOC originally assimilated, each cycle of microbial assimilation and release of DOC makes the DOC pool more recalcitrant (Ågren et al. 1996; Schiff et al. 1997), eventually leading to a decrease in the belowground C flux. The findings implicitly demonstrate that the influence of trees on microbial community composition and the belowground C-flux need to be considered in order to enable us to fully appreciate if terrestrial ecosystems will function as C sinks or sources in a future climate.

In order to maintain pre-harvest soil microbial communities I recommend that aggregate patches be placed 15-20 m apart. There has been some evidence that plants can influence the microbial community up to 20 meters into a clear-cut (suggesting aggregate retention patches need only be 40 m apart) (Churchland et al. 2012; Chapter 4), but evidence from this study shows that aggregated tree patches consistently influence microbial communities and soil C-cycling up to 10 m distant.
Figure 3.1 Soil respiration rate (mg C m\(^{-2}\) h\(^{-1}\)) in the 27- by 18-m experimental plot extending from the forested area into the clear-cut (a), \(\delta^{13}C\) values of CO\(_2\) (b), \(\delta^{13}C\) values of CO\(_2\) relative to the \(\delta^{13}C\) value of DOC (negative values indicate that CO\(_2\) was \(^{13}C\)-depleted compared to DOC, and positive values that CO\(_2\) was \(^{13}C\)-enriched compared to DOC) (c), and \(\delta^{13}C\) value of CO\(_2\) relative to the \(\delta^{13}C\) value of SOM (negative values indicate that CO\(_2\) was \(^{13}C\)-depleted compared to SOM, and positive values that CO\(_2\) was \(^{13}C\)-enriched compared to SOM) (d). The broken line represents the border between the forested area and the clear-cut area.
Figure 3.2 Microbial biomass (expressed as nmol PLFA g$^{-1}$ soil dry weight) in the 27- by 18-m experimental plot extending from the forested area into the clear-cut (a), ratio of fungal to bacterial PLFA concentration (b), $\delta^{13}$C values of the fungal biomarker PLFA 18:2$\omega6,9$ (c), weighted average $\delta^{13}$C values of bacterial PLFAs (d). The broken line represents the border between the forested area and the clear-cut area.
Figure 3.3 Weighted average $\delta^{13}$C values of bacterial PLFAs vs. $\delta^{13}$C values of SOM in the 27- by 18-m experimental plot extending from the forested area into the clear-cut (positive values indicate that bacterial biomass was $^{13}$C-enriched relative to SOM) (a), and weighted average $\delta^{13}$C values of bacterial PLFAs vs. $\delta^{13}$C values of DOC (positive values indicate that bacterial PLFAs were $^{13}$C-enriched relative to DOC) (b). The broken line represents the border between the forested area and the clear-cut area.
Figure 3.4 Path diagrams representing the C cycle and different C pools in the temperate forest soil. Numbers represent the $\delta^{13}$C value relative to Pee Dee Belemite. In both the forested (a) and the clear-cut area (b), the DOC pool has three major inputs: SOM decomposition and leaching; plant C inputs through rhizodeposition and root turnover; and microbial biomass turnover. In (a) the isotopic ratio of microbial PLFAs and respired CO$_2$ reflect the isotopic ratio of the DOC pool. In (b) however, the microbial PLFAs and respired CO$_2$ are $^{13}$C-enriched relative to the DOC by up to 3 ‰. This lack of continuity suggests the microbial community is assimilating and respiring a fraction of the DOC that does not reflect the entire pool. I suggest the microbial community is preferentially taking up labile C made available during microbial turnover, and that C produced from SOM decomposition and plant inputs constitute a smaller fraction of the C assimilated by the microbial community.
Table 3.1 List of phospholipid fatty acids (PLFAs) and their microbial associated taxonomic group.

<table>
<thead>
<tr>
<th>PLFA biomarker</th>
<th>Taxonomic group</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>i15:0</td>
<td>Gram-positive</td>
<td>O’Leary and Wilkinson 1988; Bååth et al. 1992</td>
</tr>
<tr>
<td>a15:0</td>
<td>Gram-positive</td>
<td>Zelles 1999</td>
</tr>
<tr>
<td>15:0</td>
<td>Total bacteria</td>
<td>Tunlid et al. 1989; Federle 1986; Frostegård et al. 1993</td>
</tr>
<tr>
<td>i16:1ω7c</td>
<td>Gram-negative</td>
<td>Zogg et al. 1997</td>
</tr>
<tr>
<td>10Me16:0</td>
<td>Actinobacteria</td>
<td>Kroppenstedt 1985</td>
</tr>
<tr>
<td>i16:0</td>
<td>Gram-positive</td>
<td>O’Leary and Wilkinson 1988</td>
</tr>
<tr>
<td>16:1ω9c</td>
<td>Gram-negative</td>
<td>Fritze et al. 2000</td>
</tr>
<tr>
<td>16:1ω7c</td>
<td>Gram-negative</td>
<td>Zelles 1997</td>
</tr>
<tr>
<td>16:1ω5c</td>
<td>Total microbial biomass</td>
<td>Pennanen et al. 1996</td>
</tr>
<tr>
<td>16:0</td>
<td>Common fatty acid</td>
<td></td>
</tr>
<tr>
<td>i17:1ω8c</td>
<td>Gram-negative</td>
<td>Zogg et al. 1997</td>
</tr>
<tr>
<td>10Me17:0/16:0ω6m</td>
<td>Actinobacteria</td>
<td>Kroppenstedt 1985</td>
</tr>
<tr>
<td>i17:0</td>
<td>Gram-positive</td>
<td>Bååth et al. 1992</td>
</tr>
<tr>
<td>a17:0</td>
<td>Gram-positive</td>
<td>Bååth et al. 1992</td>
</tr>
<tr>
<td>cy17:0</td>
<td>Gram-negative</td>
<td>Bååth et al. 1992</td>
</tr>
<tr>
<td>17:0</td>
<td>Total bacteria</td>
<td>Zelles et al. 1994; Frostegård and Bååth 1996</td>
</tr>
<tr>
<td>17:0ω7m/10Me18:0</td>
<td>Actinobacteria</td>
<td>Kroppenstedt 1985</td>
</tr>
<tr>
<td>18:2ω6,9</td>
<td>Fungi</td>
<td>Zelles 1997</td>
</tr>
<tr>
<td>18:1ω9c</td>
<td>Fungi and Bacteria</td>
<td>Lindahl et al. 1997; O’Leary and Wilkinson 1988</td>
</tr>
<tr>
<td>18:1ω7c</td>
<td>Gram-negative</td>
<td>Wilkinson 1988</td>
</tr>
<tr>
<td>18:1ω5c</td>
<td>Gram-negative</td>
<td>Zogg et al. 1997</td>
</tr>
<tr>
<td>18:0</td>
<td>Common fatty acid</td>
<td></td>
</tr>
<tr>
<td>18:1ω7c/10Me19:1ω7c</td>
<td>Gram-negative</td>
<td>Kroppenstedt 1992</td>
</tr>
<tr>
<td>18:0ω8m/10Me19:0</td>
<td>Actinobacteria</td>
<td>Kroppenstedt 1992</td>
</tr>
<tr>
<td>cy19:0</td>
<td>Gram-negative</td>
<td>Lindahl et al. 1997</td>
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</tbody>
</table>
Table 3.2 The range and model parameters for exponential or spherical model variograms fitted to the calculated semivariances.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model</th>
<th>Nugget (C₀)</th>
<th>Sill (C₀ + C)</th>
<th>C/C₀+C (%)</th>
<th>Range (m)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration</td>
<td>Exponential</td>
<td>19.0</td>
<td>626</td>
<td>97</td>
<td>7.1</td>
<td>0.83</td>
</tr>
<tr>
<td>δ¹³C value of CO₂</td>
<td>Exponential</td>
<td>1.2</td>
<td>11.6</td>
<td>90</td>
<td>7.4</td>
<td>0.92</td>
</tr>
<tr>
<td>δ¹³C value of DOC</td>
<td>Exponential</td>
<td>0.18</td>
<td>0.38</td>
<td>51</td>
<td>87.4</td>
<td>0.83</td>
</tr>
<tr>
<td>δ¹³C value of SOM</td>
<td>Spherical</td>
<td>0.12</td>
<td>0.23</td>
<td>50</td>
<td>10.4</td>
<td>0.89</td>
</tr>
<tr>
<td>δ¹³C value of PLFA’s¹</td>
<td>Spherical</td>
<td>1.3</td>
<td>5.6</td>
<td>77</td>
<td>142</td>
<td>0.91</td>
</tr>
<tr>
<td>δ¹³C value of bacterial PLFA’s¹</td>
<td>Exponential</td>
<td>0.14</td>
<td>2.13</td>
<td>93</td>
<td>6.4</td>
<td>0.83</td>
</tr>
<tr>
<td>δ¹³C value of 18:2ω6,9</td>
<td>Spherical</td>
<td>1.2</td>
<td>6.4</td>
<td>81</td>
<td>58.5</td>
<td>0.99</td>
</tr>
<tr>
<td>Tot PLFA (nmol g⁻¹)</td>
<td>Exponential</td>
<td>1460</td>
<td>2921</td>
<td>50</td>
<td>19.2</td>
<td>0.95</td>
</tr>
<tr>
<td>18:2ω6,9 (nmol g⁻¹)</td>
<td>Spherical</td>
<td>20.4</td>
<td>48.2</td>
<td>58</td>
<td>32.9</td>
<td>0.98</td>
</tr>
<tr>
<td>Bacterial PLFAs (nmol g⁻¹)²</td>
<td>Exponential</td>
<td>167</td>
<td>1221</td>
<td>86</td>
<td>6.3</td>
<td>0.79</td>
</tr>
<tr>
<td>Ratio Fungal: bacterial PLFAs</td>
<td>Exponential</td>
<td>0.0029</td>
<td>0.0063</td>
<td>54</td>
<td>59.6</td>
<td>0.95</td>
</tr>
</tbody>
</table>

¹Weighted average
²Sum of i15:0, a15:0, i16:0, 16:1ω7, i17:1ω8c, 10Me17:0, i17:0, a17:0, 18:1ω7, cy19:0
4 An in situ method of tracing carbon-flow from live trees into soil organisms using a stem-injection stable-isotope-labeling technique

4.1 Synopsis

Here I report on the successful application of a novel stem-injection stable-isotope-labeling and probing technique in mature trees to trace the spatial and temporal distribution of rhizosphere carbon belowground. Three 22-year-old Sitka spruce trees at the edge of a stand were injected with 6.66 g of $^{13}$C-labeled aspartic acid. Over the succeeding 30 days, soil CO$_2$ efflux, phospholipid fatty-acid (PLFA) microbial biomarkers and soil invertebrates (mites, collembolans and enchytraeids) and the $\delta^{13}$C value of each, were analyzed along a 50-meter transect from each tree into the adjacent unforested area, to determine the temporal and spatial patterns in the translocation of recently fixed photosynthates belowground. Soil $\delta^{13}$C values of CO$_2$ peaked 13-23 days after injection, up to 5 m from the base of the injected tree and was, on average, 3.5 ‰ $^{13}$C-enriched compared to the baseline. Fungal PLFA biomarker $\delta^{13}$C values peaked 2-4 days after stem-injection, up to 20 m from the base of the injected tree and were $^{13}$C-enriched by up to 50 ‰. Significant $^{13}$C enrichment in mites and enchytraeids occurred 4-6 days after injection (on average, 1.5 ‰). Stem-injection of large trees with $^{13}$C-enriched compounds is a successful tool to trace C-translocation belowground. In particular, the significant $^{13}$C enrichment of CO$_2$ and enchytraeids near the base of the tree and the significant $^{13}$C enrichment of PLFAs up to 20 m away indicates that mature Sitka spruce (Picea sitchensis) have the capacity to support soil communities over large distances.

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4.2 Introduction

The amount of carbon (C) that can be stored in and released from forests depends on how much C is translocated into the roots, taken up by soil biota, and respired back to the atmosphere. Allocation of recent photosynthates belowground can account for 25-63% of a plant’s gross primary production (Litton et al. 2007). Previous studies have shown that, on average, 45.8% of the photosynthesized C is lost through root or rhizosphere respiration during the growing season (Hanson et al. 2000) and only 25% remains in the root tissues (Högberg and Högberg 2002). This sequestered C is then rapidly used for production of fine roots rather than for storage, constituting a major C sink in forest ecosystems (Endrulat et al. 2010). Root exudation and root turnover represents 19-43% of the total fixed C (Berg et al. 1998; Heim and Frey 2004) and could serve as a C source for soil mesofauna as well as for symbiotic and free-living microbes in the rhizosphere (Högberg et al. 2008; Kaiser et al. 2010). Free-living rhizosphere microbes control an array of functional processes, but their major role is decomposition - releasing and/or retaining mineral elements through the breakdown of soil organic matter (Prescott et al. 2005). Mycorrhizal fungi and some plant-growth-promoting bacteria form symbiotic relationships with plant roots that increase the efficiency of water and nutrient acquisition, and aide successful growth of seedlings (Teste and Simard 2008). Access to a neighboring tree’s mycorrhizal network has been found to benefit seedling growth rates (Teste and Simard 2008), and large trees can act as donors of C to the shaded understory trees (Högberg et al. 1999; Leake et al. 2006).

Teste and Simard (2008) explored seedling establishment on harvested sites with retained mature trees. They found that seedling height, shoot biomass, needle biomass and nutrient uptake peaked 2.5 to 5 m from retained trees, whereas closer proximity to adult trees reduced seedling fitness. They attributed the increase in seedling growth to direct access to the mycorrhizal network of retained trees. Determining the range of influence of retained trees on harvested sites on the soil microbial community could allow forest managers to design cut-blocks that will best maintain pre-harvest soil communities and in turn, benefit seedling growth.
There may be benefits to tree seedlings if pre-harvest soil microbial communities are maintained on regenerating sites. Symbiotic microbes have the potential to increase a seedling’s access to nutrients, potentially resulting in an increase in seedling growth rate. Plant-growth-promoting bacteria (PGPB) live off root exudates in the rhizosphere. It has been proposed that they benefit the plant by creating biofilms that protect the root against pathogens (Akhtar and Siddiqui 2009). They are also thought to enhance plant growth, increase plant nutrient uptake (Adesemoye et al. 2008), and are involved in abiotic stress tolerance (Yang et al. 2009).

