SOIL MICROBIAL COMMUNITY RESPONSES TO GREEN-TREE RETENTION HARVESTING IN COASTAL BRITISH COLUMBIA

by

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ABSTRACT

Green-tree or variable retention harvesting is being increasingly adopted as an alternative to clearcutting in the Pacific Northwest, including British Columbia (BC), to maintain forest biodiversity and function and enhance aesthetic quality. Green-tree retention may also benefit the soil resource, and research is needed to understand how these silvicultural treatments affect soil nutrient availability and microbial community structure and function. The objectives of this study were to determine: (i) whether green-tree retention harvesting is better than clearcutting to retain nutrient availability and the structural and functional characteristics of the soil microbial community, and (ii) which spatial pattern of green-tree retention is superior for this purpose. Using an adjacent uncut forest to provide a baseline comparison, nutrient availability, substrate-induced respiration (SIR), enzyme activities, and phospholipid fatty acids (PLFA) were analysed in the forest floor and mineral soil of a second-growth Douglas-fir and western hemlock forest at the Silviculture Treatments for Ecosystem Management in the Sayward (STEMS) trial near Campbell River on Vancouver Island, BC, five years after aggregated retention harvesting, dispersed retention harvesting, and clearcutting. In general, there was no indication that nutrient availability and the structure and function of the soil microbial community in either green-tree retention treatment was intermediate between the clearcut and the uncut forest. Harvesting had no significant effect on NO_3^- and PO_4^{3-} availabilities and SIR rates, but it generally reduced NH_4^+ availability and enzyme activities in the forest floor. Green-tree retention harvesting caused a shift in the structure of soil microbial community, whereas clearcutting did not. Green-tree retention harvesting appeared better than clearcutting in maintaining the activities of forest floor β-glucosidase, N-acetyl-glucosaminidase, and peroxidase enzymes closer to the level found in the uncut forest, but did not offer advantages over clearcutting in maintaining soil microbial community structure. Comparing the two spatial patterns of green-tree retention, the dispersed retention treatment was superior to the aggregated retention treatment because of its ability to retain soil microbial community structure and function more evenly across the harvested site.

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LIST OF ABBREVIATIONS

°C	Temperature in degrees Celsius
µg	Microgram
µL	Microlitre
Al	Aluminium
ANOVA	Analysis of variance
B	Boron
BC	British Columbia
BEC	Biogeoclimatic Ecosystem Classification
C	Carbon
Ca	Calcium
CLPP	Community-level physiological profiles
cm	Centimetre
CMC	Carboxymethyl cellulose
Cu	Copper
CWHxm	Coastal Western Hemlock very dry maritime
DNA	Deoxyribonucleic acid
ECM	Ectomycorrhizal
Fe	Iron
g	Gram
h	Hour
ha	Hectare
K	Potassium
km	Kilometre
L	Litre
L-DOPA	L-3,4-dihydroxyphenylalanine
m	Metre
Mg	Magnesium
mL	Millilitre
Mn	Manganese
MRPP	Multi-response permutation procedures
MUB	4-methylumbelliferone
N	Nitrogen
NAGase	β-1,4-N-acetylglucosaminidase
NH_4^+	Ammonium
nm	Nanometre
NMS	Non-metric multidimensional scaling
NO_3^-	Nitrate
P	Phosphorus
Pb	Lead
PCR	Polymerase chain reaction
pH	Acidity in water
PLFA	Phospholipid fatty acid
PO_4^{3-}	Orthophosphate
PRS	Plant Root Simulator
rRNA	Ribosomal ribonucleic acid
S	Sulphur
SIR	Substrate-induced respiration
SNR	Soil nutrient regime
STEMS	Silviculture Treatments for Ecosystem Management in the Sayward
Zn	Zinc

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DEDICATION

To my beloved grandfather, Tjia Jaw Pin.

1 RATIONALE

Sustainable forest management has become an important endeavour throughout the world. Increased public concerns over clearcutting for long-term sustainability has made the traditional timber harvesting method less socially favourable. Current forest management practices are expected to incorporate a variety of non-timber objectives, such as the preservation of biodiversity, wildlife habitat, and visual quality, in addition to maintaining a sustainable timber supply (Marshall 2000). As a result, partial-cutting methods are increasingly being adopted in place of clearcutting. Green-tree retention is a partial-cutting method that leaves behind mature live trees in a dispersed or aggregated pattern on harvested sites. By retaining part of the structural attributes of forests, green-tree retention has been promoted as a method to buffer the impacts of clearcutting on forest biodiversity whilst maintaining landscape visual quality (Franklin et al. 1997).

Soil is recognised as one of the key factors influencing forest productivity (Nambiar 1996). Forest soils harbour a great diversity and abundance of microorganisms that drive key ecosystem processes, such as decomposition and nutrient cycling. The activity (function) and composition (structure) of soil microbial communities are likely to be affected by timber harvesting as it alters the soil environment and litter quality, i.e. increases the abundance of woody slash rather than less recalcitrant leaf litter and root exudates on harvested site. Changes in the structure and function of microbial communities, in turn, could potentially have immediate or lasting effects on forest ecosystem functioning. Green-tree retention may be able to buffer the impacts of clearcutting on the soil ecosystem because the retained trees may ‘lifeboat’ surviving soil biota by providing shelter and energy supply through litter fall and root exudates. Although green-tree retention is promising and has been applied in 50-60% of British Columbia’s coastal forests (Swift 2006), its benefits over clearcutting remains largely speculative and little information is available that provides guidance about the optimal retention patterns for green tree retention for

the purpose of maintaining the soil microbial community. Thus, there is a need to assess the potential of green-tree retention to maintain the structure and function of soil microbial communities closer to pre-harvest levels as compared to clearcutting.

2 LITERATURE REVIEW

2.1 Green-tree retention as an alternative to clearcutting

The coastal forests of British Columbia (BC) are amongst the most productive forests in Canada. In the region, escalating public pressure in the 1990s for multi-functional forests has initiated a shift in forest management paradigm from mainly timber production towards ecological and social sustainability (Mitchell and Beese 2002). Clearcutting, which had been the predominant harvest method in coastal BC, has now become socially unacceptable due to its association with unsightly landscapes, loss of biodiversity, and environmental degradation (Bliss 2000). Silvicultural approaches that emulate natural disturbance regimes are being increasingly favoured in place of clearcutting (Franklin et al. 1997). A partial-cutting method termed “variable retention” was proposed as a solution to achieve sustainable forestry (Beese et al. 2003). Green-tree retention is an important component of the variable retention system; although the two terminologies are commonly interchangeable. Green-tree retention involves leaving mature live trees throughout a harvested area for at least one rotation to retain the structural elements of an existing stand (Franklin et al. 1997). Residual trees can be retained in two spatial patterns (aggregated and dispersed) or a combination of both, at various levels of retention to meet specific stand management objectives (Franklin et al. 1997). According to the Forest Practices Code of BC Act, forest companies are required to retain 0-18% of the cutblock as wildlife trees (British Columbia Forest Service and British Columbia Environment 1995). The main objectives of green-tree retention are to: (i) preserve biodiversity by providing ‘lifeboats’ for the survival and dispersal of organisms after harvesting, (ii) create structural complexity that resembles a forest rather than a clearcut, and (iii) enhance habitat connectivity over the landscape (Franklin et al. 1997). Green-tree retention differs from other partial-cutting systems, such as shelterwood or seed-tree, because it focuses on ecological objectives, and regeneration is not its primary goal.

Two spatial patterns of green-tree retention are dispersed retention, in which single residual trees are uniformly distributed throughout the harvested area, and aggregated retention, in which residual trees are concentrated in patches within a cutblock. There are tradeoffs between the two spatial patterns depending upon ecological, physical, and economic objectives, such as the biology of targeted taxa, potential loss of retained structure (e.g. to windthrow), operational constraints (e.g. costs and safety), and impacts on growth of the regenerated stand (Franklin et al. 1997, Lindenmayer and Franklin 2002, Aubry et al. 2004). Aggregated retention is hypothesised to be more beneficial for organisms that require intact undisturbed forest patches (e.g. lichens associated with older forests and small mammals), whilst dispersed retention may be more suitable for management objectives that demand uniformly distributed conditions and structures (e.g. mitigation of hydrological and microclimatic impacts of harvesting) (Aubry et al. 1999). Many large-scale experiments intended for long-term studies on the effectiveness a variety of silvicultural treatments, including green-tree retention, on various biological, physical, and economic indicators as well as public perception are underway in the Pacific Northwest. Such studies include the STEMS (Silviculture Treatments for Ecosystem Management) and MASS (Montane Alternative Silvicultural Systems) trials on Vancouver Island (deMontigny 2004), DEMO (Demonstration of Ecosystem Management Options) trial in Washington and Oregon (Aubry et al. 2004), and the “Silvicultural Options for Harvesting Douglas-fir Young-Growth Production Forests” trial in Washington (Curtis et al. 2004). The STEMS trial - the location of this current study - was initiated in 2001 to compare seven silvicultural treatments: aggregated retention, dispersed retention, clearcut with reserves, modified patch cut, group selection, extended rotation with commercial thinning, and extended rotation (non-treated control) (deMontigny 2004). Findings from short-term studies on the effectiveness of green-tree retention vary significantly, depending on the indicators being studied. Rosenvald and Lõhmus (2008) performed a meta-analysis of 214 North American and European studies on green-tree retention to address whether this silvicultural treatment met the three main sustainability objectives as outlined by Franklin (1997) (above). The analysis revealed that green-tree retention reduced the

loss of above- and below-ground macro- and microorganisms compared to clearcutting in 72% of the studies, and the lifeboating objective was found to be most successful for birds, ectomycorrhizal fungi, epiphytic lichens, and small ground-dwelling animals (Rosenvald and Lõhmus 2008).

2.2 Importance of soil microorganisms in forest ecosystems

Soil microorganisms play a critical role in sustaining forest ecosystems. They drive the cycling of carbon (C) and nutrients essential for tree growth, such as nitrogen (N) and phosphorus (P), through the processes of litter decomposition and nutrient mineralisation. Litter decomposition is estimated to provide between 69-87% of nutrients required for forest growth annually (Waring and Schlesinger 1985). Although soil fauna contribute to the initial stage of decomposition by breaking down litter into smaller fragments (Dickinson and Pugh 1974), the processes of decomposition and nutrient mineralisation mainly results from the enzymatic activities of the soil microbial community, which are mainly comprised of bacteria and fungi (Prescott 2005). In forest ecosystems, the role of fungi in decomposition is most pronounced because they produce enzymes, such as peroxidase, phenol oxidase, and laccase, that attack the lignocellulose matrix in litter that are resistant to degradation by other microorganisms (Hättenschwiler et al. 2005). Although the ability to degrade cellulose is found in a wider range of fungi and bacteria, the lignocellulose matrix restricts physical and chemical access to cellulose (Béguin and Aubert 1994). Soil microorganisms can also form associations with plant roots that may be beneficial or detrimental to plant growth. For example, the association between mycorrhizal fungi and plant roots enhance water and nutrient acquisition of host plants. Soil microorganisms, especially fungi, are also involved in the formation and stabilisation of soil aggregates by producing metabolic products, such as polysaccharides that bind soil particles, as well as in the enmeshment of soil particles by fungal hyphae (Degens 1997, Oades 1993, Guggenberger et al. 1999).

2.3 Soil microbial community structure and function: relationship and methodology

Soil is known to support an immense diversity of bacterial and fungal communities (Straatsma et al. 2001, Torsvik et al. 2002, Hawksworth 2001). A single gram of forest soil has been estimated to contain 4.8×10^9 bacteria representing 6,000 different species (Torsvik et al. 1998). Although it is now clear that soil microbial communities are highly complex and diverse, little is known about the significance of such diversity in the functioning of an ecosystem. In fact, understanding the linkage between microbial community structure and function still remains a major challenge facing microbial ecology (Nannipieri et al. 2003). Members of the soil microbial community are thought to exist in overlapping niches performing the same functional roles (Stres and Tiedje 2006). This functional redundancy is believed to ensure ecosystem stability and resilience toward stress or disturbance (Stres and Tiedje 2006, Torsvik and Øvreås 2002). Factors such as site properties, temporal changes, resource diversity, biological interactions, niche diversity, and stress are thought to affect microbial diversity (Lynch et al. 2004). Diversity, in turn, may have positive effects on the functioning of an ecosystem in terms of stability, resilience, resistance to stress, and productivity (Nannipieri et al. 2003, Lynch et al. 2004). There is evidence that soil microorganisms are affected by aboveground disturbances, including timber harvesting. Harvest-induced changes in microbial community structure may ultimately alter microbial community function. For instance, a decrease in the abundance and diversity of the fungal community may impede the degradation of lignin-rich residue, decreasing the availability of nutrients (Entry et al. 1986). This could indicate that there are some non-redundant functions of the soil microbial community.

Changes in the structure and function of the soil microbial community can now be quantified by using a myriad of cultivation-independent methods. Cultivation-independent approaches are necessary for a better understanding of soil microbial ecology because only about 1% of the soil microbial community can be cultivated using standard laboratory culturing procedures (Kirk et al.

2004). Methods to measure the structural and functional diversity of soil microorganisms can be categorised into molecular, biochemical, and physiological approaches, each with its own advantages and limitations (Table 2.1). The molecular and biochemical methods typically involve the extraction of biomarker(s) from the soil community, with deoxyribonucleic acid (DNA) and phospholipid fatty acid (PLFA) being the most common compounds for the respective methods, whilst an example of the physiological methods provides community-level physiological profiles (CLPP) through soil respiration as a measure of carbon substrate use (Leckie 2005). Polyphasic approaches that aim to integrate both structural and functional aspects of microbial communities are commonly employed in microbial ecology studies to obtain a more complete picture of the soil microbial communities (Grayston et al. 2004, Grayston and Rennenberg 2006, Hernesmaa et al. 2005).

Table 2.1 Summary of commonly used culture-independent methods to measure soil microbial community structure and function.

PLFA: phospholipid fatty acid; DGGE: denaturing gradient gel electrophoresis; TGGE: thermal gradient gel electrophoresis; SSCP: single-stranded conformational polymorphism; RFLP: restriction fragment length polymorphism; ARDRA: amplified ribosomal DNA restriction analysis, T-RFLP: terminal restriction fragment length polymorphism; RISA: rRNA intergenic spacer analysis, ARISA: automated rRNA intergenic spacer analysis; FISH: fluorescent *in situ* hybridisation; CLPP: community-level physiological profiling; SIR: substrate-induced respiration (Kirk et al. 2004, Leckie 2005, Neufeld and Mohn 2006, Nannipieri et al. 2002, Nakatsu 2007).

Method	Overview	Resolution	Advantages	Disadvantages
A) Community Structure				
PLFA analysis	Assessment of community structure based on variability of membrane lipids	Medium	Targets the entire community Derived mainly from living organisms	Only a small number of fatty acids are truly characteristic for certain groups, many are ubiquitous and may be derived from other soil organisms Most variability associated with bacteria not fungi; fungal biomass might be underestimated Overlap among groups for marker PLFAs No information about species composition
Nucleic acid reassociation and hybridisation	Based on DNA complexity; rate increases when DNA complexity increases	Low	Not influenced by PCR biases Includes all DNA extracted Includes less dominant microorganisms in the community that might not be detected by PCR without fractionation	Time-consuming Two communities having similar base distributions do not necessarily have similar species composition, since different species often have the same base composition
% Guanine plus cytosine (G+C) composition	Based on base distribution in community DNA	Low	Not influenced by PCR biases, includes all DNA extracted Includes rare members of community Can be used to indicate overall changes in microbial community structure, especially when the diversity is low	Requires a large amount of DNA Depends on lysis and extraction efficiency Two communities having similar base distributions do not necessarily have similar species composition, since different species often have the same base composition

Table 2.1 Continued.

Method	Overview	Resolution	Advantages	Disadvantages
DGGE/TGGE	Separates DNA sequences based on their melting behaviour	Medium	Fast and simple; high throughput	DNA extraction and PCR biases
			High sensitivity	Only abundant species are detected
			PCR fragments can be isolated and used for sequencing	Analysis of PCR fragments over 400bp is less successful GC-rich genes are not easily analysed
				Resolution may be inadequate for diverse communities
				Difficult to exactly reproduce gel gradients
SSCP	Electrophoresis of single-stranded PCR amplicons	Medium	Same as DGGE/TGGE (above)	DNA extraction and PCR biases
			No GC clamp	Some ssDNA can form more than one stable conformation
			No gradient	
RFLP/ARDRA	Based on analysis of polymorphism of a target gene	High	Can classify at the species level	DNA extraction and PCR biases
				Banding patterns often too complex
T-RFLP	Based on analysis of polymorphism of a target gene	High	Highly reproducible, high throughput	DNA extraction and PCR biases
			Simpler banding patterns than RFLP	Target genes are identified *a priori*
			Can be automated	
RISA/ARISA	Uses natural variability of internal transcribed spacer (ITS) region to compare microbial communities among samples	Low	Highly reproducible, high throughput	DNA extraction and PCR biases
			Can be automated (ARISA)	Requires large amounts of DNA
FISH	Detects single cells in samples by whole cell hybridisation using fluorescently labelled primers	Medium	Not influenced by PCR biases	Lack of sensitivity
			Resolves species composition	Cells with low ribosome content and slow growing or starving cells might not be detected

Table 2.1 Continued.

Method	Overview	Resolution	Advantages	Disadvantages
RNA dot/slot blot hybridisation	quantifies target microorganisms and to determine the overall taxonomic composition of the microbial communities	Medium	Useful in identifying numerically dominant community members	Time-consuming Requires large amounts of RNA
Microarray	Identifies unknown nucleic acid from microbial samples by hybridisation to comprehensive collection of DNA sequences (probes)	High	Same as nucleic acid hybridisation (above) Rapid and sensitive Thousands of genes can be analysed If using genes or DNA fragments, increased specificity	Only detects most abundant species Only accurate in low diversity systems
Sequence analysis of DNA clone libraries	Catalogues species richness and community composition by sequence analysis of ribosomal RNA (or other) genes	High	Targets entire or part of community depending on PCR primers	DNA extraction and PCR biases Expensive and time-consuming to sequence full diversity from a single soil sample
B) Community Function				
Enzyme activity assay	Assesses nutrient cycling potential of soil community as an index of microbial functional diversity	Low	Simple, accurate, sensitive, and relatively rapid Relatively inexpensive A wide range of substrates are available	Measures potential, but not *in situ* activity Activities are substrate-specific, thus cannot be related to overall microbial activity of soil
CLPP using Biolog® microplates	Assesses sole carbon source utilisation to determine physiological capacity of soil community	Low	Simple, rapid, and reproducible Relatively inexpensive	Selects for fast-growing cultivable microorganisms Original soil structure disrupted
CLPP using SIR	Assesses sole carbon source utilisation to determine physiological capacity of soil community	Low	Simple and reproducible Targets active population Can use more recalcitrant substrates than Biolog	Time-consuming if using many substrates

The majority of current tools to analyse microbial community structure and function are comprised of molecular PCR-based approaches (Table 2.1). Although in general these approaches offer the advantages of: (i) being rapid, reliable, and reproducible, (ii) providing both qualitative and quantitative information on populations within a community, and (iii) allowing for the assessment of the phylogenetic diversity of soil microbial community members via comparison with sequences in database, they also have several drawbacks: (i) biases introduced during cell lysis, DNA extraction, and PCR amplification, (ii) formation of PCR artefacts, such as chimeric molecules and/or mutants, (iii) the presence of several alleles in a single bacterial strain that could lead to derivation of many different PCR amplicons from the same strain, and (iv) the inability to sufficiently separate and resolve high numbers of amplicons from complex communities (Lynch et al. 2004, Wintzingerode et al. 1997). Biases during sample preparation and PCR amplification are often highlighted as major disadvantages of PCR-based molecular techniques, because they could lead to erroneous or misleading results. For example, Gram-negative bacteria, but not Gram-positive bacteria would be lysed if the cell extraction method is too gentle, whilst both types of bacteria would be lysed, but their DNA would be sheared if the cell extraction method is too harsh, which would subsequently lead to biases in the PCR step (Wintzingerode et al. 1997). In this study, phospholipid fatty acid (PLFA) analysis was selected to assess the structure of the soil microbial community, whilst enzyme activity assays and community-level physiological profiling (CLPP) using substrate-induced respiration (SIR) were used to quantify the potential function of the soil microbial community. Molecular PCR-based methods were not chosen because of the previously mentioned drawbacks, and because the level of resolution was found to be too high for the purpose of this study.

Phospholipid fatty acid (PLFA) analysis is broad-scale community-level profiling approach that is often used to characterise the structure of soil microbial communities at the phenotypic level. PLFAs are integral constituents of all living cell membranes, and they degrade rapidly upon cell death, making them good indicators of total active biomass (Frostegård and Bååth 1996). PLFAs

are useful biomarkers because specific PLFAs are associated with different subsets of the microbial community, such as Gram-negative bacteria, Gram-positive bacteria, actinomycetes, and fungi (Frostegård and Bååth 1996, Olsson 1999). By analysing the PLFA profiles, information concerning total viable biomass and the relative abundance of certain groups of microorganisms can be derived. Indices of certain sets of PLFAs can also be used to indicate the physiological status of microbial communities. For example, the ratios of: (i) fungal to bacterial fatty acids, (ii) Gram-positive to Gram-negative bacterial fatty acids, (iii) cyclopropyl fatty acids and their precursor, and (iv) saturated to monounsaturated fatty acids have all been used to distinguish changes in microbial communities under different environmental stresses. Frostegård and Bååth (1996) used the ratio of fungal to bacterial PLFAs as an index of the relative abundance of these two main groups of microbial decomposer in soil. Soils at lower depth have been shown to have lower ratios of fungal to bacterial PLFAs and higher ratios of Gram-positive to Gram-negative PLFAs than surface soils (Blume et al. 2002, Zelles and Bai 1994). The ratios of saturated to monounsaturated, cyclopropyl to monoenoic precursors were used as an indicator of nutritional stress as these ratios were higher at greater soil depths, where oxygen and substrates are less abundant (Song et al. 2008, Fierer et al. 2003, Kieft et al. 1994). In a comparative review by Ramsey et al. (2006), PLFA analysis was found to outperform molecular and physiological profiling methods in discriminating changes in soil microbial community structure.

Whilst the assessment of soil microbial community structure provides information on the composition and abundance of the community, it provides relatively little information about the function of the organisms in soil that contribute to ecological processes (Leckie 2005). The potential of function of the microbial community can be measured based on broad physiological properties, such as enzyme activities and metabolic diversity profiles. Enzymes play crucial roles in soil ecosystems because they catalyse the biochemical transformations involved in nutrient cycling. Soil enzymes primarily originate from microorganisms, although they also derive from

plant and animal residues (Ladd 1978, Speir and Ross 1978). In addition to being intracellular, enzymes can be extracellular, secreted by organisms and existing as free enzymes in soil, attached to cell walls, or adsorbed to organic and inorganic complexes (Nannipieri et al. 2002). Extracellular soil enzymes are often the focus in ecological studies as they degrade complex, high-molecular-weight organic compounds, such as cellulose, lignin, hemicelluloses, and chitin, that could not directly enter the cell and be assimilated by microorganisms, Although more than 100 different enzymes have been identified in soils (Tabatabai and Dick 2002), the ones of primary interest in forest ecosystems are those that are directly involved in the breakdown of the most abundant biopolymers, such as lignocelluloses (e.g. peroxidase, phenol oxidase, and laccase) and chitin (e.g. chitinase), and in the transformations of critical forest nutrients - C (e.g. β-glucosidase, xylanase, cellulose, and β-galactosidase), N (e.g. protease, urease, and nitrate reductase) and P (e.g. phosphatase).

Metabolic diversity profiles, achieved by measuring microbial substrate-induced respiration (SIR) or community-level physiological profiles (CLPP), are also used to assess microbial functional diversity (Degens and Harris 1997). Substrate-induced respiration measures the amount of carbon dioxide (CO_2) evolved before and after the addition of a range of simple organic substrates commonly found in soil (Anderson and Domsch 1978). By using multiple substrates, it is possible to obtain and compare the community-level physiological profiles of different soils (Degens and Harris 1997). MicroResp™ is a relatively new SIR method developed by Campbell et al. (2003) that measures the responses of whole soil, instead of soil extracts, on a microtitre plate. The use of soil extracts has a disadvantage that it is biased towards readily extractable microorganisms which would grow rapidly within the nutrient-rich and optimum environment of the test well (Campbell et al. 2003). The substrates selected typically consist of a few ecologically relevant carbon sources found in root exudates and litter that represent different classes of chemicals, e.g. carbohydrates, carboxylic acids, amino acids, amides, and alcohols, and can also include more recalcitrant compounds, such as chitin (Campbell et al. 1997). The

MicroResp™ method has been found to offer practical advantages and be more discriminative in differentiating soil microbial communities than other approaches, such as the Biolog® plate method and the method developed by Degen and Harris (1997) that measures the amount of CO_2 produced in individual glass bottles filled with soil and carbon sources (Campbell et al. 2003, Lalor et al. 2007, Chapman et al. 2007).

2.4 Effects of harvesting on the belowground ecosystem

2.4.1 Soil properties

Forest harvesting alters the soil environment in a number of ways that may directly or indirectly affect the microbial communities and their functioning in soils. Logging operations often disturb soil by mixing or displacing the forest floor and compacting the underlying mineral soil that could potentially affect long-term forest productivity (Powers et al. 2005, Murphy et al. 2004). The forest floor is a major reservoir of organic matter, a source of nutrients for soil biota, and is where most biological processes occur (Fisher and Binkley 2000). Compaction of forest soils increases soil bulk-density and decreases porosity, affecting the water and aeration status of the soil (Greacen and Sands 1980). Soil compaction and forest floor removal have been shown to reduce microbial biomass and enzyme activities (Tan et al. 2008). Harvesting has also been shown to increase soil pH (Piirainen et al. 2002, Hannam et al. 2006), that may be attributed to reduced root respiration and cation uptake by trees (Kim 2008). The removal of canopy cover allows more solar radiation and precipitation to reach the ground, thereby elevating soil temperature and moisture variability (Keenan and Kimmins 1993, Hassett and Zak 2005). Changes in moisture and temperature have been suggested as the main factors responsible for increased decomposition that is sometimes observed following clearcutting (Prescott 1997). Harvesting also directly removes a significant amount of nutrients in harvested timber (Ballard 2000). In addition, the amounts and chemical composition of litter change, with the cessation of litter fall and deposition of relatively large amounts of logging slash on the forest floor (Covington

1981). The roots of harvested trees begin to decompose and root exudation diminishes. Through changes in soil properties and organic matter input, harvesting can have major impacts on the activity and composition of soil microbial communities (Marshall 2000).

The effects of clearcutting on soil properties have been relatively well documented, but comparatively less is known about the effects of tree retention on harvested sites. One of the mechanisms by which green-tree retention has been hypothesised to facilitate the dispersal and survival of organisms is by ameliorating forest floor microclimate (Franklin et al. 1997). Living trees left on harvested sites provide litter input and root exudates and maintain evapotranspiration. In dispersed retention the living tree-root systems are distributed over a greater area than in aggregated retention (Luoma et al. 2004), whilst in aggregated retention large portions of living root mass are concentrated in the retained patches. Thus, aggregated retention patches would have higher amounts of root exudates and litter in concentrated areas than dispersed retention trees. At the same level of retention, however, litter fall and root exudation would be more evenly spatially distributed across the cutblock in dispersed retention than in aggregated retention. Barg and Edmonds (1999) found that soil temperature and moisture in a dispersed retention (20-30 trees/ha) Douglas-fir and western hemlock forest in western Washington were generally intermediate between uncut forest and a clearcut 2-5 years after harvest. In another study at the DEMO site, soil temperature decreased with increasing levels of dispersed retention (0, 15, 40, and 100%), although soil moisture showed little variation among retention levels (Heithecker and Halpern 2006). Later, the same group of authors studied soil microclimate along a distance gradient from within 15% aggregated retention patches (56.4 m in diameter) up to 60 m from the patch edge (Heithecker and Halpern 2007). In general, they observed increasing light availability, air temperature, soil temperature, and soil moisture from the centre of the patch into the harvested area. Although soil moisture did not differ among sampling locations along the distance gradient, light availability and temperature within the forest aggregates were similar to the uncut control and were highest in the harvested area with most

changes occurring within 20m from the edge. At the DEMO site, Halpern and McKenzie (2001) found that soil disturbance and slash accumulation were slightly greater at a 15%-retention level than 40%-retention level and at the aggregated retention treatment than the dispersed retention treatment, but the differences were not statistically significant. Comparing the two spatial patterns of green-tree retention, changes in microclimate are hypothesised to be greater in the aggregated retention than dispersed retention, although these changes tend to be generalised over the harvested area in the dispersed retention, and more localised in parts of the aggregated retention (Franklin et al. 1997). The dispersed retention offers the advantage of creating more even conditions across the harvested area than the aggregated retention.

2.4.2 Soil nutrient availability

Nitrogen (N) and phosphorus (P) are the most commonly limiting nutrients for plant growth in temperate forest ecosystems (Fisher and Binkley 2000). Nitrogen is available for plant uptake as nitrate (NO_3^-) and ammonium (NH_4^+) ions, whilst P is available for plant uptake as orthophosphate (PO_4^{3-}) ion or $H_2PO_4^-$ in acidic soils (Barber 1984). Clearcutting is often associated with a flush or immediate increase in the availability of N, as indicated by high N mineralisation and leaching of nitrate (Prescott 1997, Vitousek and Melillo 1979, Piirainen et al. 2002). The flush of N was presumed to be due to faster decomposition and mineralisation resulting from increased moisture and temperature as well as reduced nutrient uptake by plant roots (Covington 1981). The rate of decomposition after harvesting, however, has been suggested to vary with regional climatic variables (Yin et al. 1989), and could be faster (Ishikawa et al. 2007), slower (Prescott 1997), or remain the same (Prescott et al. 2003). Prescott (1997) attributed the increase of N availability to reduced microbial biomass resulting from decreased C availability due to reduced litter input and subsequently lower N immobilisation by microorganisms, rather than to faster decomposition. Whilst increased soil N availability may be beneficial for the growth of seedlings, excess N not taken up by plants often leaches below the rooting zone, and could potentially affect long-term site productivity (Martin et al. 2001). The

duration of the post-harvest N flush varies among forest ecosystems. For example, even 5 years after harvest the amounts of extractable NO_3^- and NH_4^+ in a clearcut were still 9 to 10 times higher than in uncut lodgepole pine forest in Wyoming (Giardina and Rhoades 2001). In another study, the losses of N, K, and Ca increased immediately after harvest, but returned to levels comparable to uncut Douglas-fir and red alder forest within 3 years (Mann et al. 1988). Studies that compare the effects of partial-cutting and clearcutting on soil nitrogen dynamics have shown contrasting results. There is evidence that partial-cutting reduces the post-harvest N flush associated with clearcutting (Prescott 1997); this may be due to continued root uptake by residual trees and higher microbial immobilisation of N as a result of sustained rhizodeposition and litter input (Lapointe et al. 2005). Kranabetter (2004) found no difference in net mineralisation of N or mineralisable N, total N, S, and P, and exchangeable Ca, K, and Mg, in either forest floor or mineral soil 9-10 years after harvest, despite post-harvest differences in total C content and pH among the unharvested, partial-cut, and clearcut forest stands in the Interior Cedar-Hemlock zone in British Columbia. Likewise, Barg and Edmonds (1999) found no difference in N mineralisation, nitrification, or soil microbial biomass between dispersed retention (20-30 trees/ha) and clearcut treatments 2-5 years after harvest in coastal Douglas-fir stands in western Washington. This observation may be attributed to similarities in soil moisture and microbial biomass before and after harvest. Barg and Edmonds (1999) also suggested that N dynamics did not appear to be affected by proximity to residual trees, although microbial biomass was higher closer to (1 m) residual trees than farther away (6 m).

Whilst the effects of harvesting on N dynamics have been studied in depth, less is known about the effects on P availability. The cycling of P is the result of both biological and geochemical processes, which include decomposition of litter, mineralisation of P, uptake by plants or microorganisms, weathering of primary soil minerals, and adsorption and desorption of P by soil surfaces (Wood et al. 1984, Yanai 1998). Unlike N, the relatively small pool of P is fairly immobile and tightly conserved in forest ecosystems (Wood et al. 1984) because it can be easily

adsorbed by soil surfaces (Yanai 1998, Macrae et al. 2005). Gravelle et al. (2009) found no differences in total P and PO_4^{3-} concentrations among partially-cut, clearcut, and uncut second-growth grand fir, Douglas-fir, and western red cedar forests during the 5-year period after harvest. Similarly, Macrae et al. (2005) found no difference in soil P concentrations between harvested and unharvested trembling aspen and white spruce forests over a 4-year period following harvesting. Yanai (1998) monitored the leaching of P, NO_3^-, NH_4^+, Ca, and K in streamwater over a 3-year period after clearcut harvesting and in an adjacent undisturbed forest of sugar maple, yellow birch, and American beech in New Hampshire. Although the loss of P in streamwater in the clearcut was 3 times higher than that in the undisturbed forest, the amount was relatively small compared to the losses of NO_3^-, Ca, and K, which were 4 to 40 times higher in the clearcut than in the undisturbed forest, indicating that P was more strongly retained by the ecosystem. There is evidence that the pulse of soil P concentrations after harvesting is temporary and returns to levels comparable to uncut forest as stands mature (Simard et al. 2001). The dynamics of P after harvesting also depend on the forest ecosystem being studied. Lindo and Visser (2003) studied the effects of harvesting treatments (unharvested, aggregated retention, corridor partial-cut, and clearcut) on P and N availability in deciduous- and coniferous-dominated boreal forests in Alberta 2.5 years after harvest. The two forest types responded differently to the harvesting treatments. No differences among harvest treatments were found in PO_4^{3-} and NO_3^- availability in the deciduous stand, but partial-cut treatments in the conifer stand had intermediate decrease in PO_4^{3-} and increase in NO_3^- between uncut and clearcut sites. Bradley et al. (2001) found lower extractable P concentrations in shelterwood-harvested plots than either the uncut or clearcut plots in forest of western hemlock and amabilis fir near Campbell River, British Columbia 4 years after harvest.

2.4.3 Soil microbial community structure and function

In the preceding sections, timber harvesting has been shown to influence various soil physical and chemical attributes that may in turn induce changes in the composition and functioning of

the soil microbial community. The responses of soil microbial communities to clearcutting and partial-cutting treatments tend to vary among studies. Some researchers have observed changes in microbial community structure and function, whilst others have not. These variations may be attributable to differences in forest ecosystems, harvesting intensities and patterns, degrees of soil disturbance, the post-harvest time lag, and methods of analysis. Several authors have observed reductions in microbial biomass and basal respiration 3 years (Pietikäinen and Fritze 1995, Bååth et al. 1995), 4 years (Bradley et al. 2001), or 10 years (Chang and Trofymow 1996) after harvest. However, there is evidence that the reduction of microbial biomass may be temporary and could rebound to the pre-harvest level as stands age. Moore-Kucera and Dick (2008) found that microbial biomass in a 25-year-old replanted clearcut was similar to old-growth Douglas-fir forest than a more recent (10 years) clearcut. Increases (Entry et al. 1986) and no differences (Smolander et al. 1998, Pennanen et al. 1999) in microbial biomass have also been observed after clearcutting. Although microbial biomass provides a useful indication of how the soil microbial community responds to disturbances, it lacks the information on the changes in the population composition and function of the microbial community. For example, Bradley et al. (2001) found that although microbial biomass was not significantly different in partial-cut and clearcut western hemlock and amabilis fir forests, the carbon-utilisation patterns of the soil microbial community were different. This indicates that heterotrophic microbial activity may differ in partially cut and clearcut forest soils.

The fungal community has been shown to be more adversely affected by harvesting than the bacterial community, which could be explained by the absence of living roots that support growth of mycorrhizal fungi (Harvey et al. 1980). Clearcutting has been shown to decrease fungal biomass (Pietikäinen and Fritze 1995, Pennanen et al. 1999, Bååth 1980), as well as the ratio of fungal to bacterial PLFAs (Bååth et al. 1995). Bacterial biomass, on the other hand, has been shown to increase in the short-term after harvest. Lundgren (1982) observed increases in bacterial biomass, determined by microscopic counts, during the first 2 years after harvest, but

biomass decreased after the third year. Niemelä and Sundman (1977) and Sundman et al. (1978) also observed an increase in bacterial populations up to 7 years after clearcutting, but the populations decreased to numbers found in unharvested forest after 13 years. Smith et al. (2008) found a reduction in microbial biomass carbon (C_{mic}) by 18% and microbial biomass nitrogen (N_{mic}) by 25% as well as in their ratio (C_{mic}/N_{mic}) in 8-month-old clearcut sites compared to uncut forest. The ratio of C_{mic}/N_{mic} is typically positively correlated with the dominance of fungi in the microbial community (Iost et al. 2008). Kranabetter and Coates (2004) also observed decreasing C/N ratios in the order of: uncut forest > partial-cut > clearcut. Hassett and Zak (2005) found no difference in the relative abundance of fungi and bacteria among different harvest intensities (stem-only removal, whole-tree removal, whole-tree plus forest floor removal, and uncut control), although microbial biomass decreased in the harvested treatments compared to the uncut control. Hannam et al. (2006) found no effect of varying intensities of tree retention (0, 20, 50, and 100%) on microbial biomass and the relative abundances of fungi and bacteria in three forest types (deciduous-dominated, coniferous-dominated, and mixed), 4.5 to 5.5 years after harvest. However, the relative abundance of the PLFA 16:1ω5 associated with arbuscular mycorrhizal (AM) fungi and Gram-negative bacteria (Olsson 1999, Hassett and Zak 2005) was higher in the harvested plots than in uncut forest. Furthermore, microbial basal-respiration and SIR profiles obtained using 21 carbon sources also did not show differences among varying retention intensities in all three forest types. The same deciduous- and coniferous-dominated forest types were studied earlier by Lindo and Visser (2003) 2.5 years after harvest, and microbial biomass and basal respiration were lower in the clearcut than in uncut forest at both sites, indicating that these indicators had returned to pre-harvest levels between 2.5 and 4.5 to 5.5 years after harvest.

Harvesting has also been shown to cause shifts in the composition of the bacterial community. Using DNA cloning and sequencing, Axelrood et al. (2002) found the proportion of clones affiliated with *γ-Proteobacteria* in general, as well as *Pseudomonas* 16S clones were lower in

harvested plots subjected to forest floor removal and heavy compaction than those which were not subjected to these treatments, in three distinct subzones of the Sub-Boral Spruce zone in central British Columbia. The pseudomonads are a known bacterial genus in soil involved in denitrification (Killham 2001). Therefore, reductions in this bio-indicator could potentially result in decreased N cycling potential of a site. Smith et al. (2008) found differences in denaturing gradient gel electrophoresis (DGGE) and ribosomal intergenic spacer analysis (RISA) community fingerprints between an 8-month-old clearcut and an uncut spruce-dominated forest in Alberta, although further analysis of the distinct RISA bands revealed similarity in DNA sequences, which could have represented different strains of the same genus or different rRNA operons of the same strain.

Relative to other members of the microbial community, the effects of harvesting on ectomycorrhizal (ECM) fungal community have been relatively well-studied. Ectomycorrhiza is the predominant type of mycorrhizal association in temperate forest ecosystems. Many studies have consistently reported reductions in the diversity of ECM fungal community and changes in species composition as a result of harvesting. Byrd et al. (2000) found lower abundance and species richness of ECM fungi and detected significant shifts in community composition in 8-year-old lodgepole pine clearcuts than in undisturbed forests in Wyoming. Clearcutting also decreased ECM fungal diversity at a subalpine forest in British Columbia 2-3 years after harvest (Hagerman et al. 1999b). Luoma et al. (2004) tested the effects of two patterns (aggregated and dispersed) and four levels (100, 75, 40, and 15%) of green-tree retention on ECM fungal sporocarp biomass during spring and fall fruiting seasons. The number of sporocarp-producing taxa declined in all treatments, with the greatest reductions found in the 15% retention treatments, and higher levels of sporocarp production were maintained at sites with higher level of retention, despite not always being proportional with basal area retained. The 15% aggregated forest patches appeared to be more important for maintaining fruiting of a greater number of ECM fungal species than the 15% dispersed retention trees, whilst the 40% dispersed retention

treatment maintained sporocarp production better than 40% aggregated retention treatment. The authors suggested that a combination of aggregated and dispersed retention treatments may be most effective at maintaining ECM fungal community (Luoma et al. 2004).

A shadow or edge effect has been observed with regards to ECM fungi in numerous studies. For instance, ECM fungal diversity decreased with increasing distance from the edge of an Engelmann spruce and subalpine fir stand into an opening, although the decline was less notable within 2 m of the stand's edge (Hagerman et al. 1999b). Similarly, Kranabetter and Wylie (1998) found a 27% reduction in ECM fungal diversity at the forest edges and a 40% reduction in 4-year-old clearcut openings, relative to uncut western hemlock forests in northwestern British Columbia. In a later study of the same sites, 6 years after harvest, residual trees maintained ECM fungal diversity in birch seedlings that were within the rooting zone (about 5 m away from residual trees) (Kranabetter 1999). ECM fungal diversity decreased outside the rooting zone (about 25-50 m away from residual trees) by 38% in a dispersed retention treatment (1 tree/ha) and 15% in a forest stand with 60% canopy removal in gaps (Kranabetter 1999). Durrall et al. (1999) also observed reductions in ECM fungal richness with increasing distance from the forest edge into the opening 3-4 years after harvest. Similarly, Cline et al. (2005) found higher ECM fungal species richness and diversity in seedlings close (6 m) to residual trees than those farther away (16 m) in a second-growth Douglas-fir forest in Washington. These observations suggest that the residual trees are capable of providing a source of inoculum for the ECM fungal community. Jones et al. (2008) found that aggregated tree patches of varying sizes (5, 10, 20, and 40 m in diameter) retained ECM fungal communities equally for up to 10 m from the patch edge and similar to those of an uncut second-growth Douglas-fir, western hemlock forests on Vancouver Island. They recommended that numerous small patches may be more effective than a few large patches in providing ECM fungal inoculum during the first year after harvest. Although Durall et al. (1999) found that ECM fungal richness decreased with increasing harvest gap size, Hagerman (1999a) suggested that the proximity to residual trees may be more

important than the size of opening in maintaining the ECM fungal community. In this sense, at the same retention level, dispersed green-tree retention may be more appropriate than aggregated green-tree retention in maintaining ECM fungi throughout the cutblock. Twieg et al. (2007) found that the abundance and diversity of ECM fungal community were lower in 5-year-old clearcuts than in 26-year-old clearcuts, indicating that harvest effects on ECM fungi may be temporary and diminish as stands age.

In addition to changes in microbial community structure, harvesting has also been shown to induce changes in the functioning of soil microbial communities. Grayston and Rennenberg (2006) studied the effects of two forest thinning treatments (light and heavy) relative to unmanaged forest on soil microbial community structure and function at two beech forests that differ in soil and air temperature and water availability (cool-moist and warm-dry sites). The two sites responded to thinning treatments differently, with increases in microbial biomass and respiration in the warm-dry site, and decreases in these indicators in the cool-moist site. Declines in microbial biomass and respiration could be attributed to reductions in fine root biomass in the thinned treatment, resulting in reduced C availability for microbial growth (Grayston and Rennenberg 2006). Analysis of PLFA profiles and CLPP revealed separation among the thinning treatments and unmanaged forest and between the two sites. Further analysis of the CLPP data showed that the soil microbial community exhibited different preferences in carbon source utilisation after thinning, with a greater potential utilisation of acidic, basic, and neutral amino acids in the heavily thinned forest than the uncut forest. An earlier study of the same forests found high concentrations of these amino acids in beech roots in thinned stands (Fotelli et al. 2002). The authors suggested that the exudation of these amino acids in thinned stands might have selected for microorganisms that are able to utilise these compounds for growth (Grayston and Rennenberg 2006). Five years after thinning, Maassen et al. (2006) found no significant differences among treatments in microbial biomass, basal respiration, and the activities of endocellulase, exocellulase, β-glucosidase, endoxylanase, exoxylanase,

phenoloxidase, and peroxidase in both organic and mineral soil layers. However, PLFA analysis showed differences in microbial community structure between thinned and unthinned forests. The authors suggested that the changes in microbial community structure had not yet resulted in any responses in soil microbial activities, although this could also be due to functional redundancy of the microbial communities.

Hernesmaa et al. (2008) studied the effects of clearcutting, comparing soil microbial activities in a 6-year-old clearcut, recently clearcut, and uncut Scots-pine forests three times over a one-year period. Forest floor extracellular enzyme (β-glucosidase, phosphatase, N-acetyl-glucosaminidase, cellobiohydrolase, butyrate esterase, and xylosidase) activities increased 1 week after harvest and peaked between 9 and 12 months after harvest, and the 6-year-old clearcut appeared to maintain enzyme activities to levels similar to younger clearcut. In the mineral layer, there was no difference in enzyme activities among the treatments, although there was a tendency for higher enzyme activities in the uncut forest than the clearcuts. Harvesting also increased microbial utilisation of carbon substrates 1 week after clearcutting in the forest floor, and after 1 year in the mineral soil. The authors attributed the increase in microbial activities to increased N availability that may have enhanced microbial assimilation of other available substrates. Further, the authors hypothesised that maintenance of increased microbial activity in the older clearcut may have been due to vegetation growth and the formation of new root systems. Waldrop et al. (2003) found harvesting lowered forest floor extracellular enzyme activities (β-glucosidase, cellobiohydrolase, N-acetyl-glucosaminidase, phenol oxidase, and phosphatase) 3 years after harvesting regardless of the differences in post-harvest treatments (slash, mechanical chipping and piling, or broadcast burning). The authors attributed the decrease to alterations in soil moisture and reduced nutrient concentrations in the harvested sites. Similarly, Hassett and Zak (2005) found reductions in extracellular enzyme activities by 10-30% in harvested plots compared to uncut plots, 8-10 years post-harvest in aspen-dominated

stands. The decrease in enzyme activities was found to correspond to a reduction in soil microbial biomass.

2.5 Introduction to the study

In this study, I investigated whether two patterns of green-tree retention harvesting (aggregated and dispersed retention) had retained the structure and function of the soil microbial community of an uncut forest, 5 years after harvest. The study was conducted in a coastal second-growth forest of Douglas-fir and western hemlock on Vancouver Island, BC. It is part of a larger project, being undertaken as part of the STEMS project, that aims to assess the potential of green-tree retention to maintain a healthy soil ecosystem using a number soil biological indicators. In this study, soil microbial community structure and function, as well as nutrient availability were used to evaluate differences in microbial responses among harvesting treatments. PLFA analysis was used to measure changes in microbial community composition and to provide an estimate of soil microbial biomass among harvest treatments. The functioning of soil microbial communities was assessed by measuring the potential activities of five enzymes involved in the cycling of C, N, and P (i.e. β-glucosidase, phenol oxidase, peroxidase, N-acetyl-glucosaminidase, phosphatase) in addition to analysis of SIR using five carbon substrates representing some of the common compounds in litter and root exudates (chitin, glucose, carboxymethyl cellulose, serine, malonic acid). Plant root simulator (PRS™) probes were used to measure *in situ* nutrient availability of 14 ions, i.e. NO_3^-, NH_4^+, PO_4^{3-}, Ca, Mg, K, Fe, Mn, Cu, Zn, B, S, Pb, and Al. The objectives of this study are:

1. to determine if green-tree retention harvesting is better than clearcutting to retain nutrient availability and the structural and functional characteristics of the soil microbial community relative to the uncut forest

2. to investigate if residual trees in the aggregated and dispersed retention treatments are able to provide forest influence that extends into the harvested areas and determine which treatment is superior for this purpose.

To address these objectives, soil microbial community structure and function and nutrient availability were studied at the centre and edge of tree patches in aggregated retention or just beside the tree in the dispersed retention, and along a northerly transect up to 30 m into the harvested area, and randomly in the clearcut and uncut forest. The specific research questions posed within the context of this study are:

1. Does harvesting alter soil microbial community structure and function and nutrient availability?
2. Is green-tree retention harvesting better than clearcutting in terms of maintaining soil microbial community structure and function and nutrient availability?
3. Which retention pattern is more effective for the maintenance of soil microbial community structure and function and nutrient availability?
4. Does proximity to the residual trees in the green-tree retention treatments influence soil microbial community structure and function and nutrient availability?
5. How far is the zone of influence from the residual trees into the opening?
6. Does the structure and function of soil microbial community in the centre of aggregated retention patches reflect those found in the uncut forest?