Another benefit of retention trees could be to provide soil fauna with more plentiful food sources. Soil fauna play an important role in soil formation and contribute to soil structure. Some groups act as decomposers, breaking down litter and aiding in nutrient cycling, whereas others alter microbial communities, either directly by feeding on fungal hyphae, bacteria and archaea, or indirectly altering plant primary production, acting as, or protecting from, pests. Through their movement, casting, nesting and production of fecal pellets, fauna contribute to soil formation, changing soil porosity (and consequently water infiltration) and forming soil aggregates (Lavelle et al. 2006). Unrelated taxa may exploit similar resources (Ruess and Chamberlain 2010) and vary in feeding strategies and tissue turnover times. Of the three faunal groups examined in this study, chosen based on their prevalence in these forest soils, collembola, mites and enchytraeids, collembola have the shortest tissue turnover times (1.5-6 days), and tend to be generalist feeders in situ, with a preference for fungi in laboratory studies (Ruess and Chamberlain 2010). Mites are a large faunal group with variable feeding strategies and turnover times (Mitchell 1977). Enchytraeids tend to have slow turnover times (lifecycles of a year or greater), and are thought to be generalist feeders; however, previous studies have found that enchytraeids exploit litter and soil organic matter to a greater extent than rhizosphere C (Briones and Ineson 2002; Schmidt et al. 2004; Schmidt and Baldwin 2009). Studies that have directly measured C-flow from live plants into soil fauna using stable-isotope-labeling and probing typically find collembola are the most enriched of the taxa studied (Ostle et al. 2007).
In situ $^{13}$C pulse-labeling of plant C into soil organisms has been mostly limited to grassland (Johnson et al. 2002; Leake et al. 2006; Ostle et al. 2007; Denef et al. 2009; Clayton et al. 2010) and crop species such as rice (Lu et al. 2004; Wu et al. 2009) and leek (Elfstrand et al. 2008); the notable exception is Högberg et al.’s (2008, 2010) successful labeling of pine trees. Small plants and seedlings interact with their environment differently than established trees. The latter have the potential to greatly influence aboveground and belowground forest communities due to their developed root system, large canopy, and large amount of photosynthates that can be exuded through their roots. This study explores the application of a modified stem-injection technique to trace the flow of root exudate C from mature trees into soil organisms and forms part of a study assessing the importance of retention trees in maintenance of soil organisms after harvest.

Pulse-labeling of low-lying shrub or grassland is typically conducted using chamber methods and has successfully allowed tracing of $^{13}$C into arbuscular mycorrhizal fungi (Johnson et al. 2002) soil phospholipid fatty-acids (Denef et al. 2009), rhizosphere microbial DNA (Clayton et al. 2010), and collembola (Ostle et al. 2007). In order to label isolated seedlings, the “bag method” has been developed. In this method seedlings are isolated from atmospheric CO$_2$ by a gas-tight polyethylene/nylon bag sealed around the base of the stem and the bag is filled with either $^{13}$C-enriched (or depleted or $^{14}$C-enriched CO$_2$. The seedling incorporates this CO$_2$ during photosynthesis, producing labeled photosynthates (Simard et al. 1997a, b, c; Teste et al. 2009). The disadvantages of both methods described above is that the size of the plant is limited, the bags/chambers will diffuse light, and the injection of labeled CO$_2$ into the bag or head space may cause CO$_2$ partial pressure changes, which will change the rate of photosynthesis. These devices are also expensive and could be impractical in the field as they need a power supply and equipment to control the conditions within the enclosure (Talhelm et al. 2007).

Several other methods have been developed to label trees with stable isotopes. Coker (1991) applied $^{15}$N-enriched urea to the surface of Pinus radiata (D. Don) needles. The isotope was taken up within 6 hours of application and traced to needle tissue. Gessler et
al. (2003) cut and soaked Norway spruce (*Picea abies* [L.] Karst) needles, located on a girdled twig, in a solution containing aspartic acid and glutamic acid labeled with $^{14}$C and $^{15}$N. Between 22 and 26% of the isotope was taken up and transported intact within the twig. In order to study the flow of C and N from the xylem of a host plant (*Abies alba* Mill) into the xylem-tapping parasite, mistletoe (*Viscum album* L.), Escher and Rennenberg (2006) set up a perfusion system where the host xylem sap of removed twigs was replaced with $^{15}$N and $^{13}$C-enriched glutamine. They were able to trace the labeled glutamine into different sink tissues of the parasitic plant.

Few labeling studies have been conducted on trees taller than 2 m because of the large amount of isotope required to label whole trees. Carbone et al. (2007) labeled 4-m-tall black spruce trees with $^{14}$CO$_2$ using a dome-shaped portable polyethylene yurt. Högberg et al. (2008; 2010) pulse-labeled 4-m-tall pine trees using the free-air CO$_2$ enrichment (FACE) technique (Hanson et al. 2000). This technique successfully traced $^{13}$C into soil microbial cytoplasm, fungal fatty acid biomarkers, some bacterial fatty acid biomarkers and collembola (Högberg et al. 2010). Keel et al. (2012) similarly labeled a mature deciduous forest using the FACE method ($^{13}$CO$_2$ depletion) that allows for observation of C flow above and belowground at a large scale. However, Subke et al. (2009) stated that interpretation of CO$_2$ tracer data in these studies is prone to error initially after the pulse because the soil air is enriched due to the physical diffusion of the trace gas into the soil air pockets. Also, adding enriched or depleted $^{13}$CO$_2$ increases the total concentration of CO$_2$ in the atmosphere, which has been found to increase tree NPP, change C allocation within the tree and increase root exudation (Curtis and Wang 1998). This is predicted to influence soil microbial community structure and soil nutrient cycling (Paterson et al. 1997; Phillips et al. 2011b).

Consequently, other “low-cost” approaches have been developed in recent years. Stem-injection techniques have been used to successfully label 8-year-old larch trees with $^{13}$C, via tubing from battery-operated pumps (Talhelm et al. 2007), and tall maritime pine trees (20 m on average) with a multi-inlet system (Wingate et al. 2010). Direct injection of a solution of Na$_2^{13}$CO$_3$ into the xylem was used to label a small northern white cedar
tree (Powers and Marshall 2011). Similarly, large-tree stem-injection studies have successfully traced $^{15}$N into the labile organic matter within 1 m of injected 18-year-old sweet gum trees (Garten and Brice 2009) and traced $^{15}$N into grasses surrounding injected 16-year-old legume trees (Sierra and Daudin 2010). However, all these studies have only focused on assessing the partitioning of plant assimilated C and N between autotrophic (both from foliage and roots) and heterotrophic respiration; none have linked tree C allocation and the extent of its translocation into the main biotic components of the grassland or forest soil.

The aim of this study was to investigate the spatial and temporal patterns in C transfer from mature trees into the soil system in situ. Here, I tested a xylem and phloem stem-injection technique to determine to what distance recent photosynthates from mature trees influence soil respiration, dissolved organic C production, and soil microbial and mesofauna assimilation over the course of 30 days. Soil-respired CO$_2$, DOC, SOM, PLFA and fauna were measured, along with $\delta^{13}$C values of each.

4.3 Methods

4.3.1 Study site

This study was conducted in the Gisburn Forest research site, Lancashire, U.K. (54°01’30.04’’N, 2°22’57.26’’W) in September 2009. The site was originally established in 1955, harvested and replanted into a range of different tree species in pure and mixed stands in 1991 by the UK Forestry Commission and the Institute of Terrestrial Ecology (currently, Centre for Ecology and Hydrology). Its purpose was to determine the effects of silvicultural practices on soil chemistry, soil biology, soil nutrient dynamics, tree productivity and root development. The Sitka spruce (Picea sitchensis (Bong.) Carr.) installation was selected because Sitka spruce is not only indigenous to North America and grown commercially in Europe.

The soil is classified as cambic stagnogley to stagnohumic gley (Humic Gleysols, FAO; Avery 1980), is poorly drained and prone to organic matter accumulation (McNamara et
al. 2008). Three Sitka spruce trees located 15 m apart, of similar height (6-7 m), diameter at breast height (8-10 cm) and age (22 years) were selected along the edge of a plantation adjacent to an unforested plot dominated by *Nardus stricta* L., *Deschampsia cespitosa* L. and *Juncus effuses* L. (McNamara et al. 2008).

4.3.2 Stem-injection methodology

Selected Sitka spruce trees were injected with double-labeled (\(^{13}\text{C},^{15}\text{N}\)) aspartic-acid. A 2.5-cm-diameter hole was drilled 3 cm into each bole, approximately 1 m aboveground. This hole was sealed with silicone surrounding a bung with two inlet tubes, one clamped and the other attached to an inverted Nalgene bottle, secured 0.5 m above the bung (Figure 4.1). The inverted Nalgene bottle was filled with 6.66 g of \(^{13}\text{C}\)- and \(^{15}\text{N}\)-labeled aspartic acid (99.98%) dissolved in 2 L of deionized water. Each tree received 0.72 g \(^{15}\text{N}\) which was estimated to be 0.25 – 0.50 % of the N pool per tree. This is considerably less than the 5-10% that Horwath et al. (1992) reported could be injected in poplar trees, but similar to the 0.29% injected into radiata pine (*Pinus radiata* D. Don) by Proe et al. (2000). As Proe et al. (2000) reported minor areas of localised scorching, it was thought prudent to adhere to the smaller percentage of the total N pool. There was no visible evidence of damage following stem injection in this experiment.

4.3.3 Soil and gas sampling

Over the next 7 days the solution was left to drain by gravity and thus be passively incorporated by the phloem. Thereafter, gas samples were collected at the base of each tree, and along a north transect 1, 2, 5, 10, 20, 30 and 50 m into the unforested plot and to 10 m south, within the Sitka spruce plantation, 0, 1, 2, 4, 6, 9, 13, 17, 23 and 30 days during labeling. Soil respiration rates were measured *in situ* using an EGM-4 CO\(_2\) analyzer (PP systems, Amesbury, MA, USA). Soil respired CO\(_2\) was collected after 60 minutes of incubation using a closed, non-steady-state, incubation chamber described in Basiliko et al. (2009). Gas samples (14 mL each) were taken using gas-tight syringes and stored in evacuated 6 mL exetainers prior to \(^{13}\text{CO}_2\) analysis. Exetainers were over-pressured with sample to avoid contamination from atmosphere. The resulting samples
were a mixture of respired soil efflux and atmosphere, and were corrected using a Keeling plot approach (Keeling 1968, Pataki et al. 2003). Closed non-steady-state incubation chambers can cause issue with chamber headspace pressure and the sampling can create pressure differences that causes an influx of soil atmosphere around the base. These and other issues with respiratory measurements are described by Ubienza et al. (2009). Therefore, it is more appropriate to refer to our CO₂ measurement an index of respiration.

On the same sampling dates as the gas sampling, two soil cores (5-cm diameter x 10-cm deep) were collected along the same sampling regime discussed above. This soil was sieved to 2.0 mm and separated into four portions for each of pH, moisture, dissolved organic carbon (DOC) and phospholipid fatty acid (PLFA) analysis. Day 4 samples were excluded from PLFA analysis due to cost.

Soil moisture was measured by comparing soil before and after oven-drying at 75°C for 72 hours. pH (H₂O) measurement was completed according the UBC Forestry BEG protocols adapted from Avery and Bascomb (1974) and the British Standard (1995).

The δ¹³C values of the DOC were measured according to the technique described in Bengtson and Bengtsson (2007). Briefly, field-moist soil was transferred into 0.2-ml Z-spin filter units (Pall, Nanosep, Mexico), which were centrifuged at 130 000 rpm for 30 min (DiaMed, MIRKO 20). The soil water in the collection tube was transferred to an 8-mm tin cup, evaporated at 35 °C and stored in a covered container at room temperature until analysis.

4.3.4 Phospholipid fatty acid (PLFA) analysis

PLFAs were extracted from 1.2 g of freeze-dried soil according to the Frostegård et al.’s (1991) method, based upon the Bligh and Dyer’s (1959) procedure and further modified by White et al. (1979). Extracted fatty acid methyl esters were measured with an Agilent Technologies Gas Chromatograph 6890N (Santa Clara, CA, USA), interfaced with a GV Instruments GC5 MK1 combustion furnace interfaced with a GV Instruments Isoprime
ratio mass spectrometer (both Cheadle Hulme, UK). Peaks were identified by chromatograph retention times using a mass spectral comparison with the external standard bacterial acid methyl ester mix (BAME; Sigma-Aldrich, 47080-U, Oakville, ON, Canada). Each sample, including the external standard BAME, was anchored relative to an internal standard (C19:0). Sample batches were two-point calibrated with two C20:0 isotopic standards that bracketed the expected range of δ\(^{13}\)C values (C20’s equaled -30.68 ‰, standard deviation 0.02 ‰ versus VPDB, and -6.91 ‰, standard deviation 0.04 ‰ versus VPDB; Isocanoic acid methyl ester, certified reference material, Indiana University).

The following fatty acids were chosen to represent bacterial PLFAs: i15:0, a15:0, 15:0, i16:1ω7c, 10Me16:0, i16:0, 16:1ω9c, 16:1ω7c, i17:1ω8c, 10Me17:0/16:0ω6m, i17:0, a17:0, cy17:0, 17:0, 17:1ω7m/10Me18:0, 18:1ω7c, 18:1ω5c, 18:0, 18:0ω8m/10Me19:0, cy19:0 (Kroppenstedt 1985; Frostegård et al. 1993; Zogg et al. 1997). Fungal biomarkers are limited to 18:2ω6,9 and 18:1ω9c, with the latter found in some bacteria as well (Kaiser et al. 2010). Please refer to Table 3.1 for structure and biomarker specifics. Abundance of identified fatty acids is expressed as nmols per gram of freeze dried soil (nmol g\(^{-1}\)).

4.3.5 Faunal extraction

Two soil cores (PVC pipes, 6.4-cm internal diameter x 10 cm deep) were collected for faunal extractions (one core for each of enchytraeids and microarthropods) at the base and 10 m from each tree into the Sitka spruce plantation on the same sampling dates as CO\(_2\) measurements. Enchytraeids were extracted using a modified wet funnel method (O’Connor 1955), whereas Tullgren funnels (1918) were used to extract mites and collembolans. Invertebrates were collected alive in deionized distilled water, sorted by groups and freeze-dried in preparation for isotope analyses.
4.3.6 Isotope analysis

The following equation was used to calculate the $\delta^{13}$C value of soil-respired CO$_2$:

$$
\delta_{\text{sample}} = \delta_{\text{air}} + 8 \times \ln \left( \frac{C_{\text{sample}}}{C_{\text{air}}} \right)
$$

Where $C_{\text{sample}}$ and $C_{\text{air}}$ are the concentrations of CO$_2$ in the chamber headspace and ambient air respectively, and $\delta_{\text{sample}}$ and $\delta_{\text{air}}$ are the $^{13}$C/$^{12}$C isotopic mixing ratios of the chamber headspace and ambient air respectively.