Based on the literature to date, depending on regional climate and forest types, clearcutting may or may not affect soil microbial communities. However, the lack of litter input and mature living roots in the clearcut as well as differences in microclimate between a clearcut and a forest are expected to alter the composition and functioning of soil microbial community. The responses of the soil microbial community to green-tree retention will be different than those to clearcutting because of the influence of residual trees.

Thus, I hypothesised that:

1. Clearcutting will increase N availability, but will less affect P availability.
2. N and P availabilities in the green-tree retention treatments will be intermediate between a clearcut and uncut forest.
3. Enzyme activities will be lower in the clearcut, but will be retained in the green-tree retention treatments.
4. Harvesting will change microbial utilisation of carbon substrates, for example it will decrease chitin utilisation, an indicator of fungal biomass.
5. Clearcutting will decrease fungal biomass and increase bacterial biomass
6. Nutrient availabilities and the soil microbial community structure and function at sampling locations in the centre and at the edge of aggregated retention patches and beside the dispersed residual trees will be more similar to those found in the uncut forest than sampling locations in the cut area away from the retained trees.
7. Nutrient availabilities and soil microbial community structure and function will be maintained up to 10 m from the edge of the aggregated retention patches and from dispersed residual trees.
8. Nutrient availabilities and soil microbial community structure and function will be retained to a greater distance into the cut area by aggregated retention than dispersed retention
9. Nutrient availabilities and soil microbial community structure and function will be more similar among sampling locations in the dispersed retention than in the aggregated retention treatment.
10. There will be strong correlations between components of the measured biological variables (microbial community structure and function) and soil variables (moisture, pH, and nutrient availability).

3 MATERIALS AND METHODS

3.1 Study area and site description

The STEMS (Silviculture Treatments for Ecological Management in the Sayward) project is a large-scale multi-disciplinary experiment set up by the BC Ministry of Forests to test seven different silvicultural treatments: extended rotation (non-treated control), aggregated retention, dispersed retention, clearcut with reserves, modified patch cut, group selection, and extended rotation with commercial thinning (deMontigny 2004). This study was conducted in the first replicate of STEMS (i.e. STEMS 1) established in the eastern end of the Snowden Demonstration Forest (50°3'59.4"N, 125°26'21.1"W) near Campbell River on Vancouver Island, BC. The site is located within the Coastal Western Hemlock very dry maritime (CWHxm2) biogeoclimatic subzone (deMontigny 2004), which has warm, dry summers and cool, moist winters (Green and Klinka 1994). The area receives an annual precipitation of 1505 mm mostly through rainfall and has a mean temperature of 9.3 °C (Pojar et al. 1991). Soils are mainly Orthic Humo-Ferric Podzol with moder humus form and sandy-loam structure (deMontigny 2004, Pojar et al. 1991). The area has a gently rolling topography and consists of 50-80 year-old second growth forests that result from either fire or harvesting (deMontigny 2004). The stands are dominated by Douglas-fir (*Pseudotsuga menziesii*) (83%), with minor components of western hemlock (*Tsuga heterophylla*) (13%) and western redcedar (*Thuja plicata*) (4%), and the understorey vegetation mainly consists of salal (*Gaultheria shallon*) and swordfern (*Polystichum munitum*) (deMontigny 2004). Harvesting of the second-growth forest was done between July and October 2001. Retained trees consist predominantly of Douglas-fir with a minority of western hemlock. Harvesting was done by hand-felling and loader forwarding. Slash was machined piled in dispersed piles of no larger than 5 m in diameter. Reforestation of all harvested areas was done by planting Douglas-fir seedlings between March and April 2002.

For the purpose of this study, the aggregated retention, dispersed retention, clearcut with reserves, and extended rotation (uncut control) treatments were examined (Table 3.1). The target number of residual trees in the dispersed retention treatment was 45 uniformly dispersed trees per ha (deMontigny 2004). In the aggregated retention treatment, residual trees were retained in groups ranging from 0.01 to 0.15 ha in size (deMontigny 2004). The tree aggregates, however, were not uniformly spaced. In this study, aggregated retention patches of 0.02 ha (approximately 20-m diameter) were selected.

Table 3.1 Descriptions of selected treatments at STEMS 1. Data obtained from deMontigny (2004).

	Aggregated retention	Dispersed retention	Clearcut	Uncut control
Treatment size (ha)	25.5	18.2	10.9	12.0
Pre-harvest density (stems/ha)	1208	1210	747	607
Post-harvest density (stems/ha)	247	51	0	607
Pre-harvest basal area (m^3/ha)	55	55	53	53
Post-harvest basal area (m^3/ha)	10	6	0	53
Retention level (based on change in basal area) (%)	18	11	0	100
Aspect	Variable (north, west, south)	North to west	Flat to west	North
Slope (%)	0-40	0-35	0-12	10-30
BEC site series	01	01	05	01
Soil moisture regime	slightly dry to fresh	slightly dry to fresh	slightly dry to fresh	slightly dry to fresh
Soil nutrient regime	very poor to medium	very poor to medium	rich to very rich	very poor to medium
Humus form	6 cm moder	5 cm moder	4 cm moder	10 cm moder
Post-harvest standing, uprooted, and leaning trees (%)	82, 8, 10	38, 46, 15	0, 0, 0	99.5, 0.1, 0

3.2 Sampling design and soil processing

Soils were sampled between October 26 and 27, 2006, five years after harvest. Four soil cores (7-cm diameter) were collected within 1 to 2 m at each sampling location from both the forest floor (O horizon) and the top 10 cm of mineral soil (A horizon). The forest floor and mineral soil layers from each core were separated, and the sub-samples were composited by layer in separate re-sealable plastic bags. Soil samples were obtained from four random locations at least 100 m apart in the clearcut and uncut control, from the centre and edge of the four 0.02-ha (or 20-m diameter) replicate tree patches in the aggregated retention treatment, and 10 m, 20 m, and 30 m along a northerly transect from the edge the patches into the opening, and from beside four residual trees in the dispersed retention treatment and 10 m, 20 m, 30 m along a northerly transect from the trees into the opening (Fig. 3.1). There were a total of 88 samples. Soils samples were stored in an iced cooler for transport. On return to the laboratory, soils were stored at 4 °C prior to being mixed and passed through a 4-mm mesh sieve to remove rocks and plant fragments. Soils were separated into three sub-samples for different analyses. Soils designated for SIR analysis were stored at 4 °C, whereas the remaining was stored at -20 °C. Frozen (-20 °C) soil sub-samples designated for PLFA analysis were freeze-dried in batches of 12 samples for approximately 48 hours and subsequently stored at -20 °C.

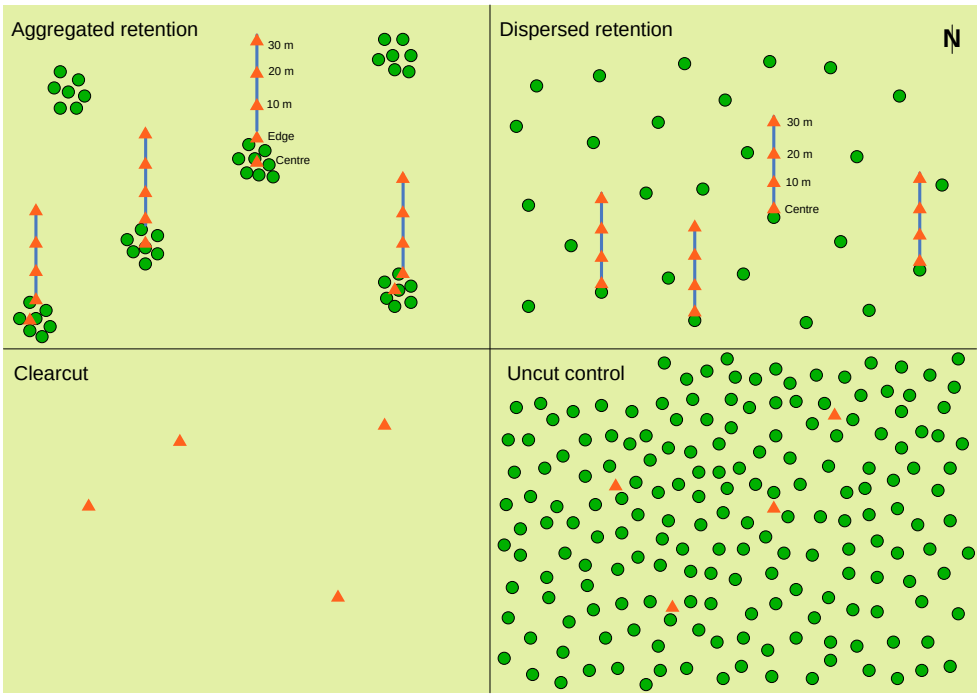

Figure 3.1 Illustration of sampling locations at the four harvest treatments STEMS 1.

3.3 Soil properties

3.3.1 pH and moisture content

Soil pH was measured following a procedure by Hendershot et al. (2008). Twenty millilitres of sterile dH_2O was added into a 30-mL sterile narrow plastic tube containing 2 g of air-dried organic soil or 10 g of air-dried mineral soil. The suspension was mixed vigorously for 30 minutes using a conical shaker, and then allowed to settle for an hour. Soil pH was measured using a pH meter by immersing the electrode into the supernatant. Soil gravimetric moisture content was determined by weighing field-moist soil samples before and after oven-drying at 105 °C for 48 hours. Soil moisture was expressed as percentage of dry weight.

3.3.2 Nutrient availability

Soil nutrient supply rates were measured *in situ* using Plant Root Simulator (PRS)™-probes (Western Ag Innovations Inc., Saskatoon, SK). The PRS™-probes consist of either anion or cation exchange membranes encapsulated in plastic stakes. The ion-exchange membranes (10-cm^2 surface area) function as ion sinks for a range of bioavailable soil nutrients, i.e. NO_3^-, NH_4^+, PO_4^{3-}, Ca, Mg, K, Fe, Mn, Cu, Zn, B, S, Pb, and Al. Four replicate pairs of anion and cation probes were buried in each soil layer (forest floor and mineral soil) within 1 to 2 m of each sampling location for ± 63 days (from October 26 and 27 to December 28, 2006). After removal, the probes were stored in an iced cooler during transport to the laboratory. Upon arrival, the probes were cleaned with a brush to remove visible soil particles, rinsed with deionised H_2O, and bulked according to sampling points by treatment. The probes were sent to Western Ag Innovations Inc. for analysis of nutrients adsorbed to the membranes. At Western Ag, the probes were eluted for one hour using 0.5 N HCl or 1M KCl. The eluate was analysed colourimetrically for levels of ammonium (NH_4^+) and nitrate (NO_3^-) using an autoanalyser. The remaining nutrients, i.e. phosphorous (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), aluminum (Al), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), and lead (Pb), were measured using inductively-coupled plasma (ICP) spectrometry/atomic absorption spectrometry(AAS)/flame emission spectrometry (FES). Nutrient supply rates generated with the PRS™-probes are reported as the amount of nutrient adsorbed per amount of adsorbing surface area per time or burial in soil (i.e. µg nutrient 10 cm^{-2} time of $burial^{-1}$) (Qian and Schoenau 2002).

3.4 Microbial community structure and function

3.4.1 Substrate-induced respiration (SIR)

Soil microbial catabolic diversity was estimated by measuring substrate-induced respiration (SIR) using the MicroResp™ protocol by Campbell et al. (2003). MicroResp™ measures CO_2

respiration from whole soil before and after the addition of ecologically relevant carbon sources. Five carbon sources were selected (Table 3.2) to represent compounds commonly found in forest litter and root exudates (Campbell et al. 1997). The 5 carbon sources and a water control were tested in 8 replicate wells on a microplate.

Table 3.2 Carbon sources used for substrate-induced respiration.

Substrate	Class
Glucose	Monosaccharide
Chitin	Polysaccharide
Carboxymethyl cellulose	Polysaccharide
Malonic acid	Carboxylic acid
D-serine	Amino acid

The MicroResp™ kit consists of four components: a 96-well deep-well incubation microplate, a 96-well CO_2 detection microplate, a rubber gasket that seals the incubation and detection plates together, and a metal clamp to ensure tight seal between the plates and equal pressure on all sides. An equivalent of 0.1 g soil dry weight was added to each well on the incubation plate. The carbon sources were prepared with an aim to supply 5 mg of carbon per gram dry weight of soil. These carbon sources (except chitin) were dissolved sterile deionised H_2O and added in a volume of 100 µL to each well containing soil samples. Chitin, which does not dissolve in water, was added in the amount of 0.0079 g per well. The detection plates contained a pink pH indicator dye - cresol red - dissolved in Noble Agar that changes colour as CO_2 is produced. The detection plates were read twice in a microplate spectrophotometer (SpectraMax® 340, Molecular Devices, USA) using the SoftMax Pro software, just before they were sealed to the incubation plates (time = 0) and after a 6-hour incubation at 20 °C. The difference between absorbance readings before and after incubation was calculated for each well. The final SIR rate is expressed as the amount of CO_2 evolved per gram of soil dry weight per hour (µg CO_2-C g^{-1} h^{-1}).

3.4.2 Extracellular enzyme activities

The potential of soil microbial functioning was assessed by measuring the activities of five hydrolytic and oxidative enzymes (Table 3.3) using a microplate approach adapted from the methods by Marx et al. (2001) and Sinsabaugh et al. (2000, 2003). Hydrolytic enzymes activities were assayed fluorimetrically using substrates linked to 4-methylumbelliferone (MUB), a fluorescent compound, whereas oxidative enzyme activities were determined colorimetrically based on the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA).

Table 3.3 Enzymes assayed in this study.

Enzyme	EC[1]	Assay substrate	Description
β-1,4-glucosidase (glucosidase)	3.2.1.21	4-MUB-β-D-glucoside	Cellulose-degrading hydrolytic enzyme
β-1,4-N-acetylglucosaminidase (NAGase or chitinase)	3.2.1.52	4-MUB-N-acetyl-β-D-glucosaminide	Chitin-degrading hydrolytic enzyme
Acid phosphatase (phosphatase)	3.1.3.2	4-MUB-phosphate	Phosphate-releasing hydrolytic enzyme
Phenol oxidase	1.10.3.2	L-3,4-Dihydroxyphenylalnine	Lignin-degrading oxidative enzyme
Peroxidase	1.11.1.7	L-3,4-Dihydroxyphenylalnine	Lignin-degrading oxidative enzyme

[1] EC = Enzyme Commission number

Frozen soil samples were individually ground to fine particles for 1 minute with a mortar and pestle that had been surface-sterilised with 70% ethanol. The ground soils were weighed (0.5 g for flourimetric assay and 0.1 g for colorimetric assay) and transferred into a 250-mL sterile screw-cap bottle. Then, 50 mL of 0.05 M sodium acetate buffer (pH 5.0) and approximately 30 glass beads were added to each bottle containing a soil sample. The bottles were shaken for one hour on a rotary shaker, after which another 50 ml of sodium acetate buffer was added to each bottle. These soil suspensions were kept at 4 °C until ready to use for up to 24 hours.

For the fluorimetric enzyme assay, substrate solutions were prepared by individually dissolving 4-MUB-β-D-glucoside, 4-MUB-N-acetyl-β-D-glucosaminide, and 4-MUB-phosphate in sterile dH_2O to concentration of 200 µM. These substrate solutions were kept at 4 °C for up to a week, except for 4-MUB-phosphate, which had to be prepared fresh. A 10-µM concentration of MUB standard solution was also prepared and stored at 4 °C for up to a week. Sixteen replicates of each enzyme assay for each soil sample (200 µL of soil suspension and 50 µL of MUB substrate) were performed different wells on 96-well black microplates (Costar microplate 3904, Corning Life Sciences, Acton, MA). The following was also added to individual wells on the same microplate: (i) 16 replicates of a standard positive control (200 µL of sodium acetate buffer and 50 µL of MUB standard), (ii) 8 replicates of a substrate negative control (200 µL of sodium acetate buffer and 50 µL of MUB substrate), (iii) 8 replicates of a quench standard (200 µL of soil suspension and 50 µL of MUB standard), (iv) 8 replicates of a soil background (200 µL of soil suspension and 50 µL of buffer), and (v) 8 replicates of a buffer negative control (250 µL of buffer). The microplates were incubated at 20 °C in the dark for 2 hours for phosphatase assay, 3 hours for glucosidase assay, and 3 hours for chitinase assay. At the end of the incubation, 20 µL of 0.5 N NaOH was added to stop the reaction. The microplates were read in a microplate fluorimeter (CytoFluor™ II, Applied Biosystems, USA) using the CytoFluor™ software, which had been set to provide excitation at 360/40 nm and emission at 460/40 nm and mixing period of 5 seconds. Potential enzyme activities were reported as the amount of substrate converted per hour per gram of soil sample (nmol h^{-1} g^{-1}). Potential enzyme activity was assumed to be zero if the calculated activity was negative.

For the colorimetric enzyme assay, 25 mM L-DOPA solution was prepared in 0.05 M sodium acetate buffer and kept at 4 °C in the dark prior to use for up to 24 hours. Phenol oxidase and peroxidase assays were performed on a separate set of 96-well clear microplates (Costar microplate 3370, Corning Life Sciences, Acton, MA). The following was added into the wells on each microplate set: (i) 16 replicates of each enzyme assay (200 µL of soil suspension and 50

µL of L-DOPA), (ii) 8 replicates of soil background (200 µL of soil suspension and 50 µL of buffer), (iii) 8 replicates of a substrate negative control (200 µL of buffer and 50 µL of L-DOPA), and (iv) 8 replicates of a buffer negative control (250 µL of buffer). For peroxidase assay, 10 µL of 0.3% H_2O_2 was also added to each well after the addition of L-DOPA. The microplates were incubated at 20 °C for 5 hours and 18 hours for peroxidase and phenol oxidase assays, respectively. After incubation, the plates were read in a microplate spectrophotometer (SpectraMax® 340, Molecular Devices, USA) at 460 nm using the SoftMax Pro software. Potential enzyme activities were expressed as the amount of substrate converted per hour per gram of soil sample (nmol h^{-1} g^{-1}). Potential enzyme activity was assumed to be zero if the calculated activity was negative. The values obtained for peroxidase activity includes phenol oxidase activity. Peroxidase activity was obtained by subtracting the activity phenol oxidase from that of peroxidase.

3.4.3 Phospholipid fatty acid analysis

The extraction of phospholipid fatty-acids (PLFAs) from soil samples was carried out following the procedure in Frostegård et al. (1993), which was based on a method of Bligh and Dyer (1959) as modified by White et al. (1979). Briefly, 1.5 g of freeze-dried mineral soil and 1 g of freeze-dried organic soil of forest floor was individually extracted in a mixture of chloroform, methanol, and citrate buffer (1:2:0.8 by volume). The extract was split into two phases by adding an equal volume of chloroform and 0.15 M citrate buffer (pH 4.0). The organic phase was recovered and fractionated through a silica column (AccuBond® II Silica Cartridges, Agilent Technologies, USA) by subsequent additions of chloroform, acetone, and methanol. The methanol fraction contains phospholipids and was retained. An internal standard, methyl nonadecanoate (Sigma N5377), was added to the methanol fraction and then the mixture was subjected to a mild alkaline methanolysis to yield fatty acid methyl esters (FAMEs). The FAMEs were analysed using a gas chromatograph (Agilent 6890N, Agilent Inc., USA) equipped with a mass selective detector (Agilent 5973N, Agilent Inc., USA). The separated fatty acids

represented as peaks on the resulting chromatogram were identified by comparing the retention time of each peak to those of a bacterial acid methyl ester (BAME) standard mix (1:20, v/v) (Supelco 47080-U) and by referring to the PLFA profile in Knief et al (2003).

The abundance of each identified phospholipid fatty acid was determined based on the area of the corresponding fatty acid peak on the chromatogram, and expressed as the concentration of fatty acids per gram of freeze-dried soil (nmol g^{-1}). The relative abundance of each PLFA used for further analyses was calculated as a percentage of total PLFAs (nmol % g^{-1}), i.e. the sum of all identified PLFAs excluding the internal standard (19:0) that can be used as an index of total viable microbial biomass in soils. The relative abundance, rather than absolute abundance, of PLFA biomarkers is most commonly reported, because it allows for comparisons of microbial community structure without the effects of differences in microbial biomass or extraction efficiencies among samples (Hebel et al. 2009).

Using standard nomenclature as described in Frostegård et al. (1993) and Zelles and Bai (1993), fatty acids were designated by the number of C atoms, followed by a colon and the number of double bonds present in the molecule. The position of the double bonds from the methyl end of the molecule is indicated by the suffix “ω”. Cis and trans configurations are indicated by “c” and “t”, respectively. The prefixes “i” and “a” refer to iso- and anteiso-branching, respectively, “10Me” denotes the presence of a methyl group on the tenth C atom from the carboxyl end of the molecule, and “cy” represent a cyclopropane fatty acid. The abundance of each microbial taxonomic group, i.e. Gram-negative and Gram-positive bacteria, total bacteria, actinomycetes, fungi, and arbuscular mycorrhizal (AM) fungi, was expressed as the sum of the proportion of the individual PLFA representative of that particular microbial group (Table 3.4).

Several PLFA indices have been used to detect changes in the composition soil microbial communities. The fungal to bacterial biomass ratio can be used as an indicator of high organic matter content and was calculated by dividing the relative abundance of total bacterial PLFAs

with fungal PLFA (Frostegård and Bååth 1996). PLFA representative of AM fungi was not included in the calculation as it is also found in Gram-negative bacteria (Hassett and Zak 2005). The ratio of Gram-positive to Gram-negative bacteria was also used to assess changes in bacterial community composition. Bacterial communities are known to alter their membrane lipids in response to environmental stresses, such as anaerobic condition, lack of nutrients, or moisture and temperature limitations (Fierer et al. 2003, Kieft et al. 1997, Bossio and Scow 1998, Zelles et al. 1994). The indices often used to evaluate bacterial community stresses were: (i) the ratio of total saturated (15:0, 16:0, 17:0, and 18:0) to total monounsaturated (16:1ω7c, 16:1ω9c 16:1ω7c, 16:1ω5c, i17:1ω8c, 18:1ω9c, 18:1ω7c, and 18:1ω5c) PLFAs, and (ii) the ratio of cyclopropyl PLFAs (cy17:0 + cy19:0) to their monoenoic precursors (16:1ω7c +18:1ω7c). Higher ratios of saturated to mono-unsaturated, cyclopropyl to monoenoic precursors, and Gram-positive to Gram-negative PLFAs, have been found with increasing soil depth, where oxygen and substrates are more limiting (Song et al. 2008, Fierer et al. 2003).

Table 3.4 Representative PLFA biomarkers chosen for taxonomic microbial groups

Taxonomic group	Signature group	PLFA biomarker	Reference
Gram-negative bacteria	Monoenoic and cyclopropane monounsaturated PLFAs	i16:1ω7c, 16:1ω9c, 16:1ω7c, i17:1ω8c, cy17:0, 18:1ω7c, 18:1ω5c, cy19:0	(Zelles 1997, Borga et al. 1994)
Gram-positive bacteria	Terminally-branched saturated PLFAs	i15:0, a15:0, i16:0, i17:0, a17:0	(Zelles 1997, Zogg et al. 1997, White et al. 1996)
Total bacteria	Multiple PLFAs	i15:0, a15:0, 15:0, i16:1ω7c, i16:0, 16:1ω9c, 16:1ω7c, i17:1ω8c, i17:0, a17:0, cy17:0, 17:0, 18:1ω7c, 18:1ω5c, 18:0, cy19:0	(Frostegård and Bååth 1996, Zelles et al. 1994)
Actinomycetes	10Me PLFAs	10Me16:0, 10Me17:0, 10Me18:0, 10Me19:0	(Zelles et al. 1994, Kroppenstedt 1985)
Fungi	Polyunsaturated PLFAs	18:2ω6,9	(Frostegård and Bååth 1996, White et al. 1996)
AM fungi	Monounsaturated PLFAs	16:1ω5c	(Olsson 1999)

Note: The following non-specific fatty acids were not used as biomarkers, but included to represent total biomass: 16:0 (common), 10Me19:1ω7c (total microbial biomass), and 18:1ω9c (Gram-positive and fungi)

3.5 Statistical analyses

3.5.1 Data screening and univariate and correlation analyses

All response variables were tested for normality and homogeneity of variances by using Kolmogorov–Smirnov and Bartlett's tests in SAS 9.1 (SAS Institute Inc., Cary, NC). Variables

that were normally distributed and had equal variances were Mg, K, Zn, B, and Al availabilities, soil pH, peroxidase activity, substrate-induced respiration (SIR) of glucose, relative abundances of Gram-negative bacteria, total bacteria, arbuscular mycorrhizal (AM) fungi, and the ratios of fungal to bacterial PLFAs and Gram-positive to Gram-negative bacteria. The rest of the data did not meet the assumptions of normal distribution and equal variances to perform parametric statistics, and attempts to normalise these variables using several transformations were unsuccessful. Thus, non-normal data were left untransformed. One-way analysis of variance (ANOVA), or Kruskal-wallis ANOVA (based on ranked data) for non-normal variables, was performed to test for significant differences in soil moisture, pH, nutrient availability, SIR rates, enzyme activities, and the composition of the soil microbial community (indicated by PLFA biomarkers) among the four treatments and at different sampling locations in both the forest floor and mineral soil (JMP 8, SAS Intitute Inc., Cary, NC). Differences were considered significant at $p < 0.05$. Post-hoc pair-wise comparisons were performed using the Bonferroni test when ANOVAs showed significance at $p < 0.05$. Correlation analysis was performed between pairs of measured variables using Spearman's rank correlation test (JMP 8, SAS Intitute Inc., Cary, NC). Associations were considered strong at $|\rho| > 0.4$.