Gas samples were analyzed via gas chromatography interfaced with an isotope ratio mass spectrometer at the Macaulay Institute, Aberdeen, Scotland (GasBench II interfaced to a Delta PlusXP continuous flow isotope ratio mass spectrometer both from Thermo; Bremen, Germany).

Gisburn forest PLFA samples were analyzed with an Agilent Technologies Gas Chromatograph 6890N (Santa Clara, CA, USA), interfaced with a GV Instruments GC5 MK1 combustion furnace interfaced with a GV Instruments Isoprime ratio mass spectrometer (both Cheadle Hulme, UK) at the Belowground Ecosystem Group Stable Isotope Facility at the University of British Columbia. An elemental analyzer (Elementar Vario EL Cube: in C, N mode; Hanau, Germany) interfaced with an isotope ratio mass spectrometer (GV Instruments Isoprime, with reference gas box; Cheadle Hulme, UK) via continuous flow, also at the University of British Columbia, was used to measure the $^{13}$C and $^{12}$C content of fauna and DOC. For each faunal sample 0.4 mg was required in order to obtain accurate isotopic readings. Depending on the size of the individuals and the species present, approximately 30-60 mites, 20-40 collembolans and 10-15 enchytraeids were required for isotopic analyses.
4.3.7 Statistical analysis

A one-level spilt-plot linear mixed-model was used to account for the potential correlation of time and distance between measurements. This data was clustered by tree, and a spatial power matrix (SP(pow)) was used to determine the covariance structure of the residuals. All statistical analyses were conducted using SAS system release 9.1 (SAS Institute Inc., Cary, NC). Statistically significant differences were $\alpha<0.05$. The SP(pow) matrix was found not to be a significant model for DOC, collembola, mites and phospholipid fatty acids: i15, a15, C15, i16:1ω7c, 17:0ω7m, 16:1ω5c and i17. Consequently a simple linear regression was used.

4.4 Results

4.4.1 Respiration enrichment

$\delta^{13}$C values of soil respired CO$_2$ were, on average, significantly greater than the baseline (pre-injection; $F_{1, 214} = 6.49, p<0.05$; Table 4.2). A significant SP(pow) for time was found ($X^2 = 71.63, df = 1, Pr<0.0001$) indicating samples were correlated over time by $0.8369^{\text{day}}$. The SP(pow) for distance was $0.03^{\text{distance}}$; so small that it can be assumed that observations were not correlated over the sampling area. $\delta^{13}$C values of respired soil CO$_2$ peaked after 2-3 weeks, on different days for the three trees (days 13, 17 and 23 for trees A, B and C, respectively; Figure 4.2). Minor peaks were observed at 20-30 m (Figure 4.2), which are likely the result of atmosphere contamination during gas sampling, due to the similarity of the isotopic ratio of those samples to that of CO$_2$ found in air.

4.4.2 Dissolved organic carbon (DOC)

Although no SP(pow) relationships were observed, when analyzed with an ANOVA DOC $\delta^{13}$C values were significantly greater than the baseline ($F=6.03, df=1, p<0.0187$) and were, on average, 1.2 % enriched. In addition, a strong trend associated with sampling date was observed ($F=1.99, df=7, p<0.0817$), with DOC $\delta^{13}$C values peaking on days 4 and 6. In contrast, DOC was not significantly different along the three transects.
4.4.3 Phospholipid fatty acids (PLFAs)

Evidence of PLFA enrichment

One day after the start of stem-injection, the $^{13}$C label was observed in the two fungal biomarkers, 18:2ω6,9 and 18:1ω9c (Figure 4.3); both fungal biomarkers were enriched by 20%, 20 m from tree B’s point of injection. Furthermore, four days after labeling 18:2ω6,9 was also enriched by 20-50%, 5 and 10 m from tree C and B’s point of injection, respectively. 18:2ω6,9 and 18:1ω9c were not significantly enriched as a result of labeling, likely due to spatial and temporal variability as well as the different labeling success between the three trees. However, it is unlikely that a 20-50% spike in $^{13}$C would occur by chance several times for the same PLFA peak over 30 plus peaks, in multiple samples. Because of this, I think they are evidence of PLFA enrichment.

Significant treatment and temporal effects

Only two bacterial PLFA biomarkers, i15:0 and a15:0, were significantly affected by the pulse-labeling stem-injection (Table 4.3), but their $\delta^{13}$C values were significantly less enriched relative to the baseline. PLFAs 16:0, a17:0, 18:2ω6,9, 18:1ω9c, 18:0ω8m and cy19:0, a significant SP(pow) temporal relationship (Table 4.1), with decreasing $\delta^{13}$C values over the sampling period. PLFA biomarkers i15:0, a15:0, 15:0, i16:1ω7c, 16:1ω5c, i17, 17:0ω7m, 17:0, and 18:1ω7c did not show a significant SP(pow) temporal relationship, but when analyzed with an ANOVA, were also significantly less $^{13}$C-enriched on later sampling dates (Table 4.3).

Significant spatial effects

Those biomarkers that exhibited significant SP(pow) spatial relationships (i.e. i16:0, 16:1ω7c, i17:1ω8c, 16:0ω6m,18:1ω7c, total Gram-negative and C18:0; Table 4.2) typically did not show any uniform directional trends. This result suggests that samples taken close together were more similar among than samples taken further apart and that the proximity to the trees did not influence the isotopic values. Only biomarker 17:0 $\delta^{13}$C
values decreased with increasing distance from the labeled trees. Although i15:0, a15:0 and i17:0 did not show a significant SP(pow) spatial relationship, their isotopic enrichment, when averaged over the entire sampling period, including pre-injection, and analyzed with an ANOVA, was significantly different due to sampling location (Table 4.3), showing greater $\delta^{13}$C values in proximity to the base of the tree.

4.4.4 Soil mesofauna

Mite $\delta^{13}$C values were significantly greater than the baseline ($F=8.31, df=1, p<0.0089$, Table 1); however, there were no significant differences in $\delta^{13}$C values of mite tissues due to location or sampling date. Enchytraeid tissues were also significantly enriched compared to the baseline (on average $1.5\%$; $F=6.65, df=1, p=0.0210$; Table 4.1) and exhibited a significant SP(pow) temporal relationship ($0.9760^{day} X^2=3.65, df=1, p<0.05$); with $\delta^{13}$C values peaking on day 6 by $6.6\%$. The isotopic enrichment of enchytraeids showed a decline with increasing distance from labeled trees, but this was not statistically significant. In contrast, there was no significant $^{13}$C enrichment in collembolans over sampling date or location.

4.5 Discussion

4.5.1 Soil respiration index

The significant difference in soil $^{13}$CO$_2$ efflux between pre- and post-injection in the Gisburn study suggests the isotope solution successfully entered into the phloem. Previous pulse-labeling studies have detected $^{13}$C label in CO$_2$ efflux after 24 hours in grasslands (Leake et al. 2006; Clayton et al. 2010) and after 2-4 days in a Scots pine forest (Högberg et al. 2008). Contrary, in this study $^{13}$CO$_2$ efflux peaked 13-23 days after stem-injection. A possible explanation for the time lag observed could be the depth of the injection sites, which may have resulted in partial absorption of aspartic acid into the xylem. Aspartic acid incorporated into the phloem is expected to follow the grain of the wood and to be allocated to a fraction of the major roots (Walia et al. 2010). Any label incorporated into the xylem would have needed to travel the whole length of the tree.
before being transferred into the phloem and subsequently exuded by the roots, resulting in a delay in the recovery of the $^{13}$CO$_2$ peak. Transportation time, up the xylem and back down the phloem, has been estimated to be about 5–6 days, but varies with season, nutrient availability (Högberg et al. 2010; Wingate et al. 2010), tree size (Powers and Marshall 2011), and changes with root versus foliage C demand (Dannoura et al. 2011). This may be why the $^{13}$C label was not detected in the soil-respired CO$_2$ along the transect until 2-3 weeks later. However, aspartic acid that was incorporated into the xylem should then be more evenly distributed throughout the phloem and, therefore, detectable throughout the entire sampling site. The different inherent rates of sugar transport in grasses versus large plants could also account for the differences in timing with previously reported $^{13}$CO$_2$ efflux recoveries (Mencuccini and Holtta 2010).

4.5.2 Microbial communities

In situ FACE studies tracing $^{13}$C from trees into the microbial community have shown that isotopic label tends to be found in ectomycorrhizal roots (Högberg et al. 2002) and fungal biomarkers, but rarely in bacterial biomarkers (Högberg et al. 2010). In this study, the $^{13}$C label was detected in fungal biomarkers 18:2ω6,9 and 18:1ω9c. Although the latter could also indicate enrichment of bacterial communities (Kroppenstedt et al. 1985), fungal enrichment is probably the main cause of the noted increase of 18:1ω9c δ$^{13}$C values as there was no evidence of other bacterial PLFA enrichment throughout the experiment. The observed spikes in $^{13}$C label of fungal PLFAs occurred on days 1 and 4, at distances 5-20 m, with enrichments of up to 50 ‰. The brevity of these spikes, when averaged over all days and distances, makes the detection of significant differences difficult. Phloem movement can range from 0.12 to 1 m per hour (Thompson et al. 1979; Barnard et al. 2007) and gymnosperms typically fall into the lower range. It is, therefore, reasonable to conclude that the phloem movement was very fast and that these spikes of $^{13}$C in the fungal biomarkers can be used as indicators of root exudation into dense mycorrhizal hyphal nets.
Conventional $^{13}$C-labeling techniques that expose the tree to either $^{13}$C-enriched or depleted CO$_2$ appear to provide more consistent temporal results than the stem-injection method presented here. In FACE studies, the isotope label is incorporated into the tree through photosynthesis; it is a less invasive method and likely provides a more accurate representation of C-flow within an ecosystem. However, the equipment required for labeling this way limits studies to small trees and seedlings, or expensive open-air FACE systems. The FACE system also has the risk of back-diffusion, confounding recovery of soil-respired CO$_2$ (Subke 2009) and risks physiological changes in the tree, caused by exposure to higher levels of CO$_2$ (Epron 2012). Stem-injection allows for targeted labeling of large trees with minimal cost, maximum incorporated label and maximum number of replicates. The draw-back of the stem-injection system could be minimized by providing multiple injection sites (if you were interested in the timing of C-flow), or by focusing on injecting into the xylem if you were interested in the spatial distribution.

It was expected that a $^{13}$C enrichment of PLFA biomarkers would occur soon after stem-injection of $^{13}$C-enriched aspartic acid. However, there appeared to be a consistent trend over time of PLFA $^{13}$C depletion from the baseline that stabilized 5-10 days after labeling. This temporal trend was observed in the two fungal PLFA biomarkers and in several bacterial PLFA biomarkers. Such changes in bacterial and fungal population $^{13}$C content has been attributed to changes in moisture regimes, particularly to drought and rewetting cycles (Ruehr et al. 2009). Rainfall events occurred in the days prior to the stem-injection, which were followed by several days of dry weather. It is likely that the microbial community relied on slightly different sources of C as a result of the abrupt changes in soil moisture, and the $\delta^{13}$C of photosynthates would change as well, becoming heavier under drier conditions.

The 17:0 bacterial biomarker was the only PLFA to show a significant decrease in $\delta^{13}$C values with increasing distance from the trees. Similarly, i15:0, a15:0 and i17:0, associated with Gram-positive bacteria, also had significantly higher $\delta^{13}$C values at the base of the injected trees. Because the differences in $\delta^{13}$C values varied along the transect throughout the whole sampling period, they likely reflect differences in C sources along
the sampling transect. This is in agreement with earlier studies investigating the spatial influence of retained trees on harvested sites on the soil microbial community. Five years after harvest the activities of key N and C-cycling enzymes, NAGase and β-glucosidase, were maintained up to 5-10 m from a retention patch of approximately 60-year-old western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) (Dewi 2009). In relation to this, Daradick (2007) determined that five months after harvest, green-tree-retention was more likely to maintain enzyme activity within 20 m of aggregated-retention patches. Both Dewi (2009) and Daradick (2007) suggested that the supply of labile C in the form of root exudates and litter from the retained trees could support this activity.

4.5.3 Soil fauna

The results of this study clearly showed significant $^{13}$C enrichment in both mites and enchytraeids, but not in collembolans, compared to the baseline. This contrasts with previous findings in grasslands (Ostle et al. 2007) and nutrient-poor boreal forests (Högberg et al. 2010), where collembola were the most responsive mesofaunal group to recent plant photosynthate inputs, suggesting that they are grazers, feeding on live mycorrhizal fungal mycelium rather than detritivores getting their nutrition from dead organic matter (Ostle et al. 2007).

Furthermore, the isotopic enrichment of enchytraeids showed a decline with increasing distance from labeled trees, whereas neither location nor sampling date had any significant effects on the $\delta^{13}$C value of mite tissues. Soil enchytraeids are thought to be generalist feeders; however, previous studies have also found that they can exploit litter and soil organic matter to a greater extent than rhizosphere C (Standen and Latter 1977; Briones and Ineson 2002), but others have noted that enchytraeids varied widely in their ability to assimilate C from living plants roots (Bull et al. 2002). Certain enchytraeid species have been found to preferentially feed on distinct organic matter fractions that may contain isotopically distinct resources (i.e. algae; Schmidt et al. 2004). These results suggest that the enchytraeid community living in the Gisburn forest is closely associated with recent rhizosphere C inputs. They are probably feeding directly on roots, root
exudates or on soil microorganisms feeding in the rhizosphere (e.g. bacteria, protozoa, algae) rather than the nearby fungi, as the fungal PLFA biomarkers were $^{13}$C-enriched 5-20 m from the base of injected trees. Interestingly, recent studies (Crotty et al. 2011) have revealed that, with some exceptions, the majority of soil invertebrates are more active consumers of bacteria than previously thought. In agreement with these observations, Waldrop et al. (2012) recently found that high bacterial and enchytraeid abundance are associated with high soil moisture and as a result, more rapid rates of C turnover occurred in those soils with higher mesofaunal grazing on bacteria. In this study, the higher $^{13}$C enrichment in both mites and enchytraeids was also coincidental with the $\delta^{13}$C value of DOC, suggesting a positive relationship between mesofaunal activities and C fluxes, which has also been observed in other systems (Osler et al. 2007; Carrera et al. 2009).

Crotty et al. (2011) also concluded that not only should the level of taxonomy applied be considered when interpreting soil biota feeding preferences, but also the soil habitat. In our studied forest system both the spatial (distance) and temporal (time) distribution of the C source also played crucial roles in determining which food sources were preferentially used and highlight the importance of investigating soil fauna feeding flexibility under different environmental conditions and geographical gradients to acquire a better understanding of ecosystem function.

### 4.6 Conclusions

This study demonstrates, for the first time, that stem-injection provides a simple way of tracing *in situ* the flow of recent photosynthate C from replicated mature trees into the below-ground components of the soil system. Although both faunal and CO$_2$ enrichment occurred within a few meters of the injected trees, the presence of enriched fungal PLFAs up to 20 m away from the labeled trees indicates that these 22-year-old Sitka spruce have the capacity to support soil communities over large distances. Methodological improvements could include increasing the number of injection sites to allow for more even labelling of the tree roots.
Figure 4.1 Stem-injection design. Arrows indicate movement of the double-labeled aspartic acid throughout the tree. Dots represent root-exuded and root-respired carbon.
Figure 4.2 Average $\delta^{13}$C (‰) value of soil-respired CO$_2$ with increasing distance from the base (distance 0 m) of each injected tree A, B and C during the course of the 30 day experiment.
Figure 4.3 Average $\delta^{13}C$ (‰) values of phospholipid fatty-acid biomarkers with increasing distance from the base of the injected tree (0 m) during the course of the 30-day experiment. Error bars represent standard errors of the three sampled trees.
Table 4.1 Average and maximum δ\textsuperscript{13}C (‰) values of the baseline (before labeling) and over the 30-day sampling period in soil-respired CO\textsubscript{2}, enchytraeids and mites.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Baseline average value (‰)</th>
<th>Sample average value (‰)</th>
<th>Maximum value (‰)</th>
<th>Distance and day of maximum δ\textsuperscript{13}C values</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO\textsubscript{2}</td>
<td>-21.33 (0.43)</td>
<td>-18.77 (0.25)</td>
<td>3.61</td>
<td>Tree base, day 17</td>
</tr>
<tr>
<td>Enchytraeids</td>
<td>-26.30 (0.25)</td>
<td>-25.82 (0.19)</td>
<td>-18.95</td>
<td>Tree base, day 6</td>
</tr>
<tr>
<td>Mites</td>
<td>-26.38 (0.25)</td>
<td>-25.92 (0.10)</td>
<td>-25.11</td>
<td>10 m from tree base, day 30</td>
</tr>
</tbody>
</table>

Values in parenthesis are standard errors of the means (Baseline: n = 24, 6 and 3 for each of CO\textsubscript{2}, enchytraeids and mites respectively; n = 240, 43, 37 for each of CO\textsubscript{2}, enchytraeids and mites, respectively).
Table 4.2 Statistical relationships of phospholipid fatty-acid biomarker $\delta^{13}$C values over space or time calculated using a spatial power matrix. Only statistically significant values are reported.

G- represent Gram-negative bacteria, G+ represent Gram-positive bacteria.

<table>
<thead>
<tr>
<th>PLFA</th>
<th>Represents</th>
<th>Relationship</th>
<th>X2</th>
<th>SP(pow)</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2ω6,9</td>
<td>Fungi</td>
<td>Temporal</td>
<td>36.16</td>
<td>0.8876^Day</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18:1ω9c</td>
<td>Fungi and bacteria</td>
<td>Temporal</td>
<td>26.31</td>
<td>0.8265^Day</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C16:0</td>
<td>Common</td>
<td>Temporal</td>
<td>10.68</td>
<td>0.7153^Day</td>
<td>1</td>
<td>0.0001</td>
</tr>
<tr>
<td>16:1ω7c</td>
<td>G-</td>
<td>Spatial</td>
<td>20.75</td>
<td>0.7804^Distance</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>i17:1ω8c</td>
<td>G-</td>
<td>Spatial</td>
<td>4.26</td>
<td>0.4208^Distance</td>
<td>1</td>
<td>0.031</td>
</tr>
<tr>
<td>18:1ω7c</td>
<td>G-</td>
<td>Spatial</td>
<td>10.42</td>
<td>0.5939^Distance</td>
<td>1</td>
<td>0.0012</td>
</tr>
<tr>
<td>Total</td>
<td>G-</td>
<td>Spatial</td>
<td>23.06</td>
<td>0.7715^Distance</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>cy19:0</td>
<td>G-</td>
<td>Temporal</td>
<td>25.73</td>
<td>0.6888^Day</td>
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<td>18:0ω8m</td>
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<tr>
<td>a17:0</td>
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<td>0.4559^Day</td>
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<td>0.0149</td>
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<tr>
<td>i16:0</td>
<td>G+</td>
<td>Spatial</td>
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<td>0.4851^Distance</td>
<td>1</td>
<td>0.016</td>
</tr>
<tr>
<td>16:0ω6m</td>
<td>G+</td>
<td>Spatial</td>
<td>10.25</td>
<td>0.6690^Distance</td>
<td>1</td>
<td>0.0014</td>
</tr>
<tr>
<td>C17:0</td>
<td>Bacteria</td>
<td>Spatial</td>
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<td>0.7113^Distance</td>
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<td>0.0029</td>
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<tr>
<td>C18:0</td>
<td>Bacteria</td>
<td>Spatial</td>
<td>3.99</td>
<td>0.3403^Distance</td>
<td>1</td>
<td>0.0458</td>
</tr>
</tbody>
</table>
Table 4.3 Statistically significant relationships of phospholipid fatty-acid biomarker $\delta^{13}$C values over space or time calculated using a three-way ANOVA. Only statistically significant values are reported. G- represent Gram-negative bacteria, G+ represent Gram-positive bacteria.

<table>
<thead>
<tr>
<th>PLFA</th>
<th>Represents</th>
<th>Component</th>
<th>F</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>17:0ω7m</td>
<td>G+</td>
<td>Day</td>
<td>2.3</td>
<td>7,132</td>
<td>0.0306</td>
</tr>
<tr>
<td>i15:0</td>
<td>G+</td>
<td>Baseline</td>
<td>36.7</td>
<td>1,95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day</td>
<td>4.4</td>
<td>6,95</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distance</td>
<td>50.8</td>
<td>1,95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distance*Tree</td>
<td>6.2</td>
<td>2,95</td>
<td>0.0029</td>
</tr>
<tr>
<td>a15:0</td>
<td>G+</td>
<td>Baseline</td>
<td>34.8</td>
<td>1,95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day</td>
<td>4.9</td>
<td>6,95</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distance</td>
<td>20.0</td>
<td>1,95</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tree</td>
<td>3.2</td>
<td>2,95</td>
<td>0.0437</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distance*Tree</td>
<td>5.9</td>
<td>2,95</td>
<td>0.0038</td>
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<tr>
<td>i17:0</td>
<td>G+</td>
<td>Day</td>
<td>5.4</td>
<td>7,132</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>i6:1ω7c</td>
<td>G-</td>
<td>Day</td>
<td>2.1</td>
<td>7,95</td>
<td>0.0559</td>
</tr>
<tr>
<td>C15:0</td>
<td>Bacteria</td>
<td>Day</td>
<td>4.8</td>
<td>6,115</td>
<td>0.0002</td>
</tr>
<tr>
<td>16:1ω5c</td>
<td>Bacteria and Fungi</td>
<td>Day</td>
<td>8.2</td>
<td>7,131</td>
<td>&lt;0.0001</td>
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</tbody>
</table>
5 *In situ* priming effects of recent photosynthates on soil organic matter decomposition along a gradient of forest soil disturbance

5.1 Synopsis

Forest ecosystems account for 50-60% of annual terrestrial net primary production and temperate forests comprise approximately 10% of terrestrial C stocks. Deforestation or reforestation can tip the terrestrial global carbon (C) budget from a net source to a net sink. Understanding how harvesting practices or soil disturbance affects soil microbial community responses to C inputs will enable us to better predict ecosystem C storage. Priming effects, which can result in enhanced soil organic matter (SOM) decomposition, arise due to microbial stimulation in the rhizosphere by the addition of labile C. Priming can increase (or decrease) rates of soil C-cycling, but the impact of this phenomenon on forest soil *in situ* is not well established. In this study I added $^{13}$C-enriched glucose to soil in a 60-year-old coastal western hemlock forest, in a clear-cut site and in a highly disturbed landing site where logs were temporarily stored before transport, and compared the short-term soil-priming effect in each. In addition to increasing level of disturbance, the sites also had decreasing concentration of roots, increasing soil temperature extremes, and increasing soil moisture extremes. A positive priming effect was observed in both the forest and the clear-cut site in response to added glucose, resulting in an increased release of old SOM-derived C into the atmosphere. Phospholipid fatty-acid atom percent (At %) $^{13}$C excess values within the forest and clear-cut site were highest in the fungal PLFA biomarker 18:2ω6,9, and decreased over time, suggesting that the fungal community was responsible for the majority of the priming effect. A negative priming effect was observed in the landing site, resulting in, at least for a short while, conservation of old SOM.

5.2 Introduction

Soil organic matter (SOM) is the largest carbon (C) pool in terrestrial ecosystems (Falkowski et al. 2000; Fontaine et al. 2003), greater than terrestrial biomass C and atmospheric C combined (Jobbagy and Jackson 2000). Formed primarily through decomposition of litter, SOM consists of a broad spectrum of plant-derived C compounds such as acid-unhydrolysable residues, often
termed lignin, cellulose, glucose and plant lipids, as well as microbial-derived byproducts and remnants of dead microorganisms (Schmidt et al. 2011). Soil organic matter accumulation depends on the balance between the supply of plant C inputs (e.g. litter and root exudates), and microbial activity, specifically decomposition, mineralization and transformation (Schmidt et al. 2011). Bulk SOM turns over every 50-100 years, with different component compounds varying in turnover time from hours to centuries (Kalbitz et al. 2003; Boddy et al. 2007, 2008; Schmidt et al. 2011). Previously, it was thought that decomposition rates of different compounds were based solely on the chemical structure of component organic compounds; more complex compounds such as lignin persisted in soil longer than simple compounds such as glucose (Melillo et al. 1982). However, recent studies have shown that structurally complex compounds can be recycled quite rapidly, and that structurally simple compounds may remain in soils for decades (Grandy and Neff 2008; Marschner et al. 2008). More recently, it has been thought that the length of time SOM persists in soil is largely due to interactions between the physiochemical and biotic environment, although chemical structure is still relevant (Sollins et al. 2006; Schmidt et al. 2011). Disturbance, climatic changes and land-use changes can all alter the soil physical, hydrological and chemical environment, indirectly influencing nutrient cycling, microbial community composition, microbial activity, and ultimately ecosystem C balance. Determining how SOM mineralization changes in response to a disturbance, such as clear-cutting, is necessary to create accurate C budgets and effective ecosystem C models.

Living roots are major contributors to microbial-derived soil C, supporting microbial communities through the deposition of C into the rhizosphere (Schmidt et al. 2011). Rhizodeposit carbon can consist of lysates, dead root cell material and root exudates (e.g. diffusates, secretions, and excretions) (Paterson 2003; Uren 2007), which consist of carbohydrates, amino acids, low-molecular-weight aliphatic and aromatic acids, fatty acids, enzymes and hormones (Grayston et al 1997; Table 1.1). Root exudates can have a large impact on soil C content. Within root-free soil there may be less than 50 µM of labile C (Monreal and Mcgill 1985; Jones 1998; van Hees et al. 2005), in rooted soil there can be up to 100 µM of labile C and in the rhizosphere it may be upwards of 500 µM (Schneckenberger et al. 2008), though these numbers will vary with soil water content. This increase in labile soil C is partially responsible for the greater (10-100 x) bacterial biomass observed in the rhizosphere (Matilla et
There is some evidence of specificity in many plant-root-microbe interactions, suggesting that strong selective pressure and competition within the rhizosphere can result in diverse root microbiomes (Podila et al. 2009, Prescott and Grayston 2013). Root exudation into soil has been found to influence the soil microbial community, and alter microbial utilization of SOM (Kuzyakov and Cheng 2001; Brant et al. 2006, Bird et al. 2011).

It is generally assumed that soil microorganisms are C-limited (Tate 1995), and that a large addition of labile C to soil will cause a spike in microbial activity, microbial biomass and enzyme production (Kuzyakov et al. 2000). Such a change is predicted to result in decomposition of older and more recalcitrant C. A priming effect is defined as the increase, or enhanced, decomposition of SOM that occurs when microbes are stimulated by the addition of labile C (Dalenberg and Jager 1989; Kuzyakov et al. 2000). As a result of C addition there is an increase in CO₂ efflux greater than that of the C added, a “positive priming effect”. If the increase in CO₂ efflux is due to an increase in microbial growth alone, and not an increase in SOM decomposition, then it is called an “apparent priming effect” (Westcott and Mikkelsen 1985; Kuzyakov et al. 2000; Kuzyakov 2010; See Appendix D). In this case it is usually the previously dormant microorganisms that respond to the added substrate (Kuzyakov et al. 2000; Kuzyakov 2010). However, if the biomass of the microbial community remains stable, and the community actively decomposes more SOM, then it is called a “real priming effect”. In some instances the addition of labile C suppresses microbial activity or results in greater C storage; this is called a “negative priming effect” (Kuzyakov et al. 2000; Kuzyakov 2010).