3.5.2 Multivariate data analyses

Multivariate analyses have been demonstrated to improve the discriminatory power of techniques used in soil microbial ecology studies (Grayston and Rennenberg 2006, Leckie et al. 2004). Thus, multivariate analyses were also performed test for differences in SIR rates, enzyme activities, and the composition of the soil microbial community among the four treatments.

3.5.2.1Multi-response permutation procedures (MRPP)

Multi-response permutation procedures (MRPP) were undertaken in PC-ORD 5.0 (MJM Software Design, Gleneden Beach, OR) to test for significant differences in soil microbial community composition and function among (i) the four harvest treatments and (ii) various distances to residual tress in the aggregated and dispersed treatments. MRPP tests were separately performed on SIR, enzyme activity, and PLFA data sets. MRPP is a multivariate non-parametric method that tests the hypothesis of no difference among two or more pre-defined groups. MRPP is conceptually similar to multivariate analysis of variance (MANOVA), but being non-parametric, the former does not require the assumptions of multivariate normality and homogeneity of variances. The algorithm used in MRPP calculates all pair-wise distances in the entire dataset, and the Sørensen (Bray-Curtis) distance measure was chosen for all analyses. The Sørensen (Bray-Curtis) distance has been increasingly preferred in community ecology, as it has the advantage of being less prone to exaggerate the influence of outliers and the results are comparable to the more traditional Euclidean distance (McCune and Grace 2002). MRPP provides a measure of within-group homogeneity (A-statistic or A) and the corresponding p-value. $A = 1$ when all items are identical within a group, $A = 0$ when the observed within-group heterogeneity equals that expected by chance, and $A < 1$ when within-group heterogeneity is larger than that expected by chance. In community ecology, A is commonly below 0.1, and $A > 0.3$ indicates fairly high separation of groups (McCune and Grace 2002). For all MRPP analyses, a p-value < 0.05 and $A > 0.1$ were considered as significant differences among groups.

3.5.2.2Non-metric multidimensional scaling (NMS)

The patterns of microbial community structure and function among different harvesting treatments and proximities to retention trees were visualised using an ordination technique known as non-metric multidimensional scaling (NMS) in PC-ORD 5.0 (MJM Software Design,

Gleneden Beach, OR) (Kruskal 1964). The ordination produces a plot in which points closer together share more similarities than points farther apart. NMS does not assume normality and linear relationships among variables, and thus has been said to be generally the best ordination method for ecological community data (McCune and Grace 2002). NMS ordination performs an iterative search for the best solution to represent a data set on a reduced number of dimensions (axes), so that the distances on the ordination plot reflect those in the original data. The final stability of each run was evaluated by examining plots of stress versus the number of iterations. Stress is a measure of the dissimilarity (distance) between ordinations in the original and in the reduced dimensional space. The final dimensionality of the dataset was chosen through NMS iterative search to minimise stress. A final stress of less than 20 has been suggested to be acceptable for ecological community data (McCune and Grace 2002).

Separate NMS analyses were performed on SIR, enzyme activity, and PLFA datasets as the main matrices, and soil moisture, pH, and nutrient bioavailability as the second matrix. The microbial community structure and function data were grouped by harvesting treatments, proximities to residual trees, and soil layers. NMS analyses were undertaken based on Sørensen's distance measure with a random starting configuration and the "medium thoroughness" autopilot mode using randomised data for a Monte Carlo test of significance of each dimensionality. In the autopilot mode, the target instability criterion was set at 0.00001, which was used to determine the dimensionality of the dataset. Fifty real data runs and 50 randomised data runs were used. The coefficient of determination (r^2) signifies the proportion of variation on the axis explained or represented by the soil variable in question (McCune and Grace 2002). Pearson and Kendall correlations (r^2 and τ values) between the ordination axes and soil variables from the secondary matrix were obtained. Soil variables which had correlation values, i.e. $|r^2|$, greater than 0.2 with the main matrix were plotted on the ordination plot. Correlations were considered strong at $|r^2| > 0.4$.

4 RESULTS

4.1 Moisture content and pH

The forest floor and mineral soil in all three harvested treatments had lower moisture content than the uncut forest (Fig. 4.1). The effect of harvesting on soil moisture, however, was more pronounced in the forest floor than in the mineral soil. Within the aggregated retention treatment, moisture content in the forest floor showed a decreasing trend from the edge of retention patches up to 20 m away, but moisture increased at 30-m distance from the patch edge (Fig. 4.2). A decrease in soil moisture was not observed across a distance gradient from residual trees in the dispersed retention treatment (Fig. 4.2). The trend for soil pH was the opposite of soil moisture. Soil pH increased in all three harvested treatments, relative to that in uncut forest (Fig. 4.3). Proximity to retention trees did not affect soil pH in either the aggregated or dispersed retention treatment (Fig. 4.4).

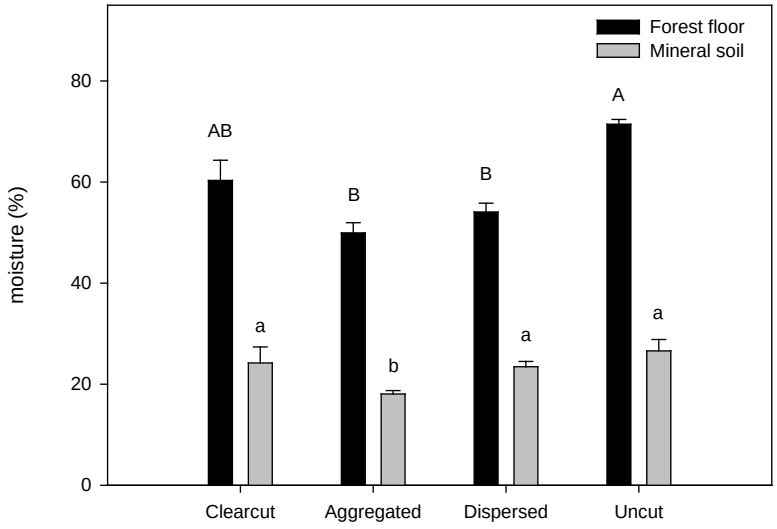

Figure 4.1 Mean moisture content of the forest floor and mineral soil in the clearcut, aggregated retention and dispersed retention treatments, and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different (upper case letters for forest floor, lower case letters for mineral soil).

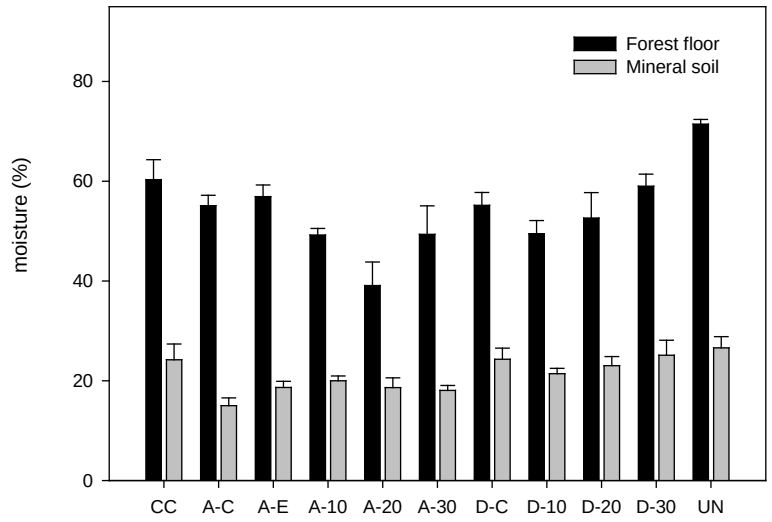

Figure 4.2 Mean moisture content of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between UN vs. A-10, UN vs. A-20, UN vs. A-30, UN vs. D-10, UN vs. D-20, CC vs. A-20, A-E vs. A-20 and D-30 vs. A-20 in the forest floor, and UN vs. A-C and D-30 vs. A-C in the mineral soil.

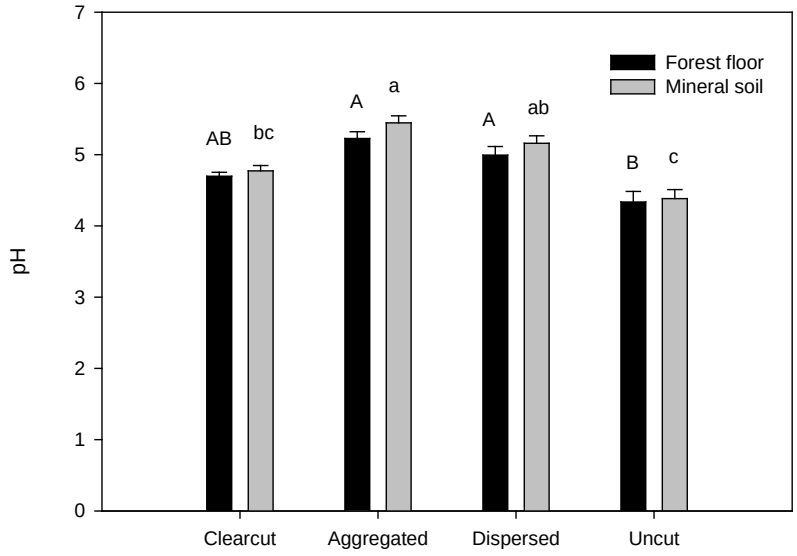

Figure 4.3 Mean pH of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different (upper case letters for forest floor, lower case letters for mineral soil).

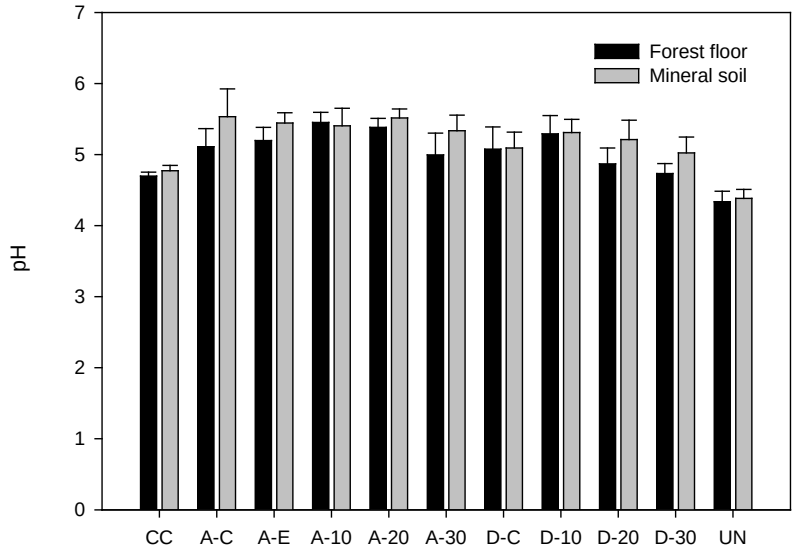

Figure 4.4 Mean pH of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between UN vs. A-10 and UN vs. A-20 in the forest floor, and UN vs. A-C and UN vs. A-20 in the mineral soil.

4.2 Nutrient availability

Comparing all four treatments in both soil layers, nitrate (NO_3^-) and ammonium (NH_4^+) availabilities were lowest in aggregated retention treatment (Fig. 4.5 and 4.7). Forest floor NH_4^+ availability was significantly higher in the uncut forest than in any of the harvested treatments. Within the aggregated retention treatment, NO_3^- and NH_4^+ availabilities in both soil layers were maintained across the distance from the centre of the retention patch up to 30 m away from the edge of the patch (Fig. 4.6 and 4.8). However, fluctuations in the availabilities of these nutrients were observed in the dispersed retention treatment (Fig. 4.6 and 4.8).

All three types of harvesting treatments increased the availability of orthophosphate (PO_4^{3-}), although the increase was not statistically significant (Fig. 4.9). Sampling locations farther away from residual trees in the dispersed retention treatment tended to have higher PO_4^{3-} availability in both soil layers than those closer to trees (Fig. 4.10). Proximity to retention trees did not seem to affect PO_4^{3-} availability in the forest floor of the aggregated retention treatment, although PO_4^{3-} availability decreased substantially from the centre to the edge of the retention patch in the mineral soil (Fig. 4.10).

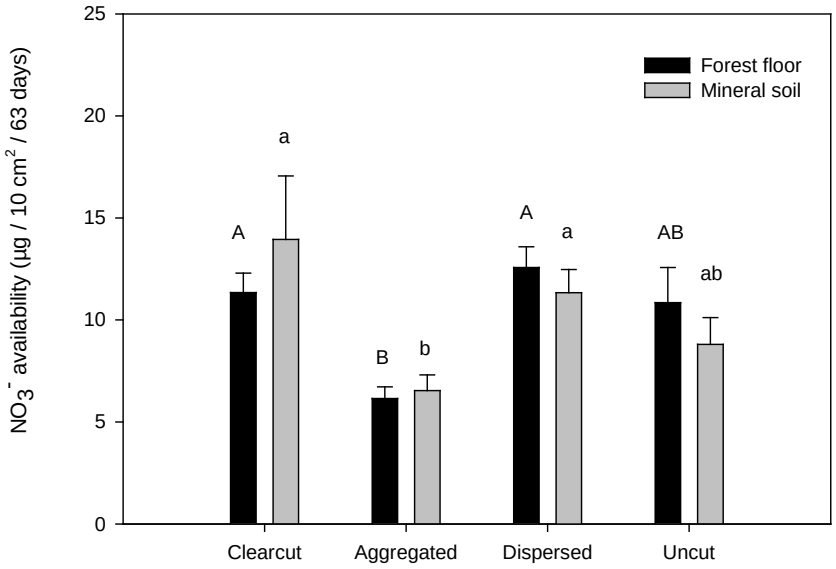

Figure 4.5 Mean NO_3^- availability of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different (upper case letters for forest floor, lower case letters for mineral soil).

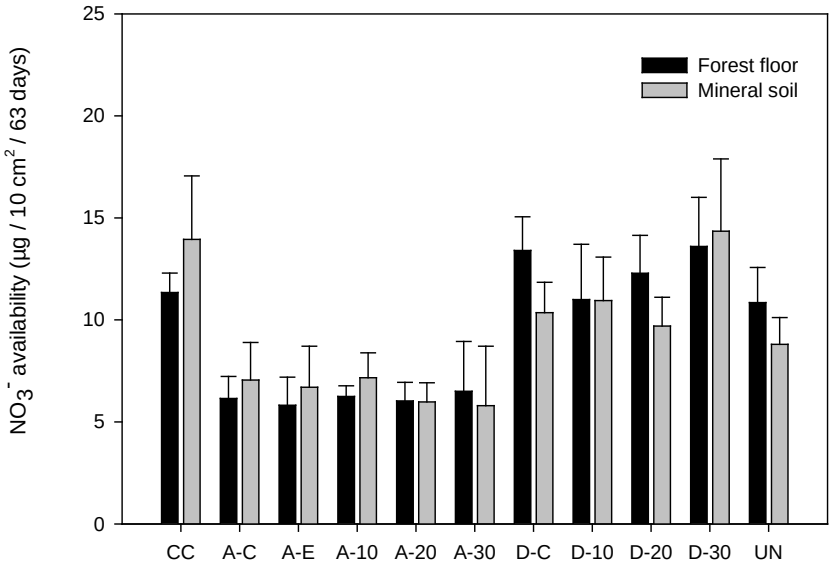

Figure 4.6 NO_3^- availability of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different among sampling locations in either soil layer.

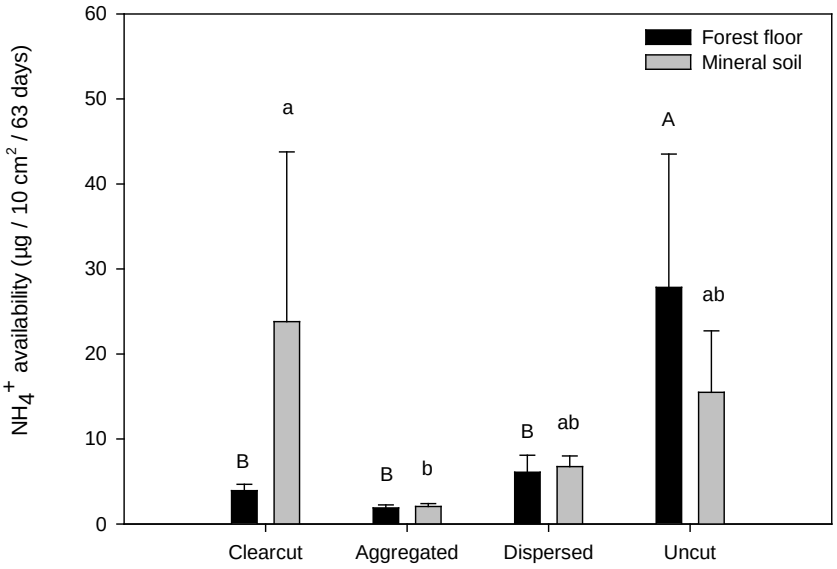

Figure 4.7 NH_4^+ availability of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different (upper case letters for forest floor, lower case letters for mineral soil).

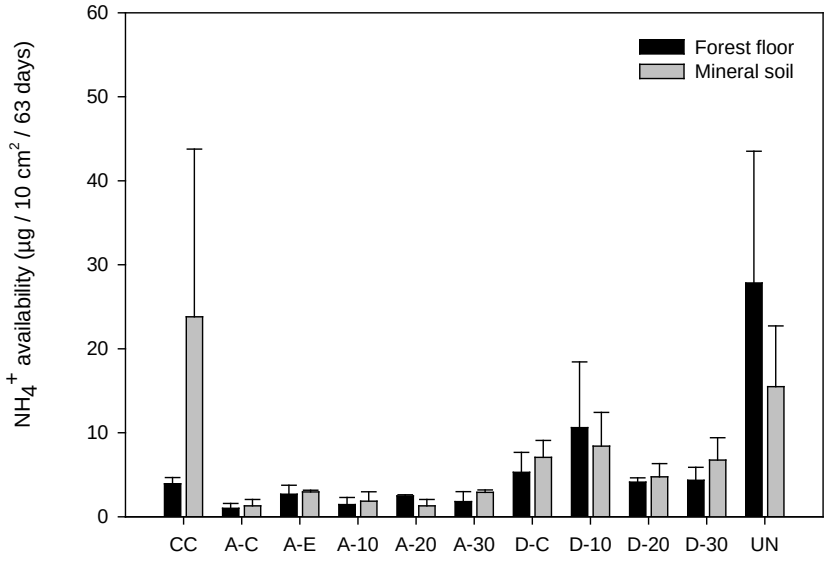

Figure 4.8 NH_4^+ availability of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between A-C vs. UN and A-10 vs. UN in the forest floor, but were not significantly different among sampling locations in the mineral soil.

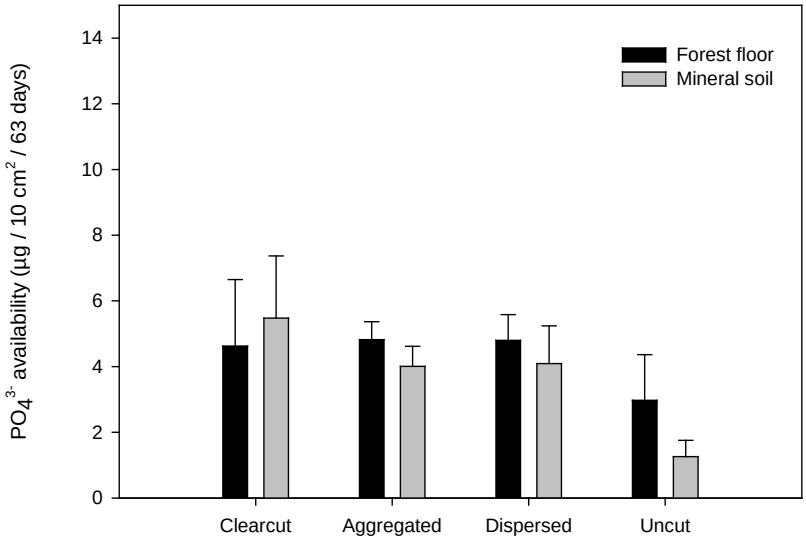

Figure 4.9 PO_4^{3-} availability of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

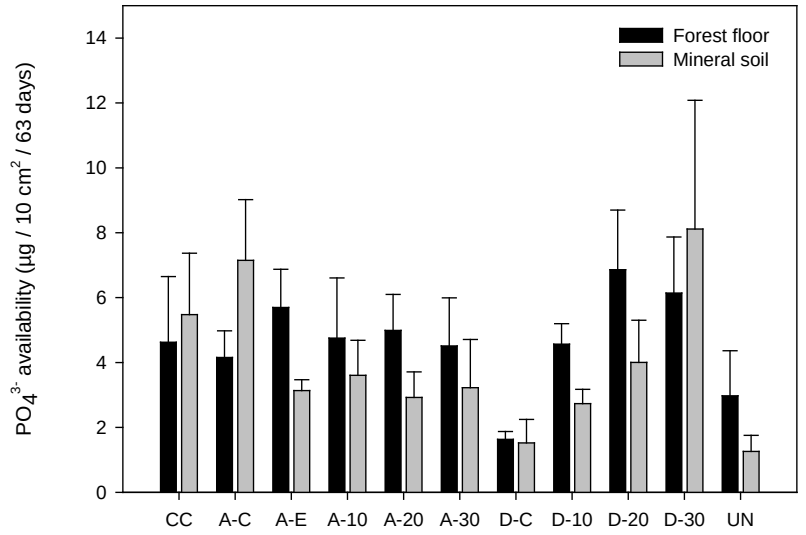

Figure 4.10 PO_4^{3-} availability of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different) in either soil layer.

The availabilities of sulphur (S), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), and aluminum (Al) were not significantly different among the four treatments in either soil layers (Table 4.1). However, all types of harvesting reduced calcium (Ca) and magnesium (Mg) availabilities and increased potassium (K) availability in both soil layers (Table 4.1).

Table 4.1 Nutrient availability in the forest floor (FF) and mineral soil (Min) at the aggregated retention, dispersed retention, clearcut, and uncut forest.