Priming effects can be rapidly induced following changes in substrate availability, and can persist for many weeks to months, in the soil (Kuzyakov et al. 2000; Blagodatskaya et al. 2007; Dijkstra and Cheng 2007) [and even up to a year (Prevost-Bourne 2010)]. The addition of straw, leaf litter, cellulose, simple sugars and root exudates have all been found to stimulate priming effects (Kuzyakov et al. 2000; Prevost-Bourne 2010; Garcia-Pausas and Paterson 2011; Guenet et al. 2012). Added ¹³C-enriched glucose is typically mineralized by the microbial community within 1-3 days (Nottingham et al. 2009; Blagodatskaya et al. 2011). Depending on the type of C added to the soil, different microbial groups initiate priming. Bacteria, specifically r-strategist bacteria, are typically the first group to immobilize and metabolize labile C added to soil.
(Paterson et al. 2007; Garcia-Pausas and Paterson 2011). This results in an immediate increase in microbial activity and microbial biomass, initiating an apparent priming effect (Blagodatskaya et al. 2007; Nottingham et al. 2009). Bird et al. (2011) added various forms of $^{13}$C-enriched organic C to grassland soil and found that Gram-negative PLFA biomarkers were the first to take up the $^{13}$C, followed by Gram-positive and fungal PLFA biomarkers. Similarly Lee et al. (2011) added $^{13}$C-enriched glucose to rice callus soil and, using DNA SIP, found the label in Arthrobacter, Pseudomonas, Actinobacteria, Basilli, $\gamma$-Proteobacteria, Chloroflexi, Sphingobacteria, Flavobacteria, Clostridia, Acidobacteria and Cyanobacteria. Ultimately, the Gram-negative r-strategist bacteria, that immobilize the added C, turn over, releasing bacterial lysates. These lysates stimulate fungi and Gram-positive, K-strategist bacteria, increasing SOM decomposition (Lundquist et al. 1999). Thus, the apparent-priming effect transitions to a real priming effect (Lundquist et al. 1999; Kuzyakov et al. 2000; Garcia-Pausas and Paterson 2011). The resulting growth of filamentous fungi can enhance access to previously inaccessible SOM, which may account for the increased fungal to bacterial ratios seen in later stages of priming effects (Lundquist et al. 1999; Bell et al. 2003). Primed rhizosphere microbial communities appear to break down older, more recalcitrant SOM, rather than newer, more labile, plant residues (Bird et al. 2011). Fontaine et al. (2011) added $^{13}$C-enriched cellulose to different layers of grassland soil incubated in the lab and determined that the mean residence time of the SOM pool decreased in the subsoil layers (where availability of C is very low) from 3130 years to 17-39 years in the surface layer (where the availability of C is high) as a result of priming effects. This demonstrates decomposition is controlled by the availability of labile C and that priming effects have the potential to drastically alter soil C-cycling and sequestration.

The majority of priming-effect studies have been carried out in laboratory settings with agricultural soil; only a few studies have been conducted on forest soil (Sulzman et al. 2005) and fewer still have been conducted in forests in situ (Brant et al. 2006; Moore-Kucera and Dick 2008a; Crow et al. 2009). In Kuzyakov’s (2010) review, he states that future studies need to be conducted in the field, as there have been issues scaling-up laboratory studies to ecosystems. For instance Subke et al. (2004), in an experiment combining girdling and $^{13}$C-enriched leaf litter placed in a Norway spruce stand, determined rhizosphere communities play an active role in priming effects; in girdled plots (where rhizosphere communities are lost) the addition of C did
not result in any additional mineralization of SOM. Kuzyakov (2010) also stressed the importance of determining which microbial groups immobilize added C, to better predict the decomposition rates and utilization of individual C pools; and stressed the importance of determining how disturbance and land-use change will influence observed priming effects as temperature, soil moisture, nutrient availability and atmospheric CO₂ concentration changes all have the ability to alter labile C availability and microbial community structure and activity. Determining the impact of disturbance, and land-use change on priming effects will give insight into the ecological significance of priming in natural ecosystems and the potential long-term effects on C cycling.

The objective of this study was to determine the impact of harvesting, and harvesting practices, on the potential priming effect of forest soils in situ. I added ¹³C-labelled glucose to soil in a 60-year-old coastal western hemlock forest, in a clear-cut area and in a highly disturbed landing area where logs were temporarily stored before transport and compared the short-term priming effect. In addition to an increasing level of disturbance, the three sites also had a decreasing concentration of roots, increasing soil temperature extremes, and increasing soil moisture extremes. I measured soil respired CO₂, dissolved organic C (DOC), soil organic matter (SOM), microbial biomass, and the δ¹³C values of each as well as ¹³C-uptake using phospholipid fatty acids (PLFAs) over the course of 24 hours.

5.3 Methods

5.3.1 Study site

Samples were collected on October 20ᵗʰ-21ˢᵗ, 2011 in the third replicate of the existing Silviculture Treatments for Ecosystem Management in the Sayward (STEMS) long-term research installation set up by the British Columbia Ministry of Forests and Range, close to Gray Lake on Vancouver Island, B.C. (50°03’43.02”N, 125°35’24.35”W). The site is in the submontane very dry maritime Coastal Western Hemlock biogeoclimatic zone (CWHxm2) (de Montigny 2004). It receives annual precipitation of approximately 1529.5 mm and has a mean temperature of 8.4 °C (Upper Campbell Lake Climate Station, National Climate Data and Information Archive, 1971-2000). Soils are mostly Orthic Humo-Ferric Podzols with moder
humus form and sandy-loam structure (de Montigny, 2004). The area is a second-growth forest, composed of 65 to 75-year-old western hemlock (Tsuga heterophylla) and Douglas-fir (Pseudotsuga menziesii) trees. In 2007 the site was harvested into aggregated tree patches approximately 0.2 ha in size, with clear-cut spaces ranging from 4.4 to 11.5 ha, in a total area of 25.5 ha. The undergrowth consisted mainly of salal (Gaultheria shallon), huckleberry (Vaccinium parvifolium) and sword fern (Polystichum munitum). The soils were a mix of mineral and organic layers in the clear-cut area due to harvesting machinery.

Three sites within STEMS 3 were selected along a disturbance gradient: an aggregated retention patch (forest) containing 65-75-year-old Douglas-fir and western hemlock trees; an adjacent clear-cut site containing vegetation, dominated by salal, sword fern and huckleberry; and a highly-disturbed landing area used to pile logs before transport. The third site was un-vegetated, the soil was compacted and the area was covered with bark. pH of soils in all three sites ranged from 3.5-5.0.

5.3.2 Experimental design

At each of the three sampling sites, in a 16-m² area, 24 PVC collars (10-cm diameter) were placed approximately 4 cm into the soil, at random and with a minimum distance of 30 cm between each collar, 24 hours before ¹³C-enriched glucose addition. Half the samples (12) were given 0.416 g of ¹³C-enriched glucose dissolved in 14 ml of water, pipetted evenly over the entire soil surface (each sample received 0.1664 g of ¹³C), the other half was given an equal amount of water. After 2, 4, 8 and 24 hours (corresponding to 10 am, 12 noon, 4 pm and 8 am the next morning) 3 samples from each site and treatment were selected for analysis. After soil respiration samples were collected (described below), all soil within the PVC collar, down to 10 cm was removed, divided into organic and mineral soil, then stored at 4 °C prior to analysis. Frequently, there was not enough mineral soil to conduct a thorough soil analysis, because the organic layer was too thick, or rocks/old roots were present. Because of this the data are incomplete and not presented.
Glucose was selected as the labile C source because it is a monomer of most plant organic polymers, and because most soil microorganisms are capable of metabolizing it (Anderson and Domsch 1978; Landi et al. 2006).

5.3.3 Soil and CO₂ analysis

All soil was sieved to < 2 mm. The δ¹³C value of DOC was measured according to the technique in Bengtson and Bengtsson (2007). Briefly, 0.5 g of field-moist soil was placed in Z-spin 0.2-µl-filter units and centrifuged at 16000 × g for 30 minutes (DiaMed, Mirko 20, Ontario, Canada). The resulting soil water in the collection tube was transferred into 8 mm tin cups, evaporated at 35°C and stored at room temperature before ¹³C analysis. Soil organic C was measured by acid-fumigating oven-dried soil to remove carbonates, which tend to skew isotopic results in mineral soil (Harris et al. 2001). Carbonate levels were found to be so low that acid fumigation was deemed unnecessary. Therefore, we used oven-dried soil to calculate SOM levels.

Gas samples for ¹³CO₂ measurement were collected after 45 minutes of incubation using a closed non-steady-state incubation chamber described in Basiliko et al. (2009). Thirty ml gas sub-samples were taken using gas-tight syringes and stored in gas-tight bags prior to ¹³CO₂ analysis in the field using a Piccaro instrument (see stable isotope analysis below). Samples were collected 2, 4, 8 and 24 hours after ¹³C-glucose amendment. The resulting gas samples were a mixture of respired soil efflux and atmosphere, and were corrected using a Keeling plot approach.

Phospholipid fatty acids were extracted from the soil samples at Lund University, Sweden. Extracted fatty acid methyl esters were identified by means of a combination of mass spectra and retention times relative to the internal standard 19:0, and an external bacterial acid methyl ester standard (BAME, Sigma-Aldrich co., 47080-0). Each sample was quantified relative to the internal standard, 19:0. The following fatty acids were chosen to represent bacterial PLFAs: i15:0, a15:0, 15:0, i16:0, i16:1ω7c, i17:1ω8c, 10Me17:0, i17:0, a17:0, 18:1ω7c, 18:1ω5c, cy19:0 (See Table 3.1; Kropfenstedt 1985; Frostegård et al. 1993; Zogg et al. 1997). Fungal biomarkers are limited to 18:2ω6,9. Abundance of identified fatty acids expressed as Mol % were calculated based on the chromatograph peak areas relative to the peak area of the internal standard 19:0.
PLFA nomenclature identifies the number of C atoms in the fatty-acid chain (e.g. 18 in 18:1ω7), the number of double bonds in the chain (e.g. 1) and the position of the first double-bonded C from the methyl end of the fatty-acid molecule (e.g. ω7).

5.3.4 Stable-isotope analysis

An elemental analyzer (Elementar Vario EL Cube: in C, N mode; Hanau, Germany) interfaced with an isotope ratio mass spectrometer (GV Instruments Isoprime, with reference gas box; Cheadle Hulme, UK) via continuous flow, at the Belowground Ecosystem Group’s Stable Isotope Facility at the University of British Columbia, was used to measure the $^{12}\text{C}$ and $^{13}\text{C}$ content of SOM and DOC.

Gas $^{13}\text{C}/^{12}\text{C}$ ratios were measured on a Picarro (Sunnyvale, California) in the field. The Picarro analyzer is based on cavity ring-down spectroscopy, which is a time-based measurement using a near-infrared laser to measure the spectral signature of CO$_2$ (see Appendix E). Briefly, a beam from a single-frequency laser diode enters a cavity, that is defined by three high reflectivity mirrors, where the beam quickly begins circulating as it reflects off the mirrors. A small fraction is allowed to leak through one of the mirrors, and once a certain threshold of leakage occurs (measured by a photo detector), the laser shuts off, and the light continues to circulate within the cavity. Due to 99.9999% reflectivity of the mirrors and due to leakage, the light intensity within the cavity slowly decreases (ring-down). When a gas sample, in our case CO$_2$, is introduced into the cavity, the gas absorbs the light and accelerates the ring-down time. The Picarro instrument then calculates, and compares, the ring-down time of the cavity with and without the introduced absorbing gas, producing precise and quantitative measurements. By tuning the laser to different wavelengths the Picarro is able to measure the target gas and their respective isotopes. Precision of $\delta^{13}\text{C}$ in CO$_2$ was: $<$0.01‰ guaranteed precision at $>$ 380 ppm CO$_2$, $< 0.25$‰ precision at 200 ppm CO$_2$, and $< 0.05$‰ typical precision $> 1000$ ppm CO$_2$.

The $^{13}\text{C}/^{12}\text{C}$ ratios of the PLFAs were analyzed at the stable isotope facility at the Department of Biology, Lund University, Sweden. The PLFAs were separated on a Thermo Scientific TRACE GC Ultra gas chromatograph, and the isotopic ratios determined by a Delta V Plus isotope ratio mass spectrometer connected to the GC via the GC Isolink and ConFlo IV interfaces (Thermo
Scientific Inc., Bremen Germany). Both $\delta^{13}C_{\text{PDB}}$ values and atomic percent $^{13}$C excess are reported. Atom percent (At %) is the absolute number of atoms of a given isotope in 100 atoms of total element. At % $^{13}$C-excess specifies the level of isotopic abundance above the control, which is considered zero. When $\delta^{13}$C values are much enriched (as they are in this experiment, particularly for the PLFAs) it is often better to present data as At %. In previous chapters, I scrutinized only the $\delta^{13}$C values, and therefore, $\delta^{13}$C value will be included in this chapter, as well as At %, for consistency and clarity.

5.3.5 Priming measurements

I used an isotopic mass-balancing equation to calculate the proportion of the respired CO$_2$ that was derived from the applied glucose, as in Bengtson et al. (2012):

\[
\text{fglu} = \frac{\text{At}^{13}C_{\text{gluCO}_2}}{\text{At}^{13}C_{\text{DOC}}} - \frac{\text{At}^{13}C_{\text{glucose}}}{99.98}
\]

Where fglu is the fraction of soil CO$_2$ efflux derived from glucose, At$^{13}$C$_{\text{gluCO}_2}$ is the measured At$^{13}$C of soil CO$_2$ efflux from soils receiving glucose additions, At$^{13}$C$_{\text{DOC}}$ is the At$^{13}$C of DOC (the C source for the microbial community) from soils not receiving glucose additions (SOM-derived), and At$^{13}$C$_{\text{glucose}}$ is the At$^{13}$C of the applied glucose (99.98 %).

To determine the relative (%) of soil organic matter mineralization I used the following equation:

\[
\text{CO}_2\text{gluRespRate} = \frac{\text{CO}_2\text{controlRespRate}}{\text{SOM mineralization}}
\]

Where CO$_2$gluRespRate is the rate of CO$_2$ respiration in treated soil, and CO$_2$controlRespRate is the rate of CO$_2$ respiration in control soil. The SOM mineralization was not measured directly, instead it was inferred from soil respiration rates and the At$^{13}$C values of DOC, respired CO$_2$ and added glucose. Consequently, it may be more appropriate to call it and estimation of SOM mineralization based on CO$_2$ efflux.
5.3.6 Statistical analysis

All statistical analysis was conducted using SAS system release 9.1 (SAS Institute Inc., Cary, NC). Data was analyzed using a 2 or 3-way ANOVA, followed by a Holm-Sidak analysis to determine differences within the sources of variation. Statistically significant differences were \( \alpha < 0.05 \).