		Clearcut	Aggregated retention	Dispersed retention	Uncut
Macronutrients (µg/10cm^2/63days)					
Ca	FF	556.77 (74.55)a	493.17 (43.95)a	654.21 (39.54)a	943.23 (123.66)b
	Min	923.85 (93.94)b	554.76 (34.80)a	787.43 (73.81)b	1049.37 (144.87)b
Mg	FF	151.33 (22.93)a	116.89 (9.36)a	148.07 (9.79)a	234.76 (30.18)b
	Min	229.32 (11.35)bc	138.53 (9.07)a	184.06 (15.29)b	265.17 (32.90)c
K	FF	344.79 (61.28)a	258.96 (19.05)ab	307.27 (22.83)a	171 (16.10)b
	Min	388.25 (56.80)a	312.27 (19.82)a	338.55 (26.08)a	141.15 (30.46)b
S	FF	15.68(2.84)a	30.45 (2.81)a	31.84 (3.34)a	28.01 (4.44)a
	Min	20.27 (2.59)a	45.45 (4.60)a	42.92 (4.33)a	35.02 (4.50)a
Micronutrients (µg/10cm^2/63days)					
Fe	FF	2.22 (0.49)a	3.32 (0.32)a	3.83 (0.36)a	3.57 (0.69)a
	Min	3.95 (0.87)a	4.75 (0.49)a	5.25 (0.32)a	4.53 (1.04)a
Mn	FF	10.38 (1.70)a	23.24 (4.49)a	23.13 (5.78)a	9.48 (2.34)a
	Min	16.01 (1.97)a	23.99 (3.96)a	23.81 (10.05)a	15.05 (5.47)a
Cu	FF	0 (0)a	0.06 (0.02)a	0.02 (0.02)a	0 (0)a
	Min	0.60 (0.60)a	0.11 (0.03)a	0.06 (0.028)a	0 (0)a
Zn	FF	1.28 (0.18)a	1.54 (0.10)a	1.86 (0.20)a	1.75 (0.34)a
	Min	2.03 (0.15)a	1.85 (0.11)a	2.22 (0.19)a	2.51 (0.43)a
B	FF	0.59 (0.10)a	0.94(0.06)a	0.85 (0.11)a	0.71 (0.11)a
	Min	0.51 (0.10)a	0.81 (0.10)a	0.72 (0.059)a	0.51 (0.08)a
Pb	FF	0 (0)b	0.62 (0.05)a	0.19 (0.04)b	0.35 (0.15)ab
	Min	0.12 (0.07)a	0.32 (0.07)a	0.23 (0.05)a	0.45(0.19)a
Al	FF	41.68 (3.04)a	47.61 (2.30)a	48.33(1.90)a	51.57 (4.46)a
	Min	52.38 (3.24)a	53.31 (1.89)a	58.28 (2.22)a	63.44 (6.23)a

Note: Values are means (n=20 for aggregated retention, n=16 for dispersed retention, and n=4 each for clearcut and uncut) with one standard error given in parentheses. Means with the same letter were not significantly different ($p<0.0083$ (=0.05/6)) (comparison is between harvest types in each soil layer).

4.3 Substrate-induced respiration (SIR)

Substrate-induced respiration (SIR) profiles of the forest floor and mineral soil showed a general similarity among the four treatments (Fig. 4.11). Multivariate (MRPP) analysis of the SIR data also indicated that the four treatments were not significantly different from each other in either the forest floor or mineral soil (A=0.0213, p=0.1352 in forest floor, A=-0.0096, p=0.6827 in mineral soil, A=-0.0009, p=0.4761 when both soil layers were combined). Further, proximity to retention trees or tree patches did not influence the SIR rates for the five carbon substrates in either the aggregated retention treatment (A=0.04183712, p=0.1912 in forest floor, A= -0.0756, p=0.9674 in mineral soil, A=-0.0233, p=0.8563 when both soil layers were combined) or the dispersed retention treatment (A=-0.0519, p=0.7978 in forest floor, A=-0.0487, p=0.8427 in mineral soil, A=-0.0326, p=0.8985 when both soil layers were combined).

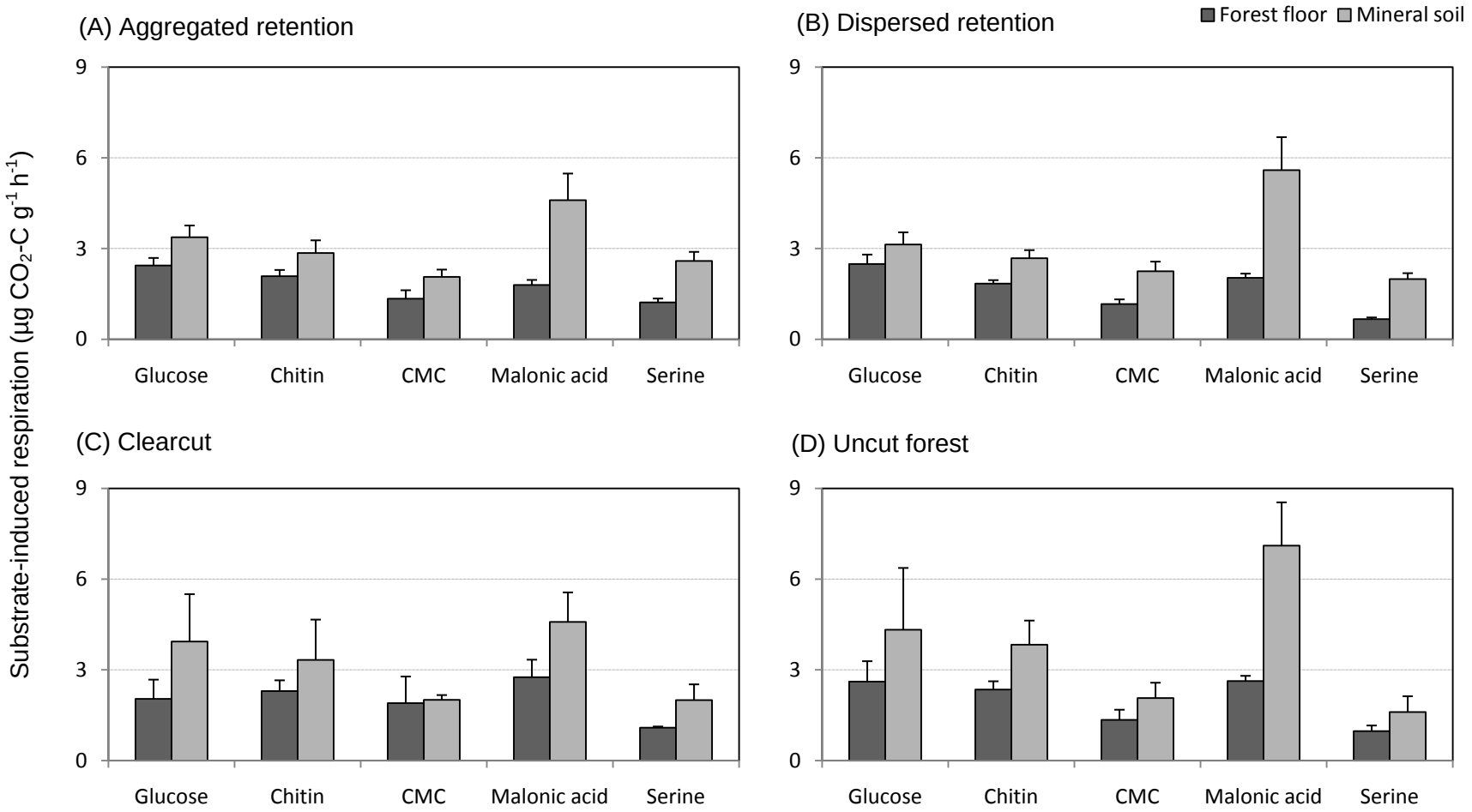

Figure 4.11 Substrate-induced respiration (SIR) profiles in the forest floor and mineral soil in the aggregated retention (A), dispersed retention (B), clearcut (C), and uncut control (D) treatments. Values are means (n=20 for A, n=16 for B, and n=4 for C and D), error bars represent one standard error. CMC: carboxymethyl cellulose.

NMS ordination of the SIR data from both forest floor and mineral soil of all four treatments showed no clustering on the ordination plot (final stress 13.00095 of and final instability 0.00001 after 74 iterations) (Fig. 4.12). Soil moisture explained 31.9% of the variance in axis 2.

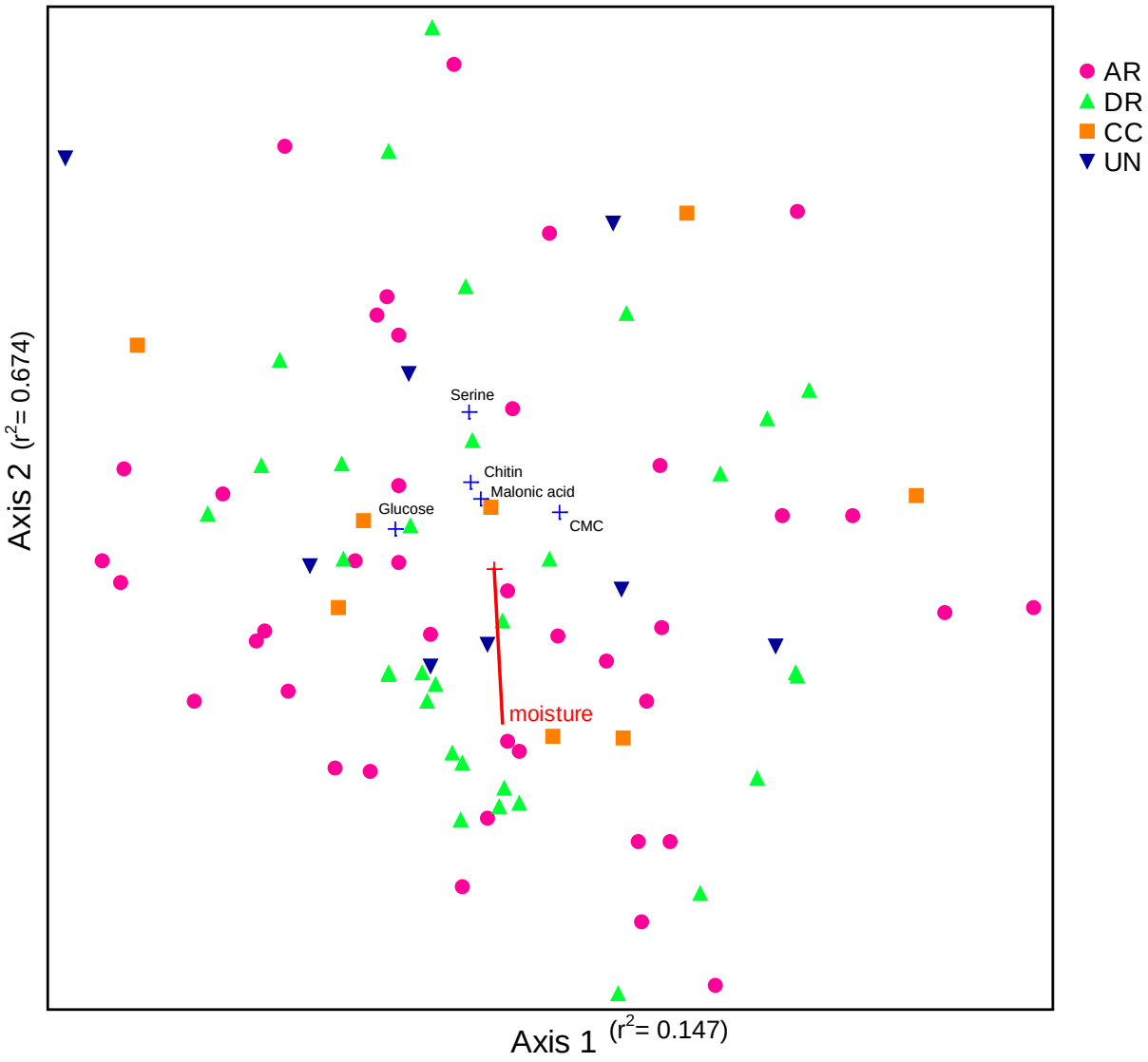

Figure 4.12 NMS ordination of the metabolic diversity profiles from both forest floor and mineral soil layers at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

Similarly, NMS ordination of the SIR data from the forest floor alone from all four treatments showed no clustering on the ordination plot (final stress 10.82634 and final instability of 0.00001 after 69 iterations) (Fig. 4.13). None of the environmental variables was strongly correlated with the ordination axes.

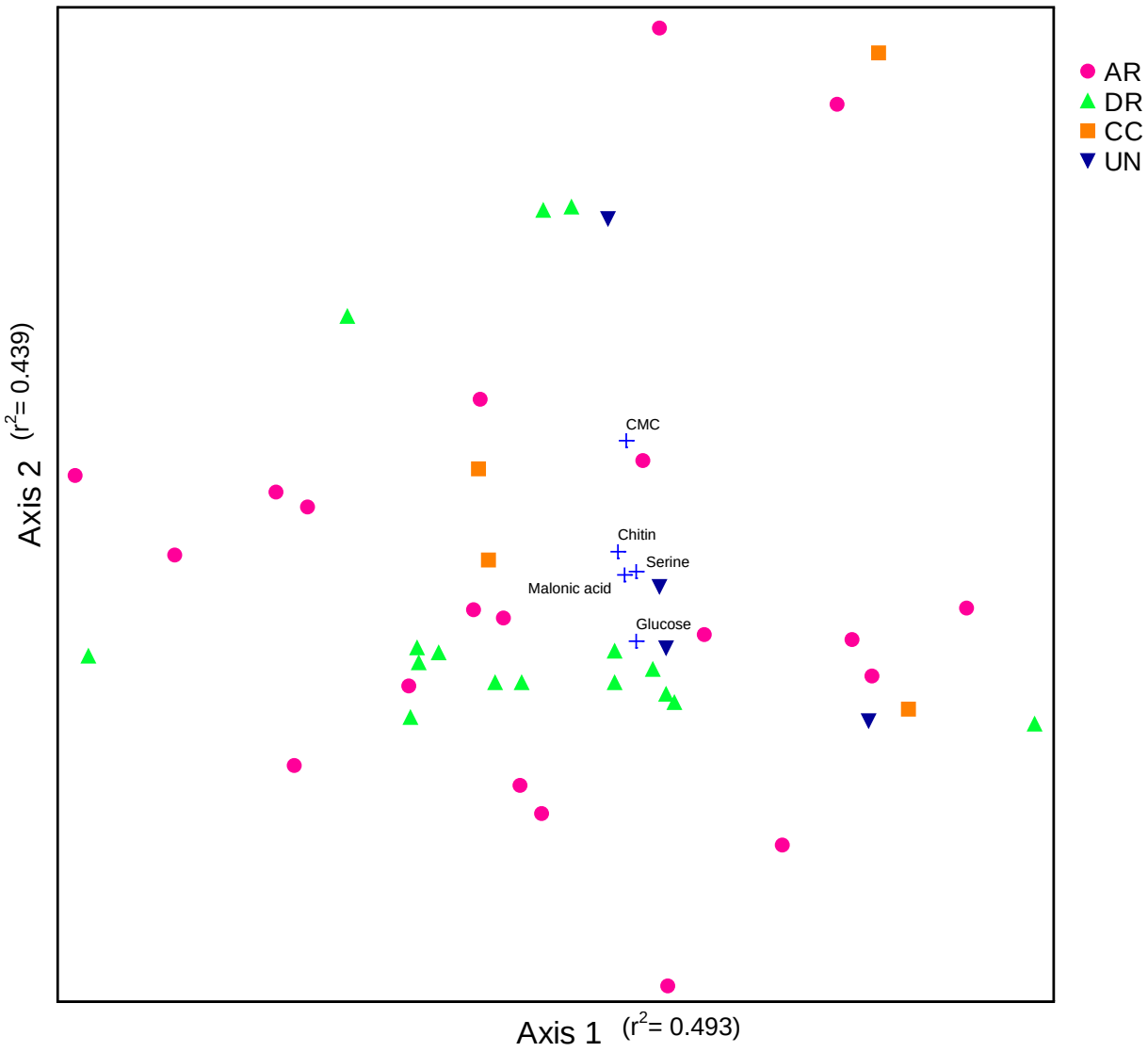

Figure 4.13 NMS ordination of the metabolic diversity profiles from the forest floor at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

NMS ordination of the SIR data from the mineral soil alone from all four treatments also showed no clustering on the ordination plot (final stress 9.58494 and final instability of 0.00001 after 115 iterations) (Fig. 4.14). The availability Fe explained 21.9% of the variance in axis 2.

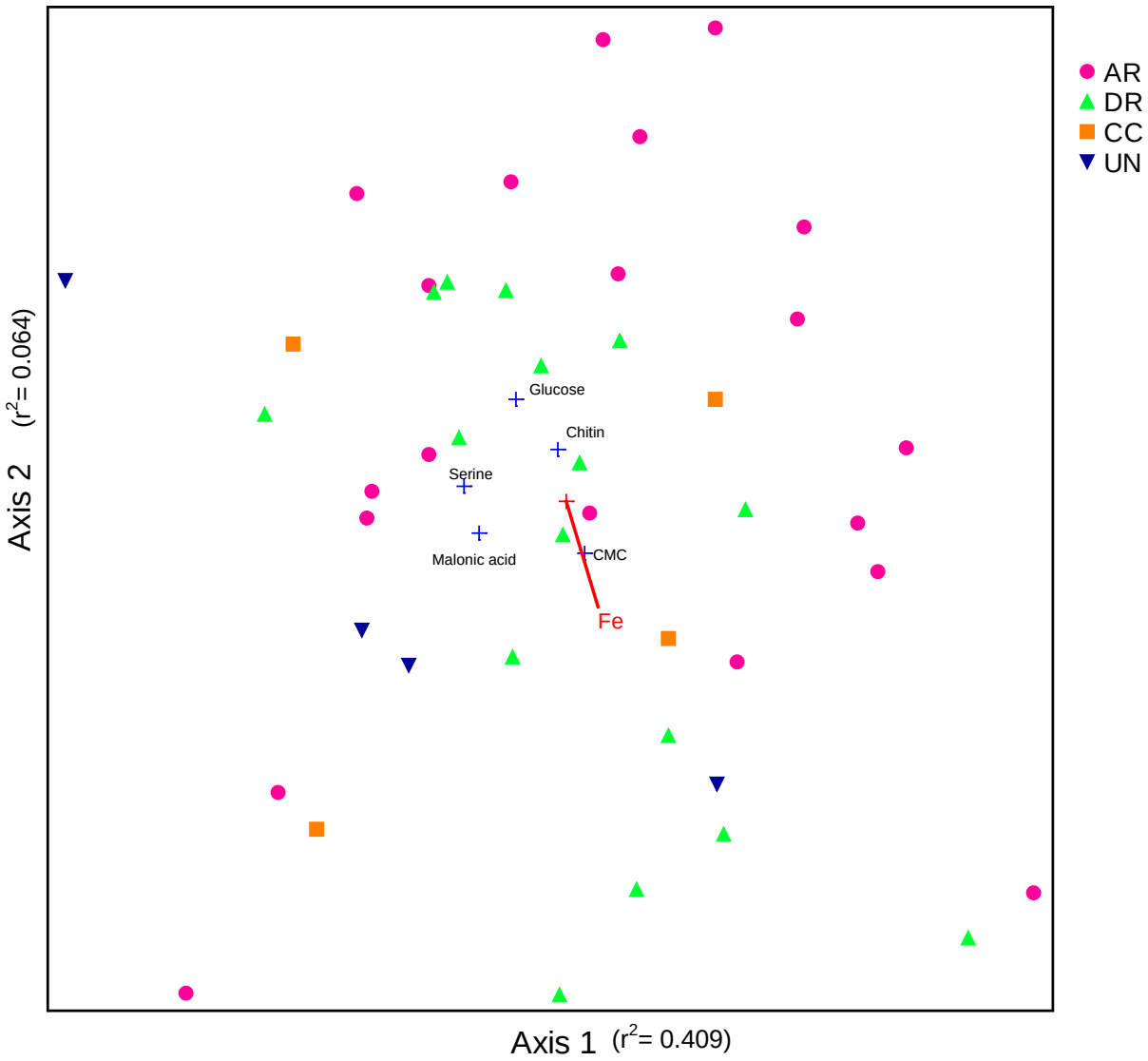

Figure 4.14 NMS ordination of the metabolic diversity profiles from the mineral soil at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

When the SIR rates for each of the five carbon sources were analysed separately using ANOVA, no significant differences were observed among the four treatments, except for serine (Fig. 4.15, 4.17, 4.19, 4.21, and 4.23).The respiration rate for serine in the aggregated retention treatment was significantly higher than in the dispersed retention treatment (Fig. 4.23). Proximity to retention trees in the aggregated and dispersed retention treatments did not significantly affect SIR rates for any carbon source (Fig. 4.16, 4.18, 4.20, 4.22, and 4.24).

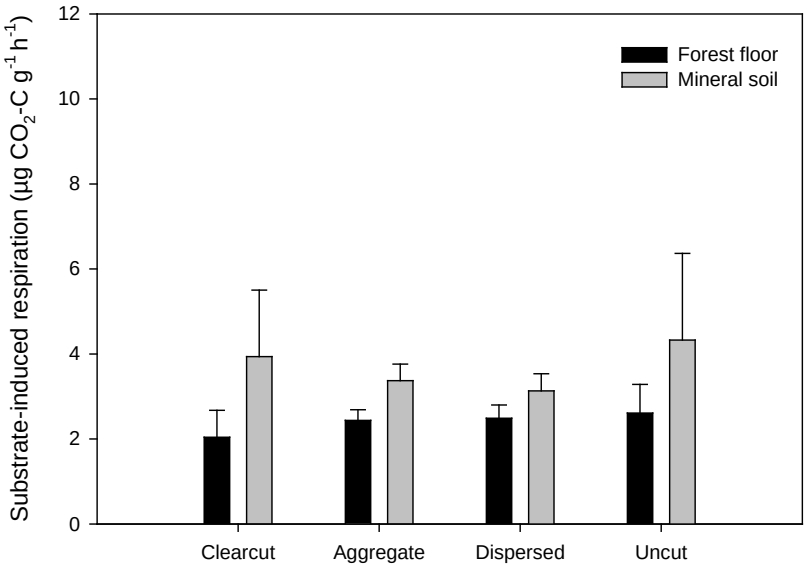

Figure 4.15 Glucose SIR rates of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

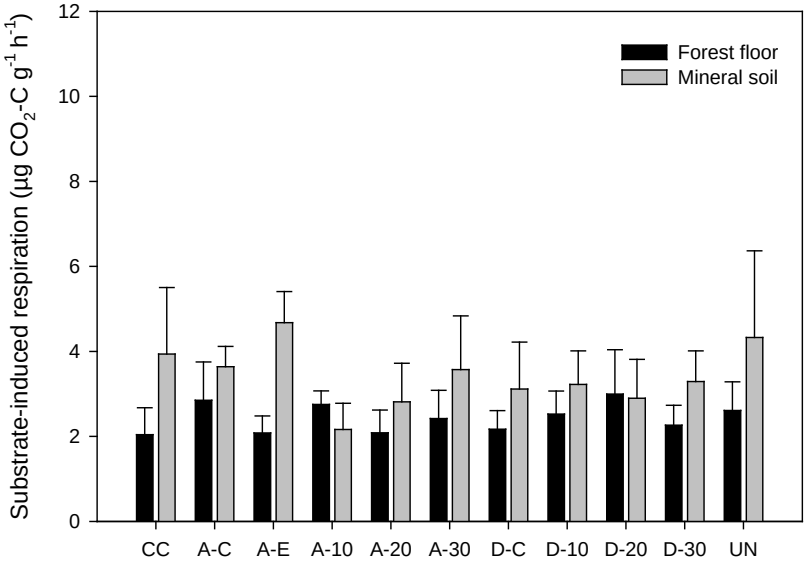

Figure 4.16 Glucose SIR rates of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

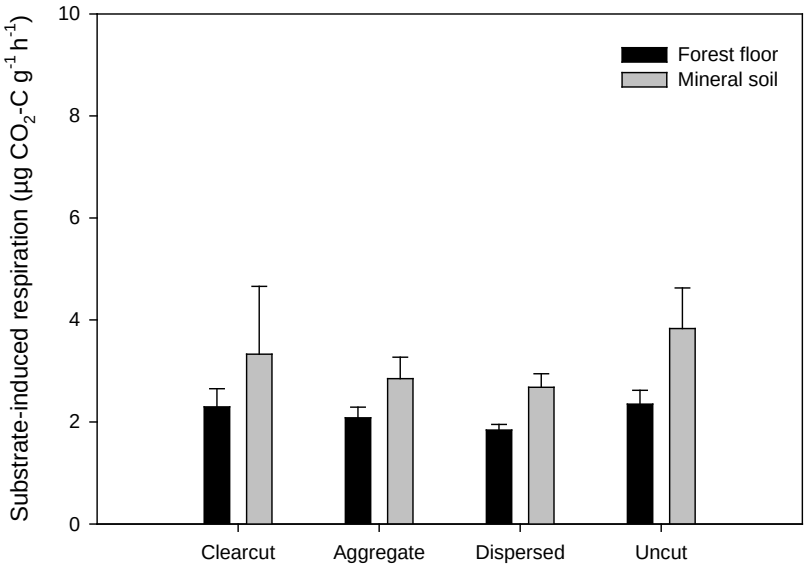

Figure 4.17 Chitin SIR rates of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