5.4 Results

5.4.1 DOC and SOM

The amendment of soil at all three sites with \(^{13}\)C-enriched glucose resulted in a significant difference between \(^{13}\)C-unamended (control) and \(^{13}\)C-amended (treatment) in DOC \( \delta^{13} \)C values (Table 5.1). The \( \delta^{13} \)C value of the DOC in the \(^{13}\)C-amended plots was up to 6 orders of magnitude greater than the control plots (Figure 5.1). There was no significant difference in \( \delta^{13} \)C values in DOC over time; however, there was a significant difference in DOC \( \delta^{13} \)C values at the different sites (Table 5.1). The \( \delta^{13} \)C value of DOC in the landing site was greater than at the forested and clear-cut sites (Figure 5.1). There was no significant difference in \% C, or total C content of DOC, due to site, treatment or sampling time.

The amendment of soils with \(^{13}\)C-enriched glucose did not result in a significant change in the \% C of SOM, except at the treated landing site, which had a higher \% C than the treated forest or clear-cut site (Fisher LSD, \( p=0.002 \)) (Table 5.1). This is likely the result of site heterogeneity; despite the treated landing sites having consistently high \% C, the values ranged from 20-55 \% C within that area. The \( \delta^{13} \)C value of SOM was significantly greater when \(^{13}\)C-enriched glucose was added to soils at all three sites (Table 5.1). In addition, the forest site had significantly greater SOM \( \delta^{13} \)C values than the bare site and clear-cut site (Table 5.1; Figure 5.2). There did not appear to be an effect with time.
5.4.2 Soil respiration

There was a significant difference in the background (control) respiration rate among the three sites and between different sampling times (3-way ANOVA, df = 2, F = 8.188, p<0.001). On average, the lowest respiration rate occurred at the landing site (35.5 mg CO$_2$-C h$^{-1}$ m$^{-2}$) while the forested site had the highest average respiration rate (48.0 mg CO$_2$-C h$^{-1}$ m$^{-2}$) (Figure 5.3). The respiration rate at the clear-cut site was intermediate to the landing and forested site (41.2 mg CO$_2$-C h$^{-1}$ m$^{-2}$). In total, 2.82 g of CO$_2$-C m$^{-2}$ was respired during the 24-hour experimental period at the landing site, while the clear-cut and forested site respired 3.42 and 3.44 g CO$_2$-C m$^{-2}$, respectively (Fig 5.3).

Addition of $^{13}$C-glucose to soils at all three sites resulted in an increase in soil respiration compared to control soils at the same site that received only water (3-way ANOVA, df=1, F= 16.21, p<0.001; Fig 5.3). The effect was most pronounced at the forested site, and least pronounced at the landing site (3-way ANOVA, df=2, F= 16.61, p<0.001, followed by Holm-Sidak, p<0.001; Fig 5.3). The rate of added glucose mineralization during the first 8 hours was similar at all three sites, but after 24 hours more glucose had been mineralized at the forested and clear-cut site compared to the landing site (Fig 5.4, Table 5.2, followed by Fisher LSD, p<0.001).

5.4.3 PLFAs

5.4.3.1 Mol %

The average mol % of PLFAs was highest in the forested site, though this difference was not statistically significant. When PLFAs were grouped into Gram-negative, Gram-positive, actinomycetes and fungi, the community appeared to have a greater proportion of Gram-negative bacteria and fungi, rather than Gram-positive bacteria and actinomycetes (Figure 5.5). The fungal to bacterial ratio was highest in the forested site and lowest in the landing, with the clear-cut intermediate. There were differences in individual PLFA composition between the sites (Table 5.3). There was significantly more of the Gram-positive PLFA biomarker i15:0 and the Gram-negative biomarker cy19:0 in the landing site relative to the forested site, with the clear-cut intermediate to these (Table 5.4). There was a significantly greater amount of the fungal biomarker 18:2ω6,9 in the forested site than in the clear-cut and landing sites (Table 5.4). In
addition, there was significantly less of the Gram-negative biomarker 18:1ω7c in the landing site, relative to the clear-cut and forested sites (Table 5.4). The actinomycete biomarker 10Me16:0 was significantly different between all sites and was highest in the forest, and lowest in the clear-cut area (Table 5.4).

5.4.3.2 At % ¹³C excess
Because the δ¹³C values of the PLFAs were so high, it is more appropriate to compare PLFA At % ¹³C excess, which compares the At % ¹³C value of the PLFA of interest in the ¹³C-amended sites versus the non-amended sites (see Appendix F for the δ¹³C values of the PLFAs). The PLFA At % ¹³C excess was greatest 2 and 4 hours after ¹³C-glucose addition (Table 5.3), greatest within the landing site, and greatest within the fungal PLFA biomarker, followed by Gram-negative bacteria, with Gram-positive and actinomycetes significantly lower (Table 5.3; Figure 5.6). There was a significant difference in average PLFA At % ¹³C excess over the sampling period within sites (Table 5.3). Two hours after ¹³C-glucose addition, At % ¹³C excess was significantly greater in the landing site than the clear-cut, with the forested site intermediate to these. This difference was not present after 4 hours, but after 8 hours At % ¹³C excess at the landing site was again significantly greater than the forested and clear-cut site, and this trend was still present after 24 hours (Figure 5.6). Within the landing site the Gram-negative bacteria had greater At % ¹³C excess, relative to Gram-positive bacteria, followed by fungi and actinomycetes (Figure 5.6). Within the clear-cut and forested site, the At % ¹³C excess value for fungi was significantly greater than all bacteria.

There was significantly greater At% ¹³C excess in the fungal PLFA biomarker, 18:2ω6,9 relative to all other PLFAs (Table 5.5). This was most pronounced at the landing site relative to the clear-cut and forested sites (Table 5.5). The PLFA biomarker 18:1ω9c, which represents both fungi and bacteria, also had significantly greater At % ¹³C excess compared to all other PLFAs (Table 5.6) (except 18:2ω6), and was significantly different for all three sites, with the landing site being highest and clear-cut being lowest (Table 5.5). The At % ¹³C excess of the Gram-negative biomarker, 18:1ω7c, was similarly high (Table 5.5, with the landing site greater than the clear-cut site and the forest site intermediate to these (Table 5.5).
5.4.4 Priming effect

Cumulative priming effects increased significantly in the forested and clear-cut sites, and decreased over time in the landing site (Table 5.2, Figure 5.7). Similarly, the relative change in estimated SOM decomposition increased over the sampling period (Table 5.2, Figure 5.8), and was greater than the control (above 100) in the clear-cut and forest sites, but was below the control levels (below 100) in the landing site (Table 5.2, figure 5.8). Glucose addition at the clear-cut and forested sites could not explain the full magnitude of the increased respiration. Instead, a part of the increased respiration was caused by an estimated increase in mineralization of SOM (positive priming effect; Fig 5.7). At the landing site, glucose additions resulted in an estimated reduction of mineralization of SOM (negative priming effect; Fig 5.7, Table 5.2). The negative priming at the landing site corresponded to a decrease in estimated SOM mineralization of between 10-30 %, while the positive priming resulted in an estimated 10 and 20 % increase in SOM mineralization at the clear-cut and forested site, respectively (Fig 5.8).

5.5 Discussion

The greater respiration rate recorded in the forested site was likely due to the greater concentration of roots at this location, compared to the clear-cut site, which had a few, mostly ericaceous roots, and the landing site, which had even fewer roots. The lower respiration rates in the landing and clear-cut sites may also have been due to a lower microbial activity. A less active microbial community in the landing-site might explain the higher $\delta^{13}C$ values of the DOC and SOM in the same area. Less of the added $^{13}C$-enriched glucose would be mineralized at the landing site if the community was less active, resulting in more of the $^{13}C$-enriched glucose remaining in the DOC. However, the microbial data does not support this. The microbial community in the landing site had higher $\delta^{13}C$ values and At $^{13}C$ excess compared to the clear-cut and forested sites. This suggests that the landing site microbial community mineralized more of the $^{13}C$-enriched glucose. However, the At $^{13}C$ excess values of the microbial community did not remain stable through the sampling period. Four hours after $^{13}C$-glucose addition, the At $^{13}C$ excess of the PLFAs decreased, particularly within the forested and clear-cut sites.
However, at the landing site PLFA At $^{13}$C excess values did not decrease, suggesting the microbial community retained the label, rather than metabolizing it.

The addition of glucose also caused an increase in respiration rate over time, an effect that was most pronounced in the forested and clear-cut sites. This difference corresponded to an estimated increase in SOM mineralization, particularly after 24 hours. In fact, the amount of respiration derived from “old” SOM was 10 and 20 % above the control for the clear-cut and forested sites respectively, indicating a positive priming effect in response to glucose addition. This, in combination with the decreasing microbial $\delta^{13}$C values and At $^{13}$C excess values over time suggests that the increase in respiration was not due to pool substitution (i.e. an apparent priming effect), but rather due to an estimated increase in mineralization of old SOM (i.e. a real priming effect). These results are consistent with other studies reporting reduced C storage in soil as a result of increased C inputs (Heath et al. 2005; Godbold 2006; Dijkstra and Cheng 2007).

However, at the landing site direct respiration of glucose explained the full magnitude of the increase in total soil respiration in response to glucose addition. In actuality, the addition of glucose seemed to cause a negative priming effect, where the soil microbial community was taking up the added $^{13}$C-enriched glucose and respiring it rather than using the additional energy to break down older, more recalcitrant SOM, resulting in an estimated decrease in old SOM mineralization by about 10-30 %. Subke et al. (2004) showed that rhizosphere communities played an active role in priming effects. Addition of pine needles to a forest soil only resulted in a positive priming effect in plots that were not girdled, whereas in plots where trees were girdled there was no additional mineralization of old SOM. Several soil-incubation studies have shown that the presence of plants can increase SOM decomposition by up to 380 % (Cheng et al. 2003). The observed difference in priming effect between the landing site and the forested and clear-cut sites may have been the result of the presence of rhizosphere microbial communities, or may be due to differences in soil bulk density. The landing site soil was more compact compared to the clear-cut and forest soil, this compaction could retard the movement of respired CO$_2$, causing a delay in the observation of the response to glucose addition. However, this does not explain why we would observe an estimated lower SOM decomposition in the landing site with glucose amended soils relative to the control, and therefore differences in bulk-density does not fully explain the differences between sites.
There were slight differences in microbial community structure between the three sites. In general, there were more fungi in the forested and clear-cut sites relative to the landing site, which would correspond to reduced mycorrhizal abundance at the landing-site (Chapter 2, 3; Churchland et al. 2013). Within the landing site there were more bacteria, specifically Gram-negative (18:1ω7c), Gram-positive (i16:0) and actinomycetes (10Me18:0). It may be that the mycorrhizal fungi dominated the microbial community within the forested site and the microbial community in the clear-cut site (evidence from Chapter 3 and 4 suggests mycorrhizae can extend 10 meters into a clear-cut) were able to quickly respond to additions of labile C because it is not an unusual occurrence in rooted soil. Root exudates can account for 6-33% of recent photosynthates (Grayston et al. 1997), and the rhizosphere microbial community has been found to respond rapidly to any labile C addition (Weintraub et al. 2007).

The relationship between microbial community diversity and ecosystem function is unclear, and is often thought to be insignificant due to functional redundancy in microbial populations (Griffiths et al. 2000; Nannipieri et al. 2003, Wertz et al. 2006, Strickland et al. 2009, McGuire and Treseder 2010). Data from Chapter 2 supports the idea that general or non-specific functions, like those associated with nitrogen cycling, are likely to be unaffected by harvesting. However, in Chapter 2 I also found evidence that harvesting impacts both the microbial community composition and ‘narrow’ specialist processes. Results presented here in this Chapter, and from Chapter 2 and 3, show a community shift from the retention patch into the clear-cut, and further changes into the landing site, specifically a transition from a fungal to a bacterial-dominated microbial community. I propose that the change in the microbial community as a result of disturbance has resulted in either the loss, or reduction, in the ability to capitalize on the added labile C, and subsequently the ability to decompose recalcitrant SOM. Paterson et al. (2011), observed such a phenomenon in bare-fallow soils, where plant-derived C was excluded for over 50 years. They suggested that the long-term selective pressures resulted in a microbial community with reduced ability to break down recalcitrant root-derived carbon. The highly disturbed landing site may have had similar selective pressures. It would be interesting to measure priming effects at this same site for a longer period of time, and to repeat the experiment after another 5 and 10 years. Clear-cut microbial communities can be quite slow to recover from disturbance (Hartmann et al. 2012), though typically are fully recovered after canopy closure
We saw no loss of estimated SOM decomposition function in the clear-cut area 4 years after harvest; however, the extensive damage to the landing soil may result in the microbial community taking much longer to recover.

Phillips et al. (2012) measured C turnover in pine forests exposed to elevated CO₂ (predicted to increase root exudation, and leaf litter) and fertilized with ¹⁵N. They found that the increased addition of C and N to the soil resulted in an accelerated turnover of root-derived C. Several other studies have suggested that higher N availability may increase the rhizosphere priming effect on SOM decomposition (Hoosbeek et al. 2006; Rasmussen et al. 2007). Conversely, we observed the greatest priming effect in the area with the lower N availability. In a previous study at the same site, N availability was lowest in the forested area, and higher in the clear-cut (we suspect the landing site would be more similar to the clear-cut than the forested site; Chapter 2; Churchland et al. 2013). This result is in accordance with several other studies that suggest priming effects are higher in nutrient-poor soils (Fontaine et al. 2003; Treseder 2008; Zak et al. 2008; Liu and Greaver 2010). In nutrient-poor soils, it is hypothesized that the low-quality C and low nutrient availability limits the amount of energy available for soil mineralization.