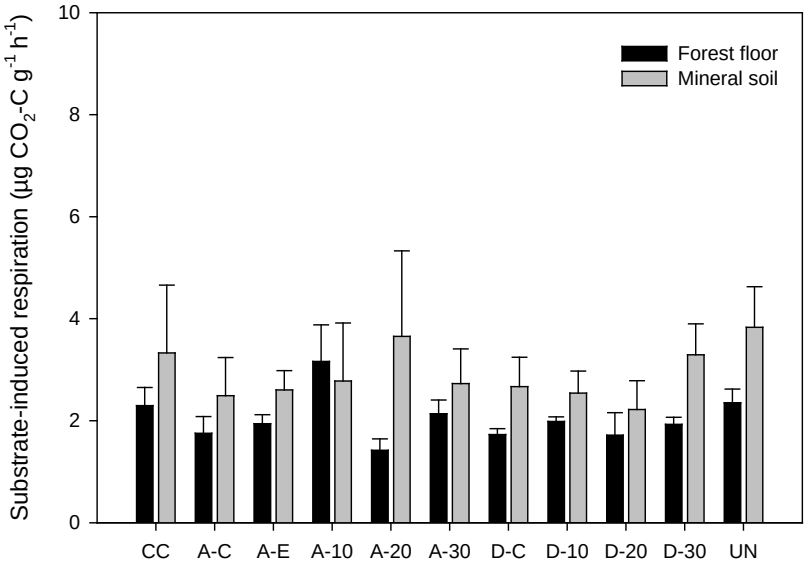

Figure 4.18 Chitin SIR rates of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

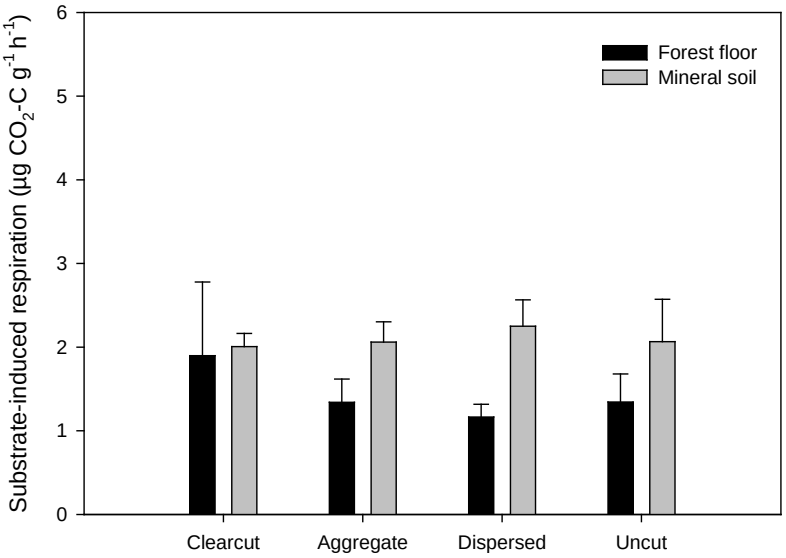

Figure 4.19 Carboxymethyl cellulose (CMC) SIR rates of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

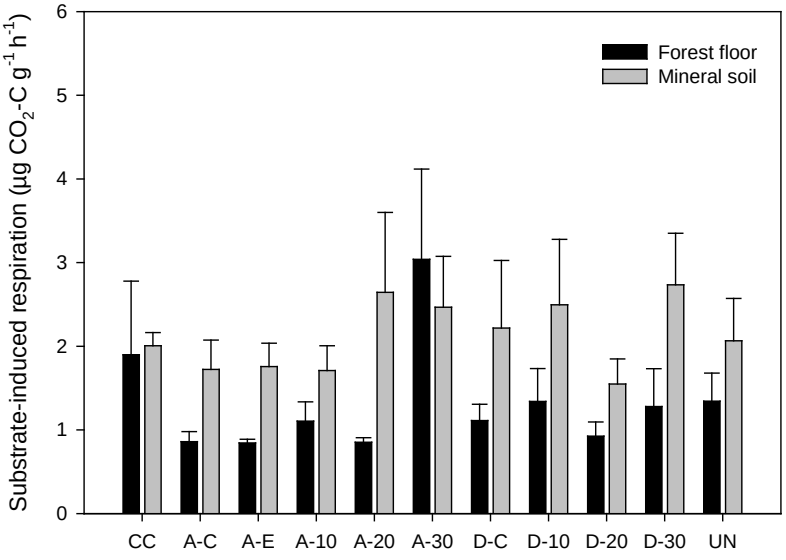

Figure 4.20 Carboxymethyl cellulose (CMC) SIR rates of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

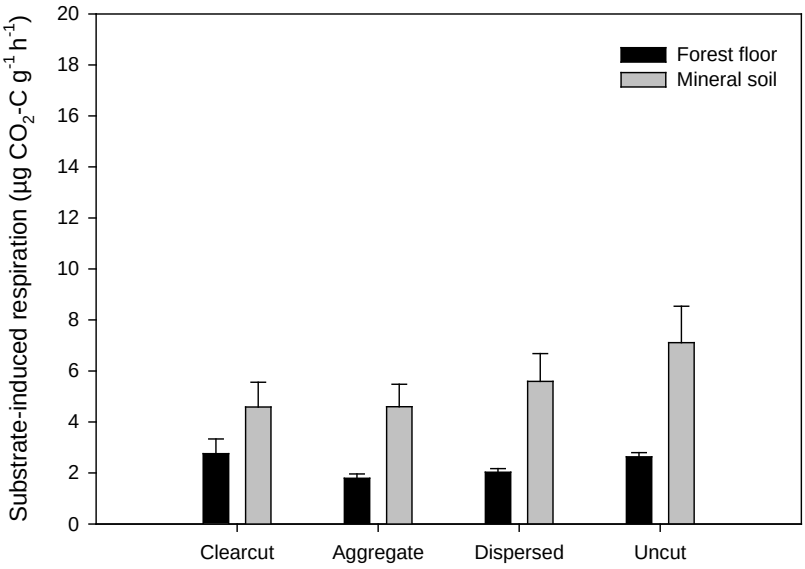

Figure 4.21 Malonic acid SIR rates of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

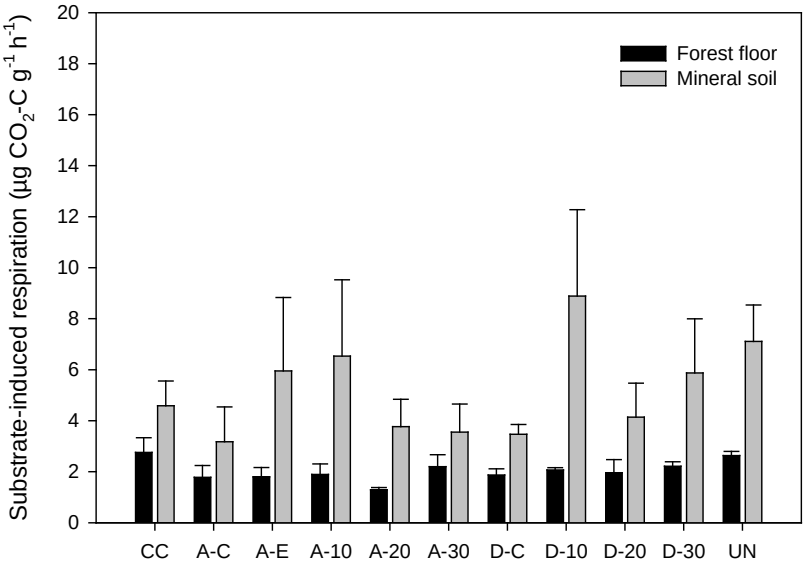

Figure 4.22 Malonic acid SIR rates of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

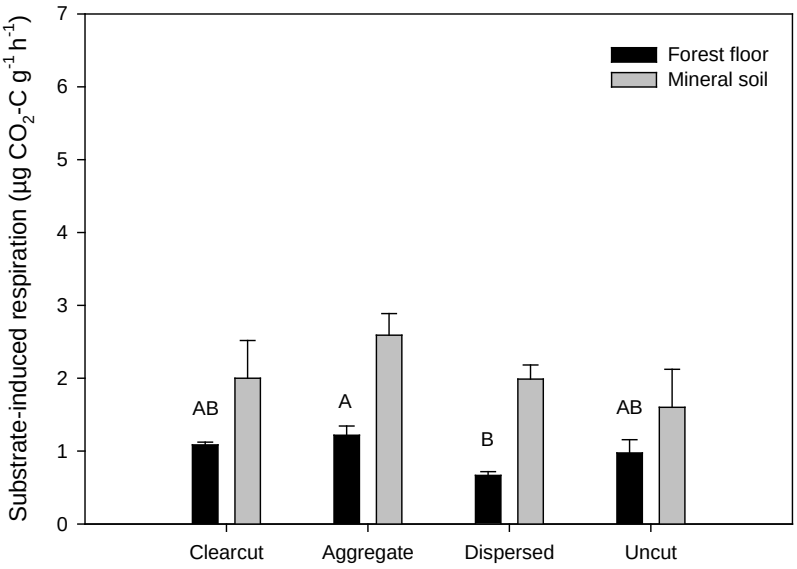

Figure 4.23 Serine SIR rates of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different. Means were not significantly different in the mineral soil.

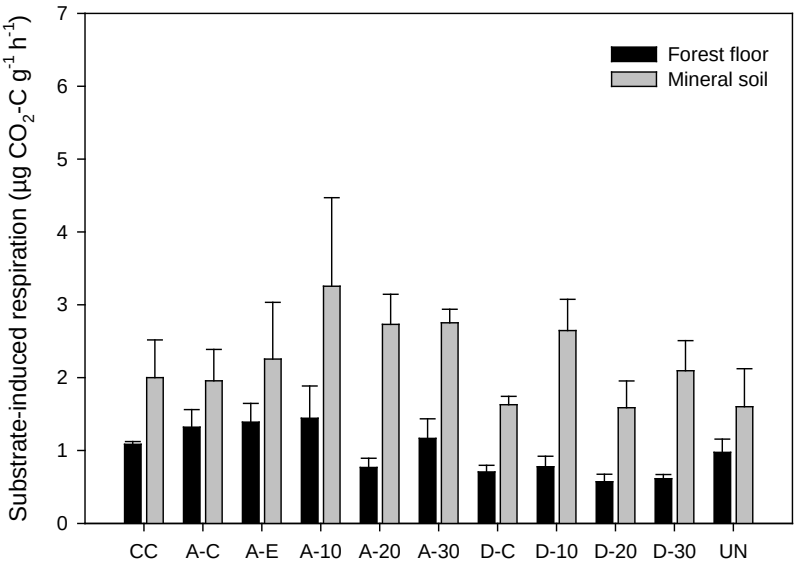

Figure 4.24 Serine SIR rates of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

4.4 Enzyme activities

There were no significant differences in the activities of all five enzymes among the four treatments, according to multivariate (MRPP) analyses (A=0.0831, p=0.0035 in forest floor, A=0.0464, p=0.0277 in mineral soil, A=0.0148, p=0.1177 when both soil layers were combined). In the aggregated retention treatment, the effects of proximity to trees on enzyme activities were only observed in the mineral soil layer (A=0.1781, p=0.0029), but not in the forest floor (A=0.0974, p=0.0522) or when both soil layers were combined (A=0.0180, p=0.2369). In the dispersed retention treatment, proximity to trees did not affect enzyme activities (A=-0.1068, p=0.9737 in forest floor, A=-0.0413, p=0.7109 in mineral soil, A=-0.0570, p=0.9894 when both soil layers were combined). Although A-values were noticeably high (>0.1) between pairs of sampling locations in the aggregated retention treatment, none of the p-values indicated a significant difference (Table 4.2).

Table 4.2 Pair-wise MRPP comparison of mineral soil enzyme activities among different proximities to tree patches in the aggregated retention treatment. An asterisk (*) indicates A>0.1, and values in bold indicate p<0.005 (=0.05/10).

		Centre	Edge	10 m	20 m	30 m
Centre	A-value	1.0000	0.1072*	0.1200*	0.1909*	0.1774*
	p-value	1.0000	0.1118	0.0096	0.0096	0.0214
Edge	A-value		1.0000	0.1687*	0.2586*	0.2736*
	p-value		1.0000	0.0314	0.0282	0.0358
10 m	A-value			1.0000	-0.0571	0.0449
	p-value			1.0000	0.9590	0.1384
20 m	A-value				1.0000	0.0409
	p-value				1.0000	0.1873
30 m	A-value					1.0000
	p-value					1.0000

NMS ordination of the enzyme activity data from both forest floor and mineral soil of all four treatments showed no tight clustering on the ordination plot (final stress of 11.15915 and final instability of 0.00001 after 96 iterations) (Fig. 4.25). Soil moisture explained 65.3% and 47.1% of the variances in axes 1 and 2, respectively, whereas the availability of Al explained 23.1% of the variance in axis 1.

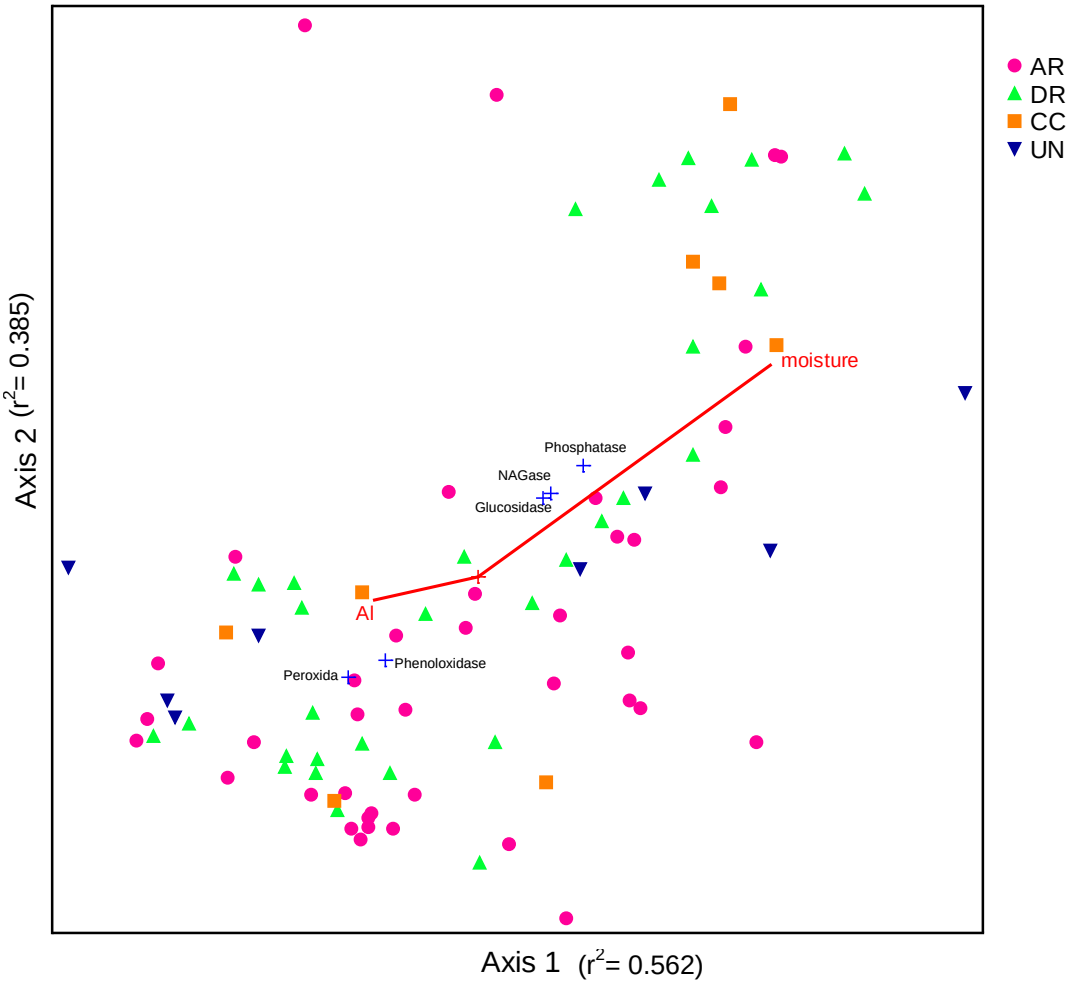

Figure 4.25 NMS ordination of enzyme activities from the forest floor and mineral soil at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

When considering the enzyme activities from the forest floor alone, samples from the clearcut treatment clustered together, whilst those from the other three treatments were scattered across the plot (final stress of 12.47587 and final instability of 0.00001 after 49 iterations) (Fig. 4.26). Soil moisture explained 26.4% of the variance in axis 2.

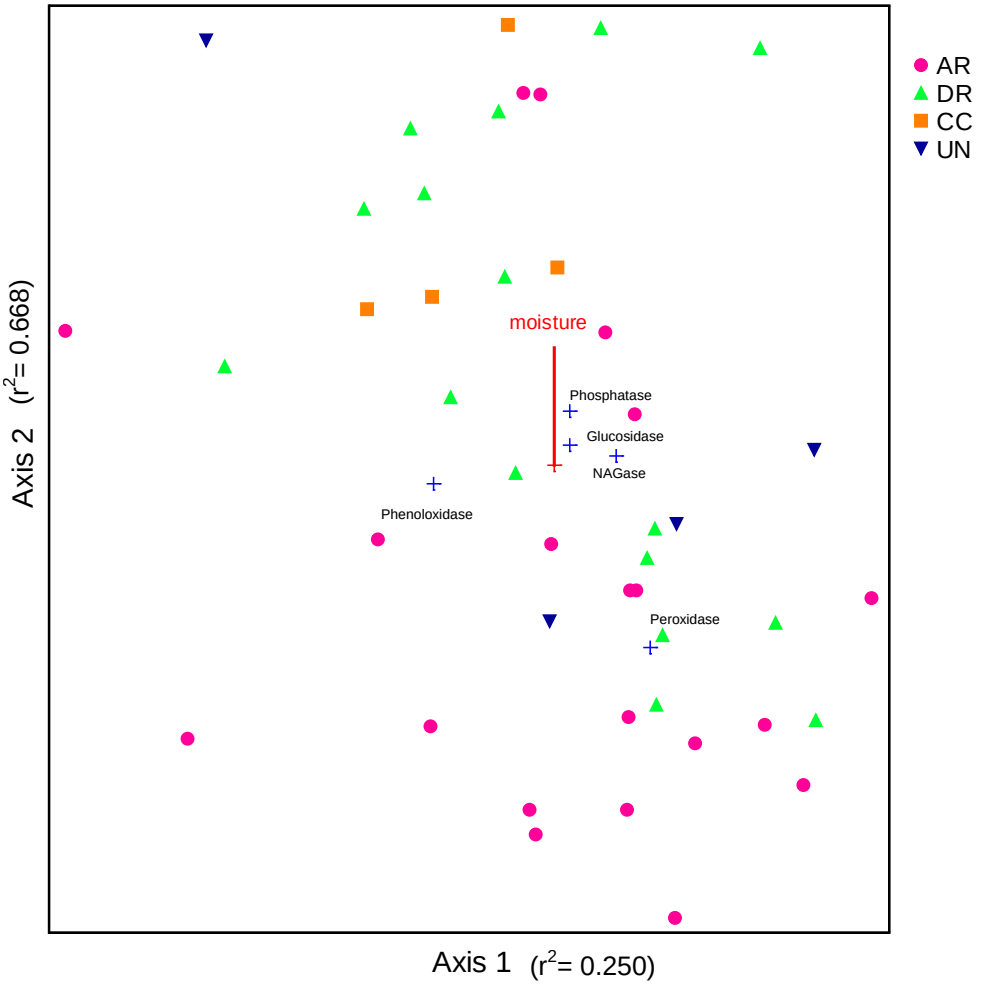

Figure 4.26 NMS ordination of enzyme activities from the forest floor at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

Attempts to perform NMS ordination on mineral soil enzyme activities were unsuccessful. A 1-D solution was produced showing the ordination points ranked on one axis according to the sample name sequence.

When the activities of each of the five enzymes were analysed separately using ANOVA, significant differences among the four treatments were observed. Although the activities of β-glucosidase and N-acetyl-glucosaminidase (NAGase) were not significantly different among the four treatments (Fig. 4.27 and 4.29) there were noticeable spatial location effects. β-glucosidase activity showed a decreasing trend with distance from the centre of tree patches in the aggregated retention treatment up to 20 m away from the patch edge in the forest floor, and up to 30 m from the patch edge in the mineral soil (Fig. 4.28). In the dispersed retention treatment, β-glucosidase activity in the forest floor was maintained at similar levels from the residual trees out into the opening, but tended to increase with distance from the residual trees in the mineral soil (Fig. 4.28). Similar to the pattern observed for β-glucosidase, NAGase activity showed a decreasing trend up to 30 m away from the patch edge in the aggregated retention treatment (Fig. 4.30). In the dispersed retention treatment, forest floor NAGase activity increased from 10 m to 30 m away from the residual trees out into the opening, but mineral soil NAGase activity remained similar across the harvested site (Fig. 4.30). In the forest floor phosphatase activity was highest in the uncut forest and lowest in the aggregated retention treatment, whilst in the mineral soil it was highest in the clearcut and lowest in the aggregated retention treatment (Fig. 4.31). In the aggregated retention treatment, phosphatase activity exhibited a decreasing trend from the centre of retention tree patches up to 30 m away from the patch edge out into the opening in both soil layers (Fig. 4.32). In the dispersed retention treatment, phosphatase activity was maintained at similar levels across the opening in both soil layers (Fig. 4.32).

Unlike the activities of hydrolytic enzymes (i.e. β-glucosidase, NAGase, and phosphatase), the activities of oxidative enzymes (i.e. phenol oxidase and peroxidase) were generally higher in the

mineral soil than in the forest floor. In the forest floor, phenol oxidase activity was highest in the uncut forest, whilst in the mineral soil it was highest in the clearcut (Fig. 4.33). Forest floor phenol oxidase activity fluctuated more in the aggregated retention treatment than in the dispersed retention treatment (Fig. 4.34). Forest floor peroxidase activity in the aggregated and dispersed retention treatments was more similar to the level found in the uncut forest than in the clearcut, whilst mineral soil peroxidase activity in the aggregated and dispersed retention treatments was more similar to the clearcut than the uncut (Fig. 4.35). These differences, however, were not statistically significant. In the aggregated retention treatment, a decrease in peroxidase activity was observed from the centre of the retention tree patches up to the patch edge in the forest floor, and from the patch edge to a distance of 10 m from the patch edge in the mineral soil (Fig. 4.36). Peroxidase activity in the dispersed retention treatment was more uniform across the harvested sites than in the aggregated retention treatment (Fig. 4.36).

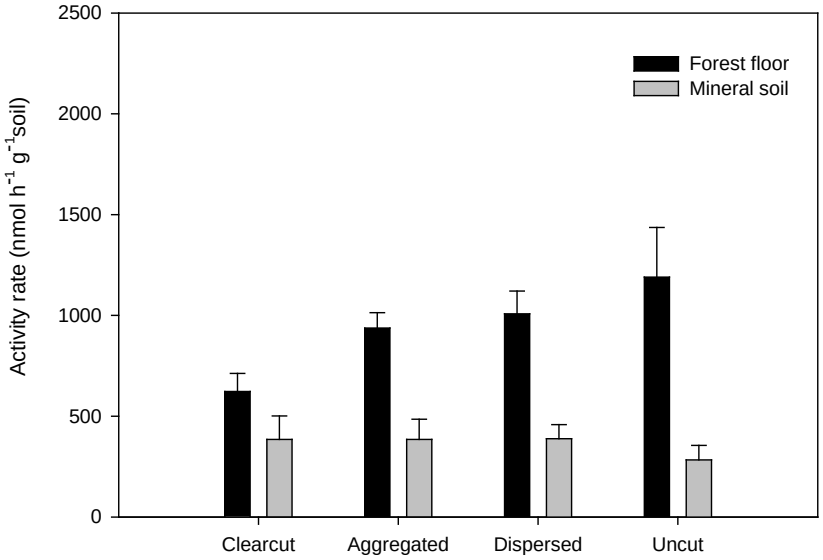

Figure 4.27 β-glucosidase activity of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

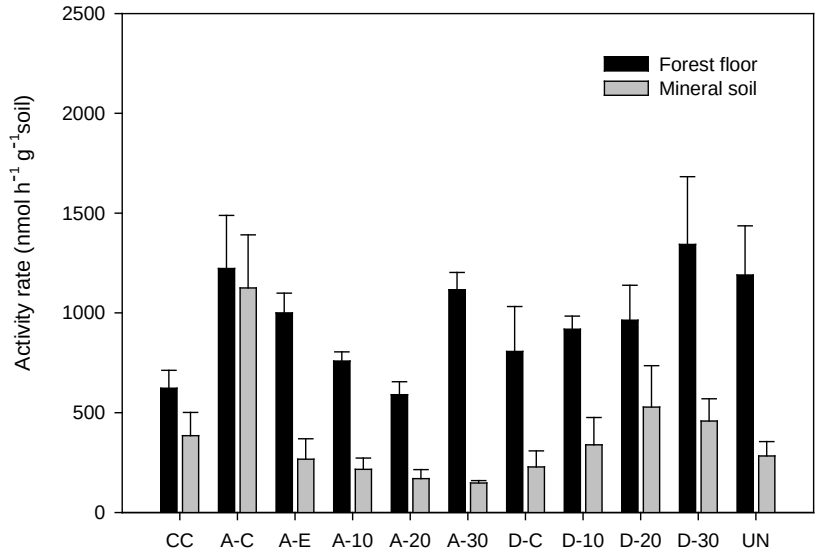

Figure 4.28 β-glucosidase activity of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in the forest floor, but were significantly different between A-C and all other sampling locations except D-20 in the mineral soil.

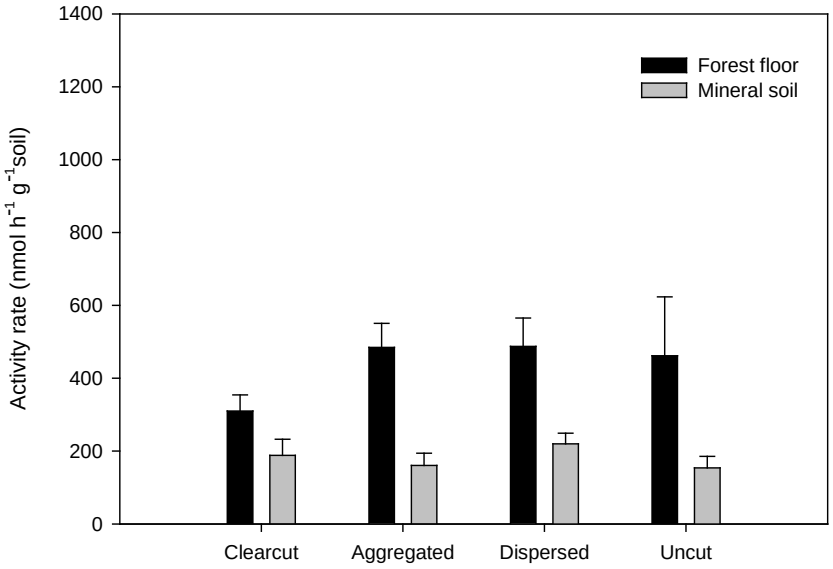

Figure 4.29 NAGase activity of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

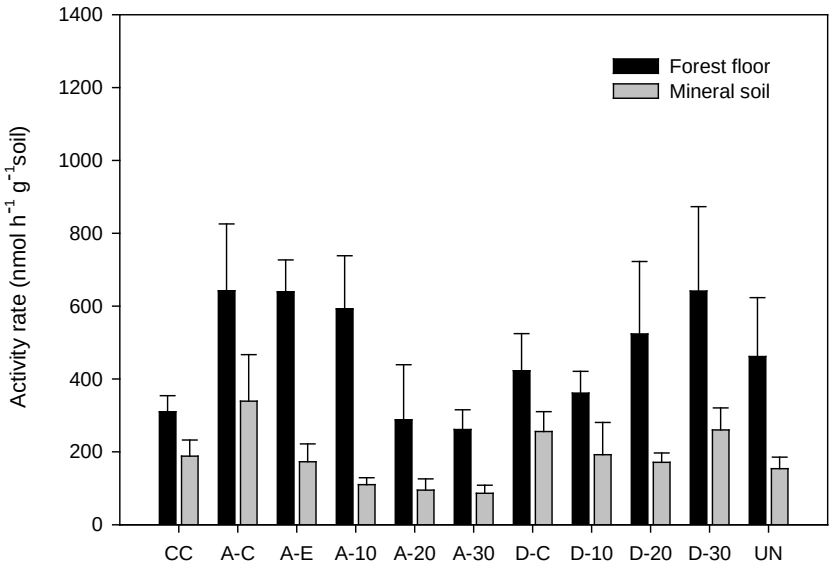

Figure 4.30 NAGase activity of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

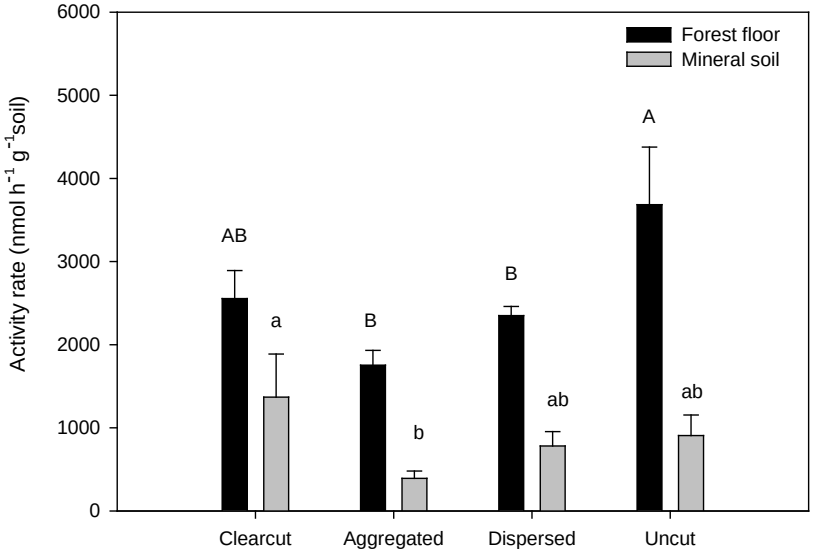

Figure 4.31 Phosphatase activity of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different (upper case letters for forest floor, lower case letters for mineral soil).

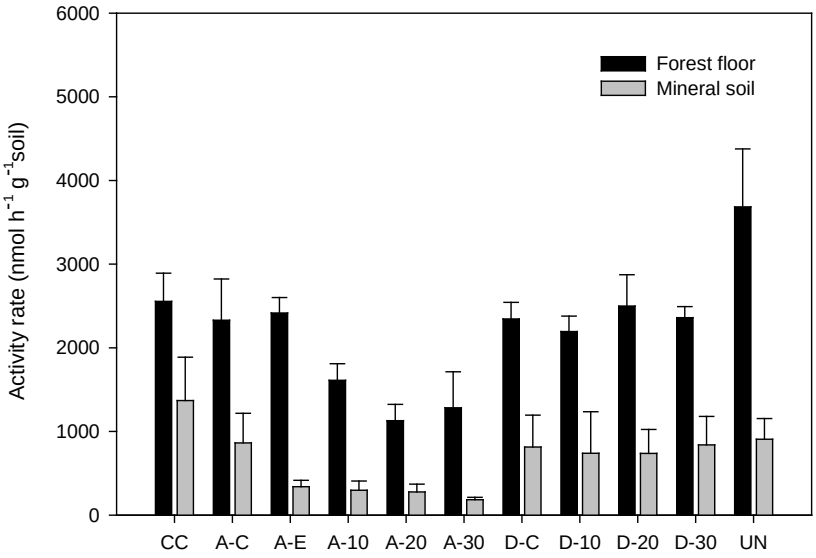

Figure 4.32 Phosphatase activity of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between UN vs. A-10, UN vs. A-20, and UN vs. A-30 in the forest floor, but were not significantly different in the mineral soil.

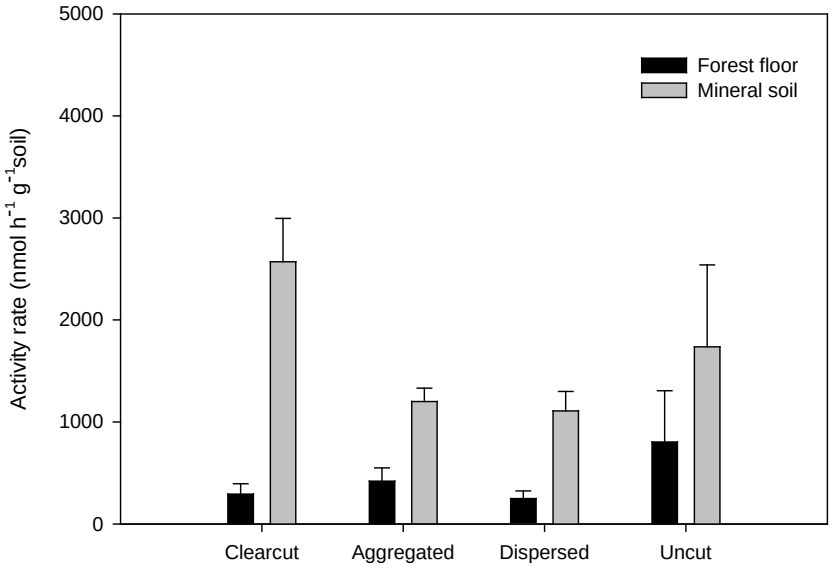

Figure 4.33 Phenol oxidase activity of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

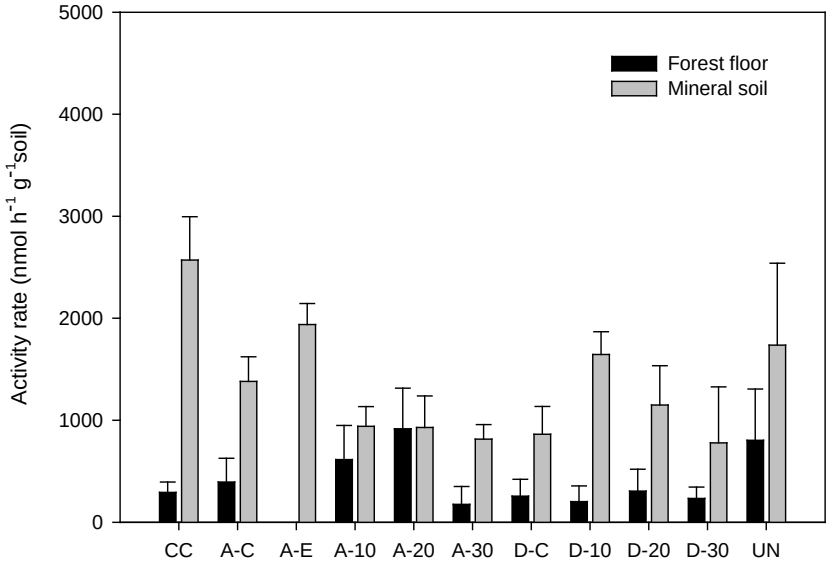

Figure 4.34 Phenol oxidase activity of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

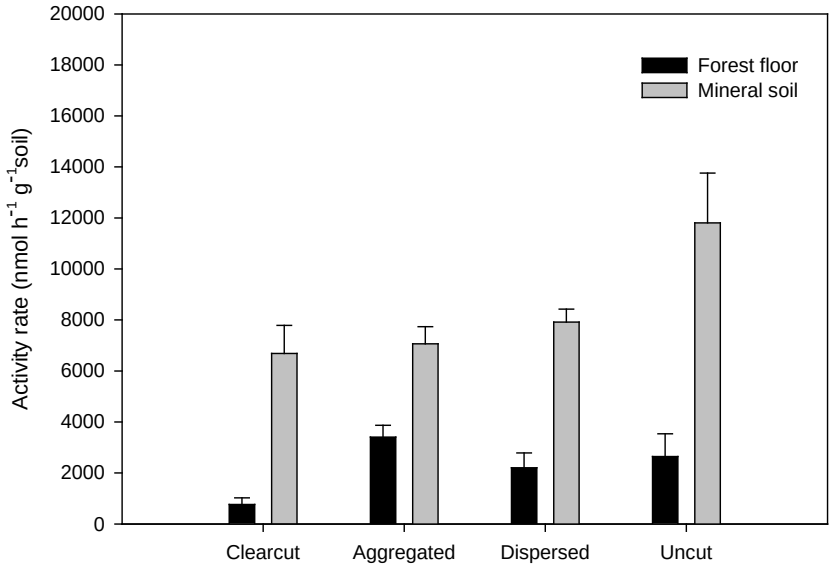

Figure 4.35 Peroxidase activity of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

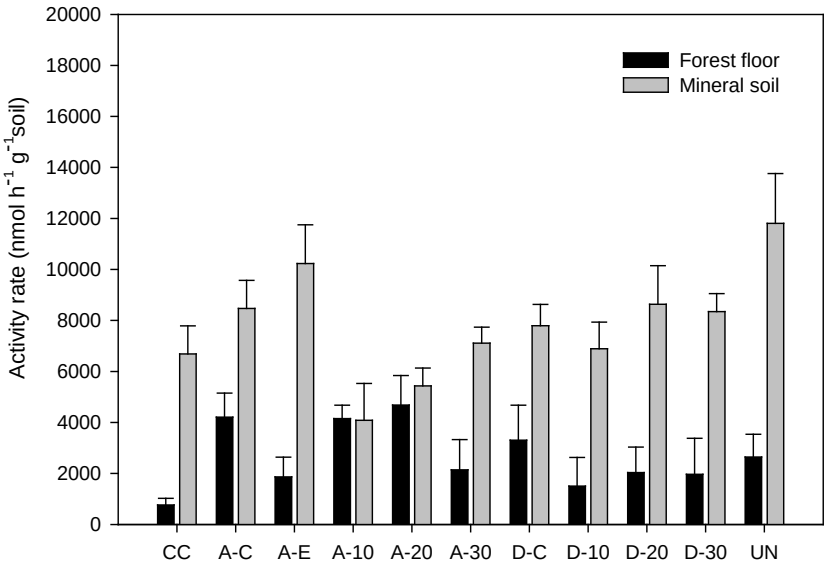

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Figure 4.36 Peroxidase activity of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in the forest floor, but were significantly different between UN vs. A-10 and UN vs. A-20 in the mineral soil.

4.5 Phospholipid fatty acid (PLFA) analysis

4.5.1 PLFA profiles

In general, forest floor and mineral soil PLFA profiles showed a similarity among the four treatments (Fig. 4.37). However, the patterns of certain PLFAs (e.g. 16:0, 16:2ω6,9, 18:1ω9c, and 18:1ω7c) were more similar in the dispersed and the aggregated retention treatments and in the clearcut and uncut control (Fig. 4.37). Multivariate (MRPP) analyses of PLFA profiles showed that harvesting significantly affected soil microbial phospholipid fatty acid composition (A=0.1331, p=0.0000 in the forest floor, A=0.126, p=0.0000 in the mineral soil, and A=0.1097, p=0.0000 when both soil layers were combined). Post-hoc pair-wise comparisons among the four harvest treatments consistently showed no significant difference between the clearcut and uncut control treatments and between the aggregated and dispersed retention treatments (Table 4.3, 4.4, and 4.5). Forest floor and mineral soil PLFA composition was significantly different between the green-tree retention treatments and both clearcut and uncut control (Table 4.4 and 4.5).

Proximity to retention trees did not significantly affect soil phospholipid fatty acid composition in either the aggregated retention treatment (A=0.0264, p=0.2283 in forest floor, A=0.0256, p=0.2341 in mineral soil, A=0.0191, p=0.1437 when both soil layers were combined) or dispersed retention treatment (A=0.0033, p=0.4206 in forest floor, A=-0.0066, p=0.5024 in mineral soil, A=0.0114, p=0.2655 when both soil layers were combined).

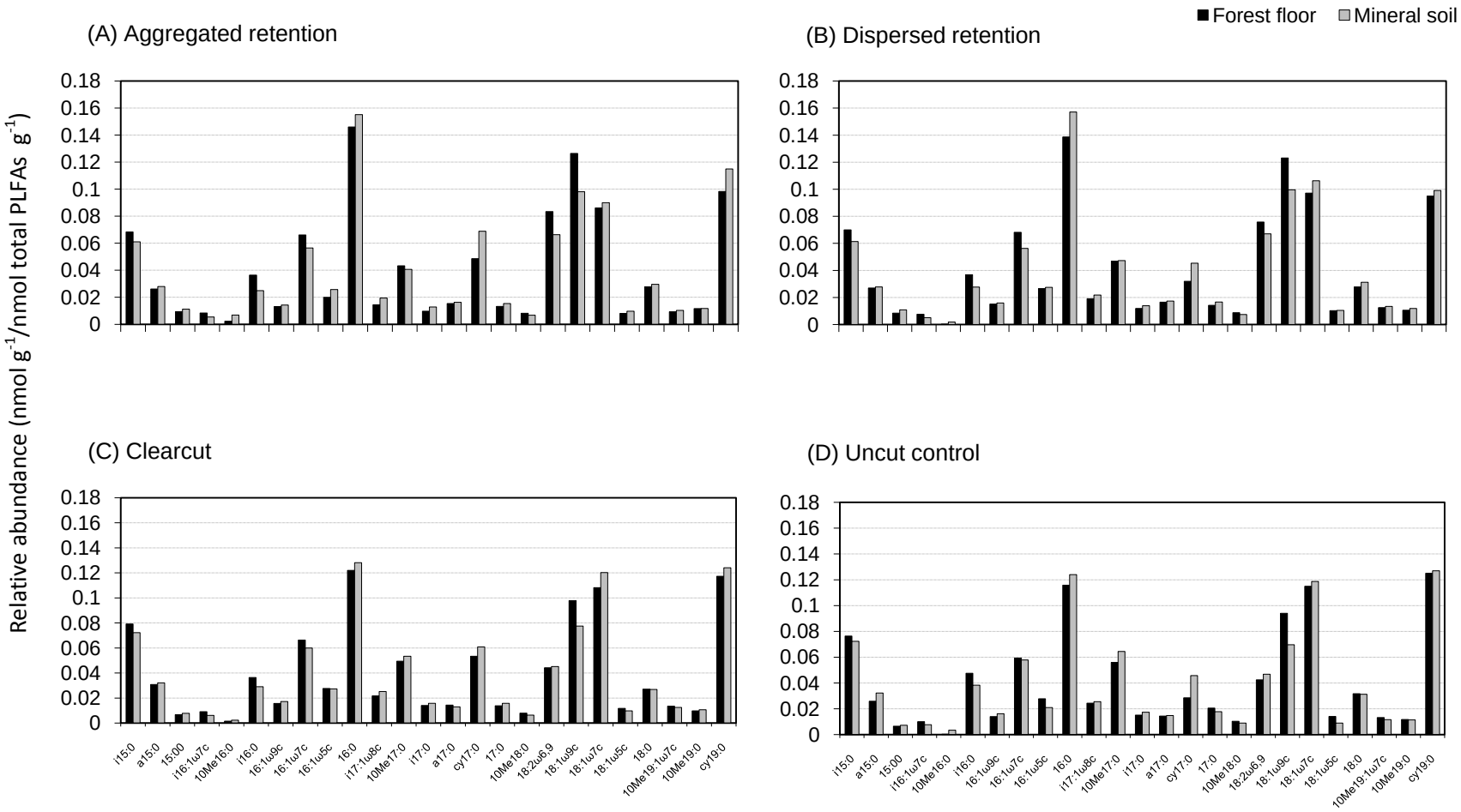

Figure 4.37 Forest floor and mineral soil PLFA profiles in the aggregated retention (A), dispersed retention (B), clearcut (C), and uncut control (D) plots. . Values are means (n=20 for A, n=16 for B, and n=4 for C and D).

Table 4.3 Pair-wise MRPP comparison of PLFA patterns among harvesting treatments in both forest floor and mineral soil. An asterisk (*) indicates A>0.1, and values in bold indicate p<0.0083 (=0.05/6).

		Clearcut	Aggregated retention	Dispersed retention	Uncut control
Clearcut	A-value	1.0000	0.0702	0.0896	0.0468
	p-value	1.0000	**0.0000**	**0.0000**	0.0270
Aggregated retention	A-value		1.0000	0.0346	0.0927
	p-value		1.0000	**0.0000**	**0.0000**
Dispersed retention	A-value			1.0000	0.1079*
	p-value			1.0000	**0.0000**
Uncut control	A-value				1.0000
	p-value				1.0000

Table 4.4 Pair-wise MRPP comparison of PLFA patterns among harvesting treatments in the forest floor. An asterisk (*) indicates A>0.1, and values in bold indicate p<0.0083 (=0.05/6).

		Clearcut	Aggregated retention	Dispersed retention	Uncut control
Clearcut	A-value	1.0000	0.0937	0.1219*	0.0853
	p-value	1.0000	**0.0002**	**0.0021**	0.0686
Aggregated retention	A-value		1.0000	0.0318	0.1163*
	p-value		1.0000	**0.0070**	**0.0000**
Dispersed retention	A-value			1.0000	0.1378*
	p-value			1.0000	**0.0009**
Uncut control	A-value				1.0000
	p-value				1.0000

Table 4.5 Pair-wise MRPP comparison of PLFA patterns among harvesting treatments in the mineral soil. An asterisk (*) indicates A>0.1, and values in bold indicate p<0.0083 (=0.05/6).

		Clearcut	Aggregated retention	Dispersed retention	Uncut control
Clearcut	A-value	1.0000	0.0759	0.0975	-0.0089
	p-value	1.0000	**0.0005**	**0.0047**	0.5091
Aggregated retention	A-value		1.0000	0.0555	0.1003*
	p-value		1.0000	**0.0005**	**0.0001**
Dispersed retention	A-value			1.0000	0.1223*
	p-value			1.0000	**0.0012**
Uncut control	A-value				1.0000
	p-value				1.0000

4.5.2 Soil taxonomic microbial community composition using PLFA biomarkers

Harvesting also affected the taxonomic composition of the soil microbial community (A=0.1768, p=0.0000 in forest floor, A=0.1178, p=0.0003 in mineral soil, and A=0.1397, p=0.0000 when both soil layers were combined). Similar to the results obtained from multivariate analysis on PLFA profiles (previous section), post-hoc pair-wise comparison analyses also revealed that microbial community taxonomic composition 5 years after clearcutting was not significantly different to that in uncut forest, and both green-tree retention treatments had a significantly different microbial community composition than that found in clearcut and uncut forest (Table 4.6, 4.7, and 4.8).

Proximity to retention trees did not significantly affect the taxonomic composition of soil microbial community in either the aggregated retention treatment (A=0.0253, p=0.2937 in forest floor, A=0.0426, p=0.2248 in mineral soil, A=0.0335, p=0.1224 when both soil layers were combined) or dispersed retention treatment (A=-0.0313, p=0.6306 in forest floor, A=-0.0088, p=0.4818 in mineral soil, A=0.0111, p=0.3055 when both soil layers were combined).

Table 4.6 Pair-wise MRPP comparison of microbial community composition among harvesting treatments in both forest floor and mineral soil. An asterisk (*) indicates A>0.1, and values in bold indicate p<0.0083 (=0.05/6).

		Clearcut	Aggregated retention	Dispersed retention	Uncut control
Clearcut	A-value	1.0000	0.1007*	0.1744*	-0.0064
	p-value	1.0000	**0.0000**	**0.0000**	0.4985
Aggregated retention	A-value		1.0000	0.0096	0.1128*
	p-value		1.0000	0.0961	**0.0000**
Dispersed retention	A-value			1.0000	0.1894*
	p-value			1.0000	**0.0000**
Uncut control	A-value				1.0000
	p-value				1.0000

Table 4.7 Pair-wise MRPP comparison of microbial community composition among harvesting treatments in the forest floor. An asterisk (*) indicates A>0.1, and values in bold indicate p<0.0083 (=0.05/6).

		Clearcut	Aggregated retention	Dispersed retention	Uncut control
Clearcut	A-value	1.0000	0.1527*	0.2259*	-0.0645
	p-value	1.0000	**0.0003**	**0.0005**	0.8400
Aggregated retention	A-value		1.0000	0.0082	0.1472*
	p-value		1.0000	0.2145	**0.0003**
Dispersed retention	A-value			1.0000	0.2121*
	p-value			1.0000	**0.0009**
Uncut control	A-value				1.0000
	p-value				1.0000

Table 4.8 Pair-wise MRPP comparison of microbial community composition among harvesting treatments in the mineral soil. An asterisk (*) indicates A>0.1, and values in bold indicate p<0.0083 (=0.05/6).