Most studies measuring SOM decomposition due to priming effects in the presence of plant roots have been conducted on grasses or shrubs, and in the short term (Helal and Sauerbeck 1984; Liljeroth et al. 1994; Cheng 1996; Cardon et al. 2001; Cheng et al. 2003; Bader & Cheng 2007), although the priming effect can persist for many months (Dijkstra and Cheng 2007), or even years (Prevost-Bourne et al. 2010). Several studies have explored forest soil priming effects in situ (Subke et al. 2004; Brant et al. 2006, Moore-Kucera and Dick 2008a; Crow et al. 2009; Fontane et al. 2011). Unlike this study, previous studies added recalcitrant C or additional litter and observe the soil respiration response. Moore-Kucera and Dick (2008a), Crow et al. (2009) and Subke et al. (2004) all added ¹³C-enriched needle litter and found an increase in recalcitrant SOM mineralization, which could account for 11.5-21.6 % of annual CO₂ efflux from the litter-amended plots (Crow et al. 2009). Despite the difference in the type of C amendments, the increase in soil respiration in the Crow et al. (2009) study, as a result of litter addition, is similar to the 10-20 % increase in SOM-mineralization I observed in the ¹³C-enriched glucose amended forested and clear-cut sites. Previous studies that have added more labile forms of C to forest
soil, such as cellulose (Fontaine et al. 2011) and glucose (Brant et al. 2006), found significant positive priming effects, and found that soil fungi incorporated the majority of the added label and were thought to be responsible for the priming effect. Similarly, I found that fungi in the clear-cut and forest site had the greatest At % $^{13}$C excess relative to the bacteria. This suggests that the fungal community is in part responsible for the observed priming effects.
Figure 5.1 The average $\delta^{13}$C value of DOC within the treated plots (dark grey) and the control plots (light grey) over 24 hours. F = forested site, CC = clear-cut site, L = landing site. The vertical bars represent the standard error of the means (n=3). Some soil samples were so small that DOC could not be calculated; consequently, some samples do not have error bars. Glucose was added at 8 am, therefore 2, 4, 8 and 24 hours after addition corresponded to 10 am, noon, 4 pm and 8 am the following morning.
Figure 5.2 The average $\delta^{13}$C value of SOM within the treated plots (dark grey) and the control plots (light grey) over 24 hours. F = forested site, CC = clear-cut site, L = landing site. The vertical bars represent the standard error of the means (n=3). Some soil samples were so small that SOM could not be calculated; consequently, some values do not have error bars. Glucose was added at 8 am, therefore 2, 4, 8 and 24 hours after addition corresponded to 10 am, noon, 4 pm and 8 am the following morning.
Figure 5.3. Average cumulative respiration ($^{12}$C- and $^{13}$C- CO$_2$), in both $^{13}$C-enriched glucose-amended (treatment) and unamended (control) samples at the three sites, over 24 hours. The vertical error bars represent standard error (n=3).
Figure 5.4. Total respiration induced by glucose (mg C) over the entire sampling period at the three sites. The vertical error bars represent standard error (n=3).
Figure 5.5. Average mol % of Gram-negative, Gram-positive, actinomycete and fungal PLFAs at the three sampling sites over 24 hours.
Figure 5.6. Average At % $^{13}$C excess of actinomycetes, fungal, Gram-negative and Gram-positive PLFAs at the three sampling sites over 24 hours.
Figure 5.7. Cumulative priming effects at the three sites over the entire sampling period. The vertical error bars represent standard error (n=3).
Figure 5.8 Relative additional SOM mineralization as a result of $^{13}$C-enriched glucose amendment at the three sites over the entire sampling period. Vertical error bars represent standard error (n=3).
Table 5.1. Results of two-way analysis of variance for DOC, SOM and cumulative respiration. α<0.05. Only statistically significant differences are reported.

<table>
<thead>
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<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
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</tr>
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<td></td>
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</tr>
<tr>
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<tr>
<td></td>
<td>Site</td>
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<td>10.3</td>
</tr>
<tr>
<td></td>
<td>Site x Treatment</td>
<td>2</td>
<td>10.2</td>
</tr>
<tr>
<td>% C or SOM</td>
<td>Site x Treatment</td>
<td>2</td>
<td>8.8</td>
</tr>
</tbody>
</table>
Table 5.2 Results of two-way analysis of variance for soil respiration and priming effects. 
α<0.05. Only statistically significant differences are reported.

<table>
<thead>
<tr>
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<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<td></td>
<td></td>
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<td>Cumulative glucose-derived respiration (mg CO₂-C m⁻²)</td>
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<td></td>
<td></td>
</tr>
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<td>97.0</td>
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</tr>
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<td>0.3</td>
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<td>Cumulative priming (mg CO₂-C m⁻²)</td>
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Table 5.3. Results of three-way analysis of variance for PLFAs. α<0.05. Only statistically significant differences are reported.

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<th>Dependent variable</th>
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Table 5.4 Abundance (mol%) of PLFAs in the landing, clear-cut and forest site. SE represents standard error of the mean (n=24). For each PLFA, similar letters indicate the individual treatments are not significantly different (Holm-Sidak ANOVA, α>0.05).

<table>
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Table 5.5 At % $^{13}\text{C}$ excess of PLFAs in the landing, clear-cut and forest site. SE represents standard error of the mean (n=24). For each PLFA, similar letters indicate the individual treatments are not significantly different (Holm-Sidak ANOVA, $\alpha>0.05$).

<table>
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<th>SE</th>
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6 Conclusions

This thesis explored the impact of live trees retained on harvested forest sites on soil microbial community composition and function over several different harvesting regimes. Specifically, I examined the spatial and temporal movement of root-exuded C through the ecosystem using $^{13}\text{C}$ natural abundance stable-isotope-ratio analysis, as well as stable-isotope labeling and probing techniques. This allowed me to determine the range of influence of recently photosynthesized tree-carbon on soil microbial community composition, as well as the impact of the presence (or absence) of labile C sources on soil C-cycling.

Live trees retained on harvested sites are thought to maintain pre-harvest microbial community structure and nutrient availability due to their ability to maintain microclimate conditions under their canopy, through shading, and due to their ability to supply C to soil organisms, through above and below-ground litter, and through continuous root exudation of labile C (Carlson and Groot 1997; Franklin 1997; Lee et al. 2002). Access to tree seedling-specific mycorrhizae and other plant growth-promoting bacteria may, in part, be necessary for seedling success (Teste and Simard 2008). Retained trees on harvested sites are thought to act as hubs for the species-specific soil microbial communities necessary for seedling success (Franklin 1997). Previous work has shown the potential of variable-retention harvesting to maintain pre-harvest enzyme activities (Daradick 2007; Dewi 2009), ECM sporocarps richness/abundance (Luoma et al. 2006; Jones et al. 2008) and microbial community structure (as defined by PLFAs) (Kranabetter and Wylie 1998; Hagerman et al. 1999). Variable-retention harvesting also has the potential to mitigate the effects of clear-cut harvesting on nutrient availability, specifically nitrate (Knight et al. 1991; Parsons et al. 1994; Prescott et al. 2003; Hope et al. 2003; Redding et al. 2003; Jerabkova et al. 2011). However, nutrient (Bock and VanRees 2002; Carmosini et al. 2002; Jerabkova et al. 2006; Theil and Perakis 2009) and microbial community responses (Kropp and Albee 1996; Buée et al. 2005; Teste et al. 2012; Bach et al. 2013) are inconsistent. Through observation of the microbial community, nutrient availability, and microbial C sources in different green-tree-retention regimes, I was able to determine which design best maintains pre-harvest ecosystem function in Chapter 2.
The range of influence tree roots impart on the soil microbial community, and the harvesting regime that best maintains pre-harvest microbial community structure, are key knowledge gaps in variable-retention harvesting (Gustafsson et al. 2012). To understand how forest ecosystems respond to a changing environment, it is necessary to address the fate of plant-allocated C belowground, determine how plants influence the abundance and flow of C to different groups of soil microorganisms, and determine how retained trees influence soil C-cycling. Plants allocate approximately one-third of recently photosynthesized C belowground, though it can increase to two-thirds depending on the species and climatic conditions (Litton et al. 2007). The C allocated belowground supports the growth and activity of roots, mycorrhizae and rhizosphere microbial communities (Read 1991; Curtis et al. 2002; Read and Perez-Moreno 2003). How this C is allocated between root biomass, root respiration, mycorrhizal biomass, mycorrhizal respiration and root exudates varies (Goodale et al. 2002; Lipson et al. 2005; Kandeler et al. 2008; Castro et al. 2010), and has been historically difficult to assess. In Chapter 3, I explored the spatial influence of an aggregated-retention patch on soil microbial activity community composition, and examined the sources of C for the microbial community through the analysis of $^{13}$C/$^{12}$C natural abundance stable-isotope ratios.

Plants create and maintain a mosaic of environmental conditions due to differences in nutrient demands, transpiration, and above/belowground C inputs. This translates to spatial variation in $\delta^{13}$C values, due to different C inputs, age of plants and activity of soil microorganisms and fauna. Little is known about how the proximity to plants, specifically large trees, translates into corresponding spatial variations in the activity and abundance of different groups of microorganisms. However, evidence is emerging that fungal versus bacterial dominance in the soil could have profound effects on the belowground C-cycle (Jastrow et al. 2007; Cheng 2009; Strickland and Rousk 2010; Drake et al. 2011; Zak et al. 2011), and it has been suggested that compositional shifts in soil saprotrophs are the drivers of ecological response to environmental disturbance (Zak et al. 2011). Results from Chapters 2 and 3 show evidence of significant shifts in microbial community composition as a result of harvesting, though individual-tree influences on the microbial community are not well understood. Through the development of a novel stem-injection stable-isotope-labeling
and probing technique I was able to tease out the range and timing of C-flow from individual live trees into the soil microbial and faunal community, and determine which microbial groups are most dependent on tree C for growth and metabolism, in effect, establishing which microbes will be most affected by harvesting (Chapter 4).

It is generally assumed that soil microorganisms are C-limited (Tate 1995), and that large additions of labile C cause spikes in microbial activity, microbial biomass and enzyme production (Kuzyakov et al. 2000). Such a change is predicted to result in decomposition of older and more recalcitrant C. A priming effect is defined as the increase or extra decomposition of SOM that occurs when microbes are stimulated by the addition of labile C (Dalenberg and Jager 1989; Kuzyakov et al. 2000; Kuzyakov 2010). Priming effects can be induced rapidly following changes in substrate availability, and can persist for many weeks to months in the soil (Kuzyakov et al. 2000, Blagodatskaya et al. 2007; Dijkstra and Cheng 2007). The addition of plant residues, simple sugars and root exudates have all been found to stimulate priming effects (Kuzyakov et al. 2000; Prevost-Bourne 2010; Garcia-Pausas and Paterson 2011; Guenet et al. 2012). Depending on the type of C added to the soil, different microbial groups initiate priming. The majority of priming effect studies have been carried out in laboratory settings with agricultural soil; only a few have been conducted with forest soil (Sulzman et al. 2005), and fewer still have been conducted in situ (Brant et al. 2006; Moore-Kucera and Dick 2008a; Crow et al. 2009). In Kuzyakov’s review (2010), he states that future studies need to be conducted in the field, as there have been issues in scaling-up laboratory studies to ecosystems. In situ studies have found that priming effects are most pronounced in nutrient-poor soils (Fontaine et al. 2003) and in pH near-neutral soils (Blagodatskaya and Kuzyakov 2008). Kuzyakov (2010) also stressed the importance of examining which microbial groups are immobilizing added C and how disturbance and land use change will influence observed priming effects. In Chapter 5, I added $^{13}$C-enriched glucose to forest soil in situ, traced the flow of C into the microbial community and measured their response to this labile C addition. Determining the impact of disturbance and land-use change on the potential priming effect of forest soil will give insight into the ecological significance of priming in natural ecosystems and the potential impact on C-cycling.
6.1 Main findings

In Chapter 2, I explored the impact of different green-tree-retention harvesting regimes on microbial community composition, microbial C use and nutrient availability. Four different treatments were compared: a dispersed-retention area, an aggregated-retention patch, a clear-cut edge within 9 m of the aggregated retention patch, and a clear-cut area. Unlike previous studies, I did not observe elevated nitrate in the harvested areas; instead, ammonium was elevated. Availability of N and other nutrients were surprisingly similar between the dispersed-retention area and the aggregated-retention patch. In general, despite being in close proximity to the aggregated retention patch, microbial community structure and nutrient availability in the clear-cut edge were more similar to the clear-cut than to the forest, contrary to my hypothesis that clear-cut edges would resemble forested sites. This result contradicts previous studies in which clear-cut edges tended to resemble forest sites (Hope et al. 2003; Jerabkova et al. 2006; Theil and Perakis 2009). The microbial community, however, was different in the clear-cut, the clear-cut edge and the dispersed-retention area relative to the retention patch, mostly due to a significantly lower abundance of fungi coupled with an increase in bacteria, specifically Gram-negative bacteria. This shift in the microbial community composition in the dispersed-retention area did not appear to have a major impact on microbial functioning and nutrient availability, indicating that this harvesting practice has the potential to maintain general microbial function. There may, however, be one major exception – Mn was twice as high in the retention patch (and clear-cut edge) as in the clear-cut and dispersed-retention areas. Mn levels in soil are either controlled by production and activity of specific Mn-reducing bacteria that live in close association with mycorrhizal fungi, or alternatively Mn levels are controlled by the presence and abundance of Mn peroxidase, which concomitantly removes Mn from solution. The shift to a bacterial-dominated community in the clear-cut and dispersed-retention area likely resulted in the loss, or reduction, of certain narrow functions (those performed by a small number of specialized microorganisms).

In Chapter 3, I explored the movement of C from live trees retained on clear-cut sites into the soil microbial community through analysis of $^{13}$C natural-abundance stable-isotope values in
the microbial community, soil-respired CO₂, DOC and SOM. In order to achieve this I set up a 100-point grid along a transition from an aggregated-retention patch into a clear-cut area and used geostatistics to visualize the differences in δ¹³C values in C sources. Recent tree photosynthate C was the dominant C source for the microbial community in the retention patch and for up to 10 meters into the clear-cut area, and was a particularly important C source for the fungal community. At distances greater than 10 meters from retention trees, the abundance of the fungal PLFA biomarker decreased and the δ¹³C value of this signature biomarker increased, reflecting a shift from mycorrhizal to saprotrophic fungi. The lack of recent photosynthate in the clear-cut area likely caused a shift in microbial community composition. This supports the hypothesis that the fungal community would shift from a mycorrhizal dominated to a bacterial and saprotrophic fungal dominated community in response to a decrease in plant-derived C. Concurrently, in the harvested area there was ¹³C enrichment of the bacterial PLFA biomarker and soil respired CO₂, relative to their measured C source, DOC. I suspect this is the result of the microbial community tightly recycling microbial-derived C during microbial turnover. Because microbial biomass is such a small fraction of the total C in the soil, I did not see an increase of δ¹³C values in the DOC and SOM. These results support Chapter 2’s assertion that fungi are dependent on carbon from trees, specifically recent tree photosynthates, for growth. However, Chapter 2 describes the “transition area” as similar to a clear-cut, whereas these results clearly show that the aggregated-retention patch maintained pre-harvest microbial community structure up to 10 meters into a clear-cut, demonstrating that plants influence the structure and function of soil microbial communities, and act as important regulators of belowground C flux and soil C cycling.

In Chapter 4, I developed a method to examine the spatial and temporal patterns of rhizosphere C transfer from mature trees to the soil microbial and faunal community in situ. Using a novel stable-isotope stem-injection labeling technique I traced the flow of ¹³C and ¹⁵N-labelled aspartic acid, which I injected into the phloem and xylem of three 22-year-old Sitka spruce trees, into the soil microbial and faunal community. δ¹³C values of respired CO₂ peaked 13-23 days after injection, up to 5 m from the base of injected trees. Fungal PLFA biomarkers peaked 2-4 days after stem-injection, up to 20 m from the base of injected trees.
Significant $^{13}$C enrichment in mites and enchytraeids occurred 4-6 days after injection (on average, 1.5 %). These findings support the hypothesis that the $^{13}$C enrichment of all DOC, fauna and PLFAs would be observed from the base of the trees up to the canopy edge. However, the influence of trees on the soil microbial community extended well beyond the canopy. This study shows that stem-injection of large trees with $^{13}$C-enriched compounds can be used as a tool to trace C-translocation belowground. In particular, the significant $^{13}$C enrichment of CO$_2$ and enchytraeids near the base of the tree and the significant $^{13}$C enrichment of PLFAs up to 20 meters from the trees, indicates that mature Sitka spruce trees have the capacity to support soil communities over large distances.

In Chapter 5, I explored the potential priming effects of recent photosynthate C on SOM decomposition in disturbed forest soil. Addition of litter to forest soil has been shown to cause positive priming effects over several months (Brant et al. 2006; Moore-Kucera and Dick 2008a; Crow et al. 2009). However, no studies have added labile C to forest soil to study priming effects in situ. In this experiment I added $^{13}$C-labelled glucose to three forest soils that had been exposed to three levels of disturbance: an aggregated-retention site (clear-cut 65 years earlier), a clear cut-site (clear-cut 4 years earlier) and a site that was used as a log-landing during the clear-cut harvesting (un-vegetated), within which the soil was compacted and tended to have an accumulation of tree bark. Over the 24 hours of incubation there was an increase in soil respiration in response to added glucose at all sites. The $^{13}$C-glucose was taken up by all PLFA groups, although it appears that the fungi incorporated a greater proportion, relative to their abundance in the forested and clear-cut sites, whereas in the landing site it was taken up preferentially by Gram-negative bacteria. Addition of glucose in the forested and clear-cut sites increased SOM mineralization 10-20 % above the unamended soils, with a positive priming effect being evident 24 hours after addition. Despite the increase in soil respiration, the landing site had reduced SOM mineralization with glucose addition, being 10-30 % below unamended soil, resulting in a negative priming effect after 24 hours. The greater fungal $^{13}$C enrichment corresponded with the positive priming effect, suggesting that the fungal community plays a role in forest soil priming effects. This contradicted the hypothesis that the landing site would experience the highest priming effect. Although it is unlikely that such a large amount of labile C would be added to
the soil in such a way naturally, it is interesting to compare the potential responses of the
different soil types to the added C. In a changing climate, it is predicted that the greater
atmospheric CO$_2$ concentration will increase litter production and root exudation. It appears
that those sites with a large concentration of roots will be more likely to respire old C in
response to added C (at least initially).

6.2 Limitations and future directions

Of the findings presented in this thesis, there are a number of areas where methodological
improvements and advances in testing techniques could improve the strength of my
assertions. Many of these short-comings are discussed in context within their respective
Chapter section. Here, I will point out some of the limitations of the study designs and
discuss further improvements that could be made as well as potential future studies that will
address these issues.

6.2.1 Scale of spatial and temporal analysis

The spatial analysis of forest C sources and their use by different members of the soil
microbial and faunal community in Chapter 3, aimed to explore the transition from an
aggregated-retention patch into the clear-cut, and improve upon the comparative analysis of
the variable-retention areas conducted in Chapter 2. Although this was accomplished in part,
it is difficult to assess the impact of an aggregate-retention patch (90 m$^2$) on the microbial
community which has been found to be spatially-related at the millimeter scale within forest
soil (Wardle 2006; Nielsen et al. 2012). Comparing my results (tens of meters, plant-scale) to
a grid at the ecosystem scale (hundreds of meters to kilometers), to a grid at the microbial
scale (millimeter to centimeter ) would be a comprehensive means to address these issues,
and it would be interesting to see how, or if, the spatial relationship changes. Do we still see
the benefit of an aggregated-retention patch on the microbial community when looking at the
landscape scale, or would variations in topography be responsible for changes in soil
respiration, microbial community composition and nutrient availability? Would the influence
of living trees still be observed if we examined the impact of an individual-tree fine root on
the microbial community? I suspect we would see spatial relationships relative to the scale
measured, though certain factors such pH, soil temperature and soil moisture, will influence soil microbial diversity and activity (Orchard and Cook 1983; Fierer et al. 2009; Lauber et al. 2009). Although several studies have examined these relationships at coarse scales (meters to kilometers), far fewer have examined microbial communities at smaller scales \textit{in situ} (Wardle 2006; Nielsen et al. 2012).

Most of the studies were conducted in spring (Chapter 2 and 3) and autumn (Chapter 4 and 5). The timing was selected based on evidence that root exudation is greatest in the spring, before bud break, and the autumn, during leaf senescence. However, seasonal differences will cause changes to soil temperature and soil moisture as well as tree photosynthesis and C allocation, which could have impacted the results. Future studies could be repeated in multiple seasons to observe and account for any seasonal effects.

6.2.2 Stem-injection techniques

In Chapter 4 I examined the effect of a single tree providing C sources for the soil microbial and faunal communities through the introduction of an isotopically-labeled compound via stem-injection. The stem-injection technique itself requires modification. At present a single injection site is used to label the entire tree, which results in only a fraction of the roots receiving the label. Multiple injection sites around the bole of the tree should improve this. Alternatively, injecting into the xylem (which we believe occurred by accident in Chapter 4) would result in whole tree labeling and the $^{13}$C label would be allocated among roots according to source strength. However, in order to get a large signal response in the microbial community a large amount of label will have to be injected, which would increase the cost. Issues with seasonal timing of injection are also a concern. Injection should be conducted in the fall when the phloem transport is to the roots, rather than transporting sugars for bud-break, although for conifer trees, anytime after bud set is optimal. In future studies, it would be interesting to trace the movement of the labeled C for a longer period of time; this would also give insight into the movement and long-term dynamics of root-exuded C. The application of this stem-injection method, in combination with other stable-isotope-probing techniques (e.g. the bag method), with each technique using a different C isotope.
(\textsuperscript{13}C or \textsuperscript{14}C), could tease apart the movement and flow of C in forest ecosystems with even more clarity. Several studies have successfully conducted dual \textsuperscript{13}C and \textsuperscript{14}C stable-isotope probing, though they have been limited to smaller trees. One example of how stem-injection stable-isotope-labeling techniques could be applied, is to prove or disprove the concept of large trees donating C to seedlings via a common mycorrhizal network. Simard et al. (1997b) examined the bidirectional transfer of C between paper birch and Douglas-fir seedlings. Using the bag method each seedling was labeled with either \textsuperscript{13}CO\textsubscript{2} or \textsuperscript{14}CO\textsubscript{2}. More recently there has been some evidence that large trees within forests donate C to seedlings through mutual interconnected mycorrhizae. Stem-injection labeling of large trees, and further sampling of neighboring seedlings could prove or disprove this concept (Simard et al. 1997 a, b, c).

6.3.3 \textit{In situ} priming effects

The priming experiment described in Chapter 5 successfully demonstrated a positive priming effect in the forest and clear-cut soil 24 hours after \textsuperscript{13}C-labeled glucose addition. However, addition of carbon to forest soil can lead to a priming effect that lasts for months to years (Brant et al. 2006; Dijkstra and Cheng 2007; Crow et al. 2009). The C added in previous studies was more recalcitrant (pine litter), suggesting there could be long-term effects of C addition to the C balance of the forest ecosystem. This study could be improved through consistent addition of a much smaller about of labile C rather than a pulse of C greater than one would observe in nature and through observation of the priming effects over the long term. This could be achieved by using a porous microthread (simulated root) that would slowly leak labile C, simulating root exudation. This would not only allow for the observation of the effects of increased root exudation on different C pools, but it would also allow us to determine which microbes benefit the most from the C addition over the long-term. Then the effects of increased root exudation could be observed over several seasons. If such an experiment was maintained over the long-term it might then be possible to observe potential changes in the microbial community and the soil C balance that would occur in response to greater root exudation, which is a predicted consequence of predicted increases in atmospheric CO\textsubscript{2} (Phillips et al. 2011b).
6.3.4 Microbial analysis

In order to determine to the genus or species level which microbes are dependent on recent root exudates, DNA stable-isotope-probing techniques could be used. This would enable a greater understanding of community composition changes. However, this technique has flaws. For accurate results, DNA-SIP requires $^{13}$C enrichment of at least 100 per mil (which was not possible for the majority of studies described in this study). It also has the tendency to favor extraction of DNA from microorganisms whose nucleic acids contain more cytosine and guanine, due to the extra purine ring causing variation in buoyant density (Dumont and Murrell 2005). However, knowledge of community composition changes may not reflect changes in the functionality of the soil microbial community as there is a great deal of functional redundancy in microbial communities. Alternatively, DNA stable-isotope-probing analysis, in combination with either RNA gene analysis (to assess the active portion of the microbial community), substrate-induced-respiration or enzymatic analysis, could provide information on the consequences of harvesting on the soil microbial community composition as well as provide information on potential losses of function. This may be particularly relevant to Chapter 2 and 5, both of which report evidence that harvesting, and soil disturbance resulted in microbial community changes and decreased soil nutrient cycling.

6.3 Management implications

British Columbia’s Forest Practices Act (http://www.for.gov.bc.ca/code/), Canada’s National Forest Strategy (http://www.ccfm.org/english/coreproducts-nscf.asp), and Canada’s Biodiversity Strategy (http://www.eman-rese.ca/eman/reports/publications/rt_biostrat/intro.html) all emphasize the importance of maintaining forest biodiversity, ecological integrity and sustainable use of forest resources. In order to retain pre-harvest abundance of soil microorganisms and fauna after harvest, it has been recommended by the forest practices act that coarse woody debris is retained on site and that removal of the soil organic layer is minimized. Coarse woody debris retained in coastal western hemlock and Douglas-fir forests has been found to lower soil pH, increase available aluminum and increase available iron (Spears and Lajtha 2004). Current guidelines
do not sufficiently consider the importance of soil organisms. The research reported in this thesis addresses the impacts of variable retention harvesting on the soil microbial community, with the goal of establishing which harvesting method best maintains pre-harvest microbial diversity, microbial activity and soil nutrient availability.

Although there is evidence that retaining live trees on clear-cut sites benefits above-ground biodiversity (Siira-Pietikäinen et al. 2001), very few studies have examined the effects belowground with any accuracy. The methods developed in this research have given us the ability to not only measure the direct impacts of trees on the belowground microbial and faunal community, but have also allowed us to measure the spatial aspect of C-flow and tree influence. Application of this knowledge to current harvesting practices will enable better management methods to maintain pre-harvest belowground biodiversity.

The knowledge gleaned from the spatial analysis (Chapter 3) determined that live trees act has hubs of microbes allowing seedling access to species-specific microbes if they are within the range of tree root influence. However, comparisons between different variable-retention harvesting methods (Chapter 2) suggested that even under variable-retention, community composition will change, and some functionality may be lost. The greater the disturbance, the greater the impact on microbial function (Chapter 5). This information has important management implications for harvested sites in the Pacific Northwest. Seedlings located 2-5 m from the base of a tree have been found to have greater success when located near the edge of a neighboring tree canopy (Jones et al 2008), though their success has not been measured over the long-term. Seedling success was presumed to be due to greater access to the tree’s mycorrhizal network. Through use of the stem-injection stable-isotope-labeling technique (Chapter 4) and observation of stable-isotope natural abundance (Chapter 3), I was able to determine that live trees retained on a clear-cut site can support fungal communities 9-20 m away from their base. These results suggest GTR, specifically dispersed-retention harvesting, better retains soil microbial community function compared to clear-cut sites, but soil microbial community structure was not retained in any of the harvesting regimes analyzed.
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Appendices

Appendix A This figure compiles data from surface horizons of 20 long-term field experiments (up to 23 years) in temperate climates, using $^{13}$C-labeling to trace the residence time of bulk SOM and of individual molecular compounds. For clarity, outliers were excluded and they tentatively included fire-derived organic matter. Data points: thin horizontal lines= 10$^{th}$ and 90$^{th}$ percentiles; box= 25$^{th}$ and 75$^{th}$ percentiles; central vertical line= median. From Schmidt et al. (2011).
Appendix B This figure shows the major components of the C budget in forest ecosystems, and the partitioning of gross primary production into aboveground biomass (annual net primary production of wood/foliage), aboveground respiration (wood, foliage) and total belowground C flux (root biomass + root respiration + mycorrhizal biomass + mycorrhizal respiration + rhizosphere respiration + exudates). The values in parentheses are 10th and 90th percentiles of C partitioning. Biomass foliage was calculated in the majority of studies as litter-fall, change in foliar biomass, or both. Biomass wood was calculated using tree diameter and volumetric equations. Aboveground respiration was calculated using gas exchange and scaling techniques, and several techniques were use to measure belowground flux, but was most often calculated by measuring soil surface CO₂ efflux via chambers. The conservation of mass or C balance technique was employed to scale to the ecosystem level. Adapted from Litton et al. (2007).
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Appendix D This figure displays the sequence of processes for apparent (aPE) and real (rPE) priming effects: 1. Input of available organics by rhizodeposition (exudation). 2. Activation of microorganisms (mainly r-strategists) by available organics (activation). 3. Activation of K-strategists. 4. Production of extracellular enzymes that degrade SOM by K-strategists. 5. SOM decomposition and production of available organics and mineral nutrients. 6. Uptake of nutrients by roots. From Kuzyakov (2010).
Appendix E  Schematic of Picarro CRDS analyzer showing how a ring-down measurement is carried out (http://www.picarro.com/technology/what_is_crds).
Appendix F \( \delta^{13} \text{C} \) values of PLFAs in the landing, clear-cut and forest site. SE represents standard error of the mean (n=24). For each PLFA, similar letters indicate the individual treatments are not significantly different (Holm-Sidak ANOVA, \( \alpha > 0.05 \)).

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