		Clearcut	Aggregated retention	Dispersed retention	Uncut control
Clearcut	A-value	1.0000	0.0731	0.1399*	-0.0447
	p-value	1.0000	0.0116	**0.0066**	0.6925
Aggregated retention	A-value		1.0000	0.0103	0.1009*
	p-value		1.0000	0.1931	**0.0019**
Dispersed retention	A-value			1.0000	0.1721*
	p-value			1.0000	**0.0022**
Uncut control	A-value				1.0000
	p-value				1.0000

NMS ordination on microbial community composition data from both forest floor and mineral soil layers showed samples from the clearcut and uncut treatments clustered together, on the bottom left of the plot, and those from the aggregated and dispersed retention treatments also clustered in the centre of the plot (final stress of 5.90300 and final instability of 0.00001 after 59 iterations) (Fig. 4.38). Soil pH explained 26% of the variance in axis 2.

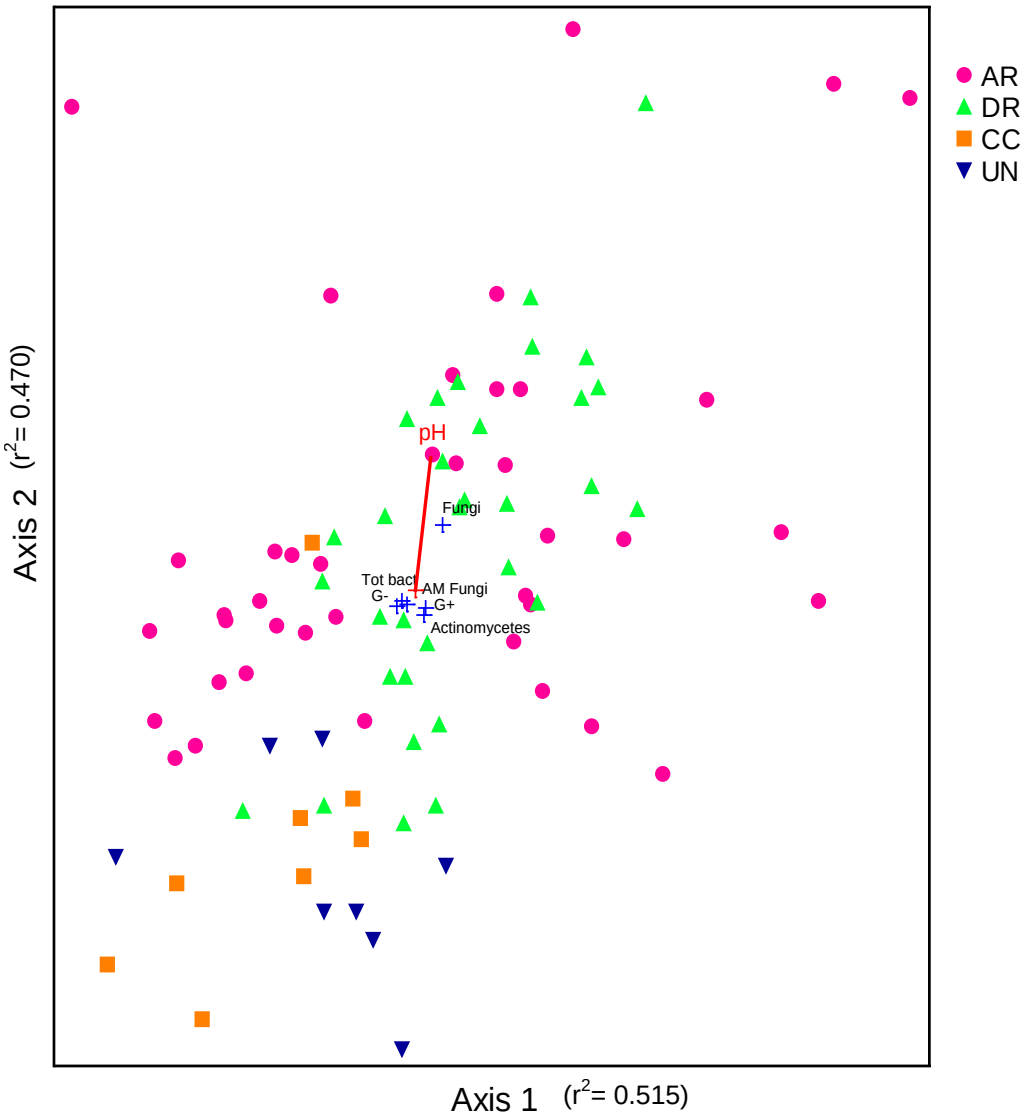

Figure 4.38 NMS ordination of the soil microbial community composition from the forest floor and mineral soil at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

When only the soil microbial community composition data from the forest floor were considered, NMS ordination again showed clustering of samples from the clearcut and uncut treatments, as well as from the aggregated retention and dispersed retention treatments (final stress of 4.71141

and final instability of 0.00001 after 59 iterations) (Fig. 4.39). Soil pH explained 19.7% and 15.8% of the variances in axes 1 and 2, respectively, whereas soil moisture explained 12.2% and 17.1% of the variances in axes 1 and 2, respectively.

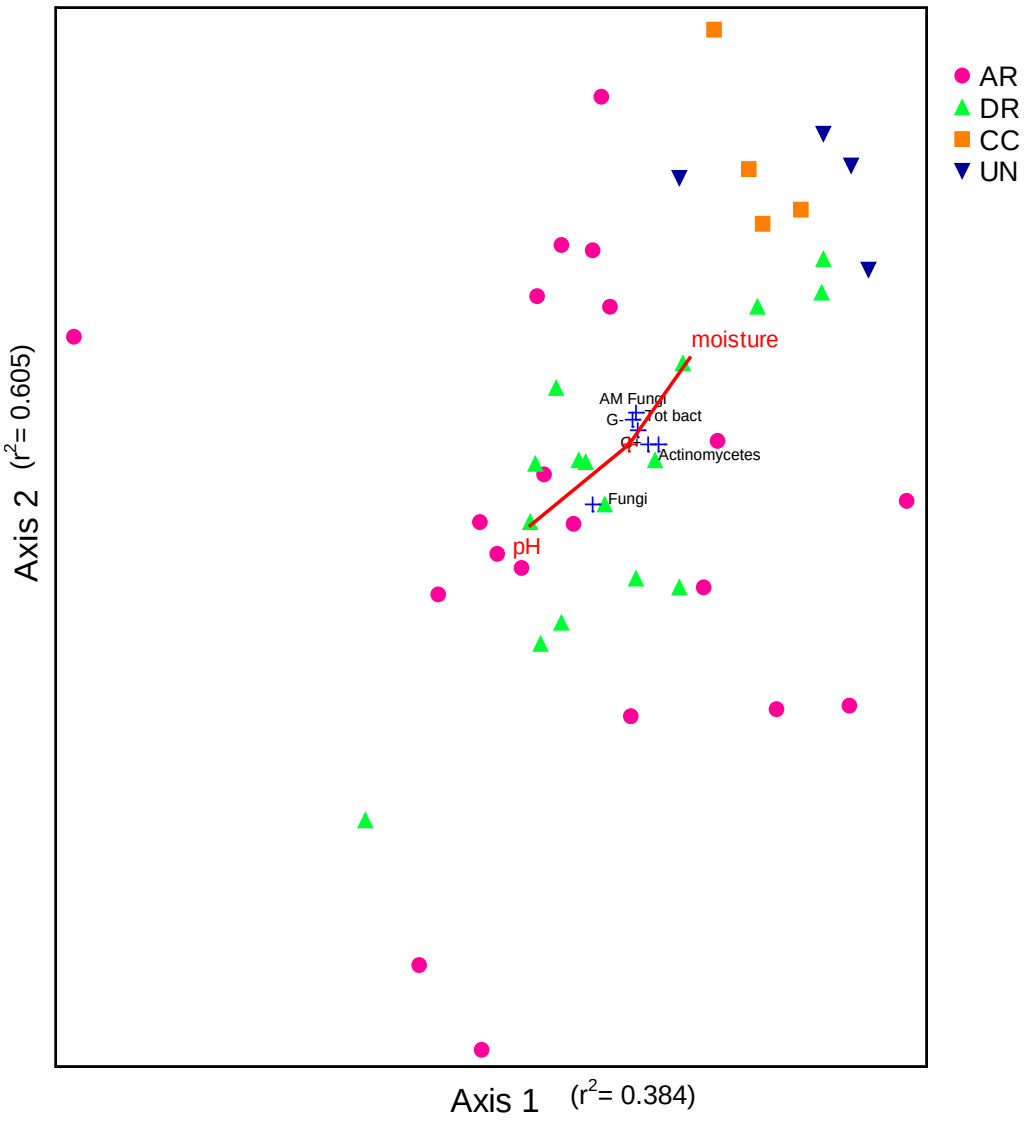

Figure 4.39 NMS ordination of the soil microbial community composition from the forest floor at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

When only the soil microbial community composition data from the mineral soil were considered, NMS ordination also showed clustering of samples from the clearcut and uncut treatments, as well as from the aggregated retention and dispersed retention treatments, but the clustering was not as strong as in the forest floor (final stress of 5.60668 and final instability of 0.00001 after 101 iterations) (Fig. 4.40). Soil pH explained 36.3% of the variance in axis 2.

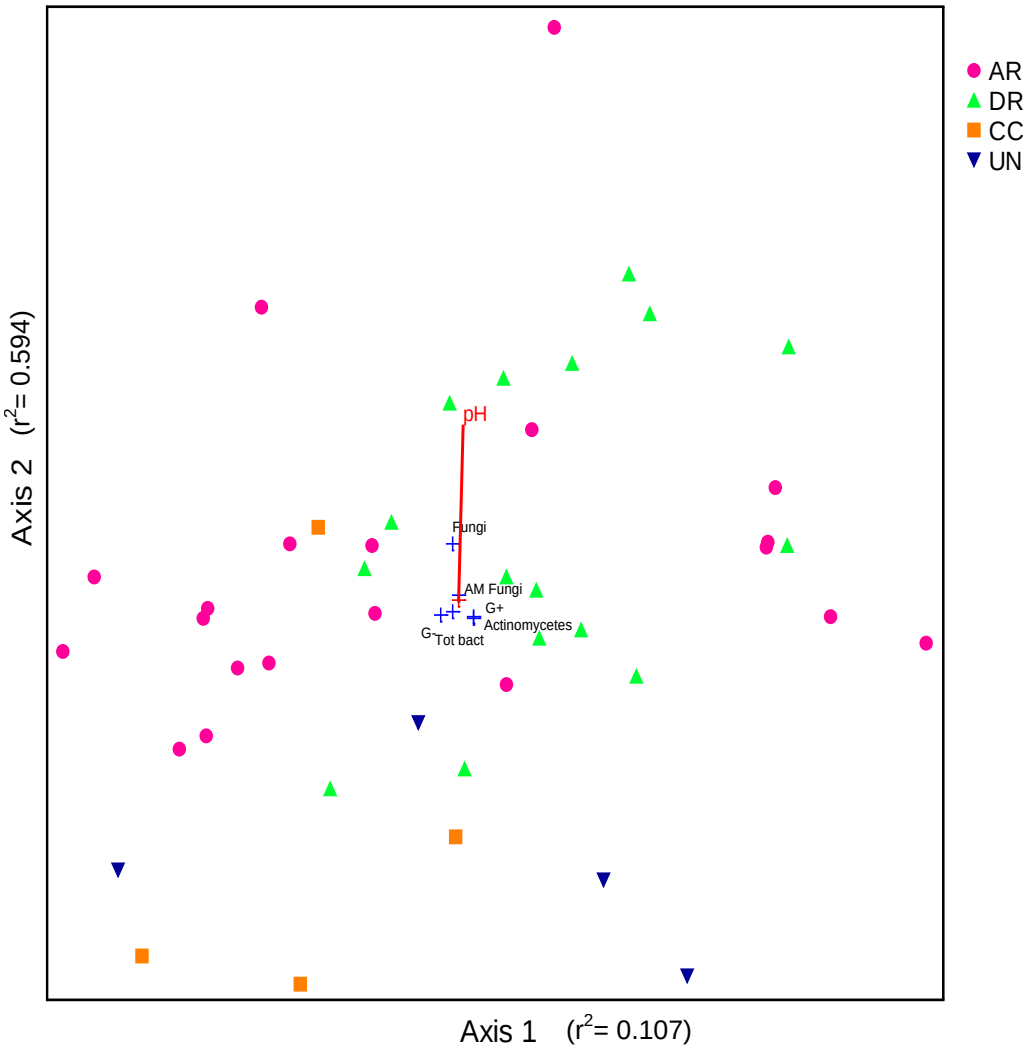

Figure 4.40 NMS ordination of the soil microbial community composition from the mineral soil at the aggregated retention (AR), dispersed retention (DR), clearcut (CC), and uncut control (UN) plots at STEMS 1.

4.5.2.1 Total biomass

Harvesting did not significantly affect total microbial biomass in the forest floor, but total microbial biomass in the mineral soil was significantly lower in the aggregated retention treatment than the clearcut (Fig. 4.41). In the aggregate retention treatment, total microbial biomass in the forest floor decreased with distance from the retention patches up to 20 m into the opening, whilst the fluctuations in total microbial biomass in the forest floor of the dispersed retention treatment did not exhibit any particular trend (Fig. 4.42).

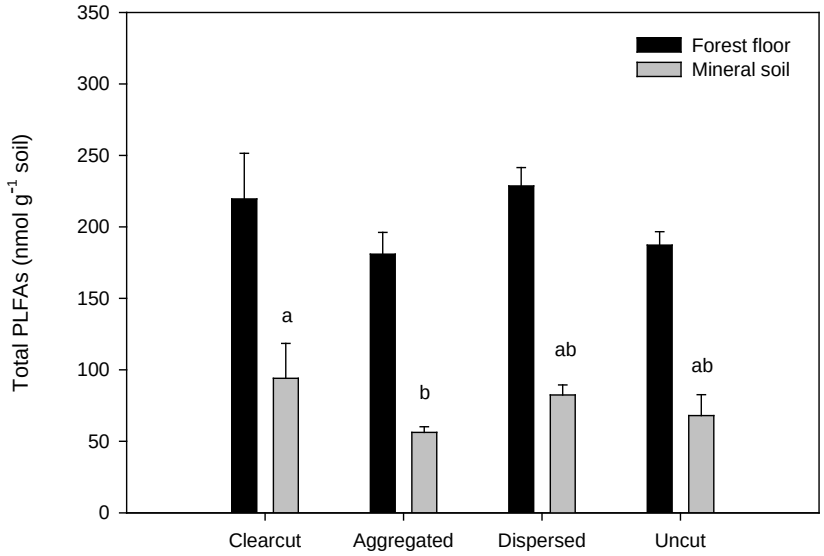

Figure 4.41 Total microbial biomass of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different. Means were not significantly different in the forest floor.

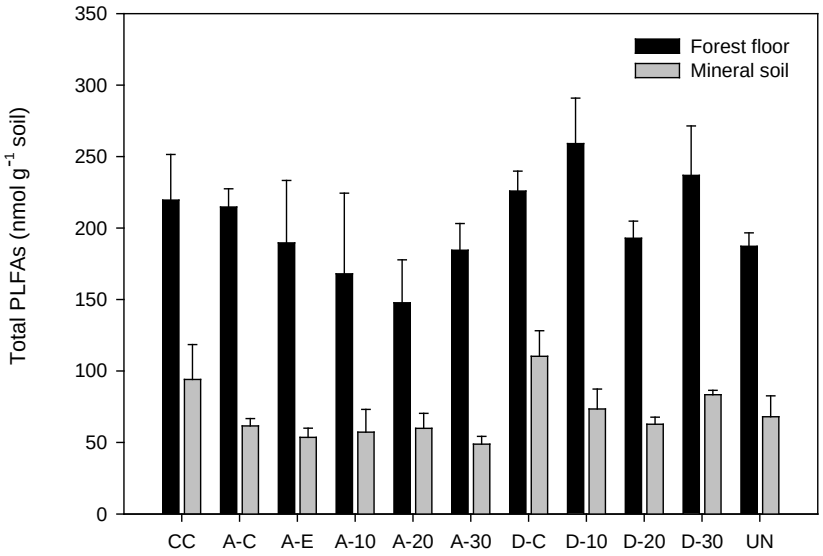

Figure 4.42 Total microbial biomass of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

4.5.2.2 Bacterial PLFAs

Whilst no effect of harvesting on the relative abundance[1] of Gram-positive bacteria was observed in the forest floor, the abundance of Gram-positive bacteria in the mineral soil was significantly reduced by green-tree retention harvesting, but not by clearcutting (Fig. 4.43). In the forest floor of the aggregated retention treatment, the abundance of Gram-positive bacteria decreased at the edge of retention tree patches, but gradually increased with distance into the opening (Fig. 4.44). The abundance of Gram-negative bacteria was highest in the forest floor and mineral soil of the clearcut (Fig. 4.45), but showed no significant difference among different sampling locations (Fig. 4.46). The abundance of actinomycetes was not significantly different among the four treatments in either soil layer, although the highest abundance was found in the uncut forest (Fig. 4.47). In the forest floor of the aggregated retention treatment, the abundance of actinomycetes was lowest at 10 m and highest at 30 m beyond the edge of retention tree patches (Fig. 4.48). Overall, bacteria (total) were more abundant in the clearcut and uncut forest than in the aggregated and dispersed retention treatment (Fig. 4.49). The abundance of bacteria (total) was not significantly different among different sampling locations (Fig. 4.50).

[1] Relative abundance will be referred to as 'abundance' for simplicity throughout this thesis

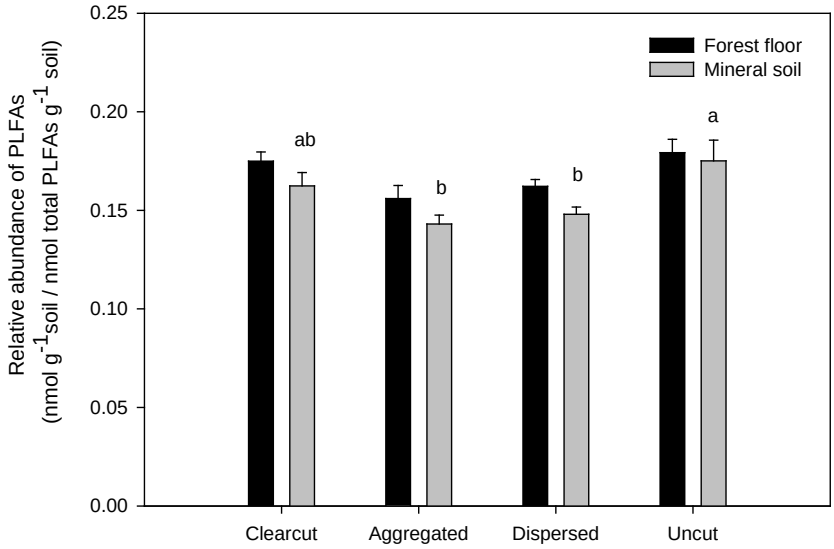

Figure 4.43 Relative abundance of Gram-positive bacteria of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means connected with the same letter are not significantly different. Means were not significantly different in the forest floor.

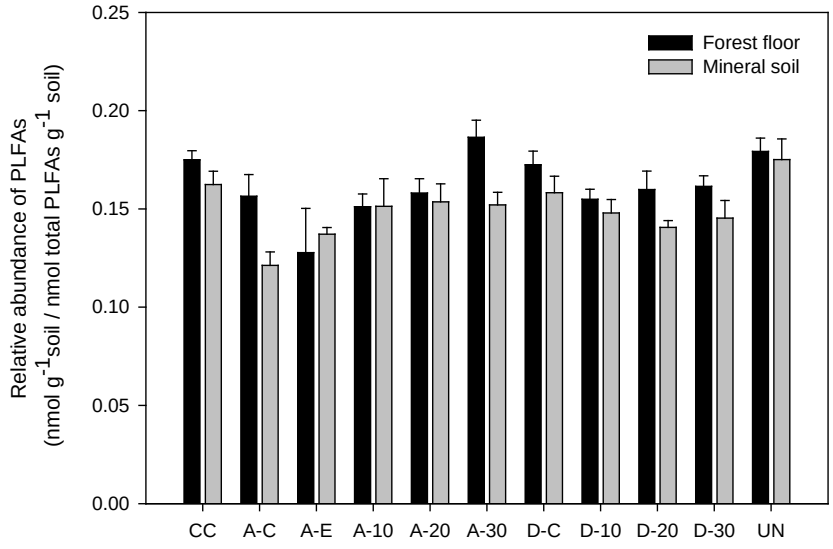

Figure 4.44 Relative abundance of Gram-positive bacteria of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between A-30 vs. A-E and UN vs. A-E in the forest floor, and between UN vs. A-C and CC vs. A-C in the mineral soil.

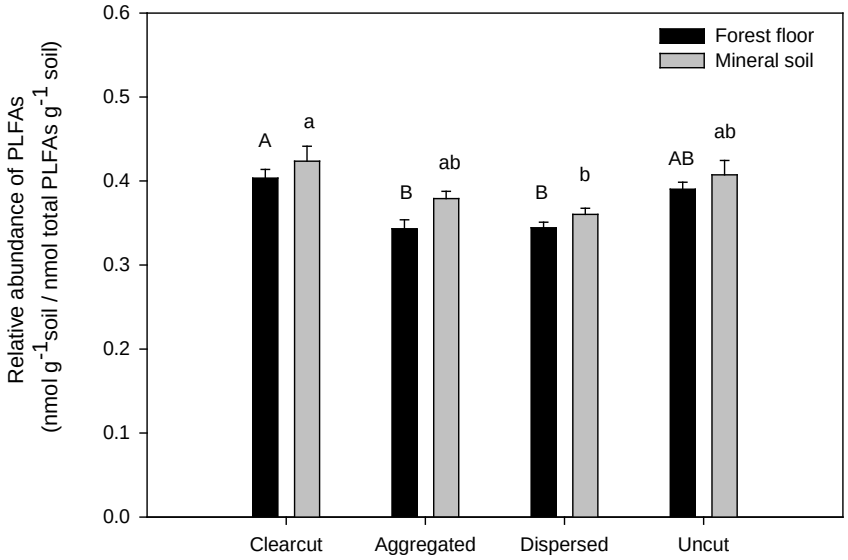

Figure 4.45 Relative abundance of Gram-negative bacteria of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter are not significantly different (upper case letters for forest floor, lower case letters for mineral soil).

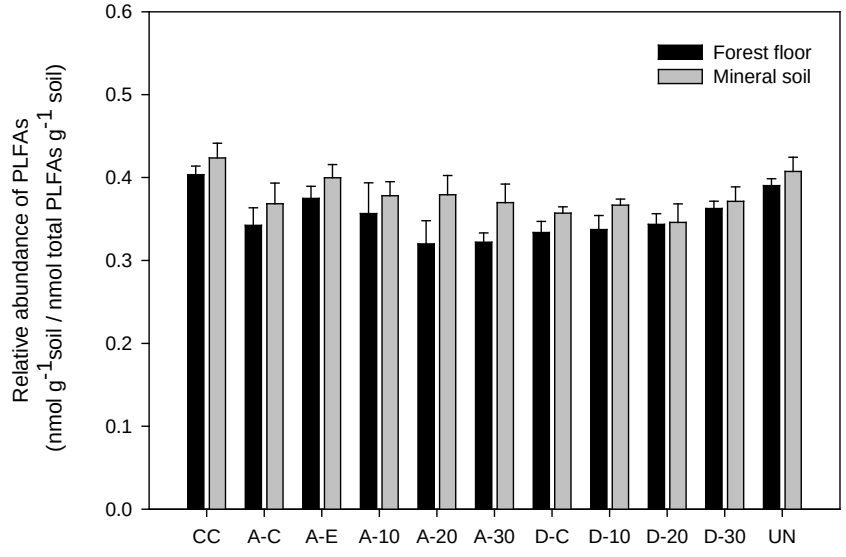

Figure 4.46 Relative abundance of Gram-negative bacteria of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

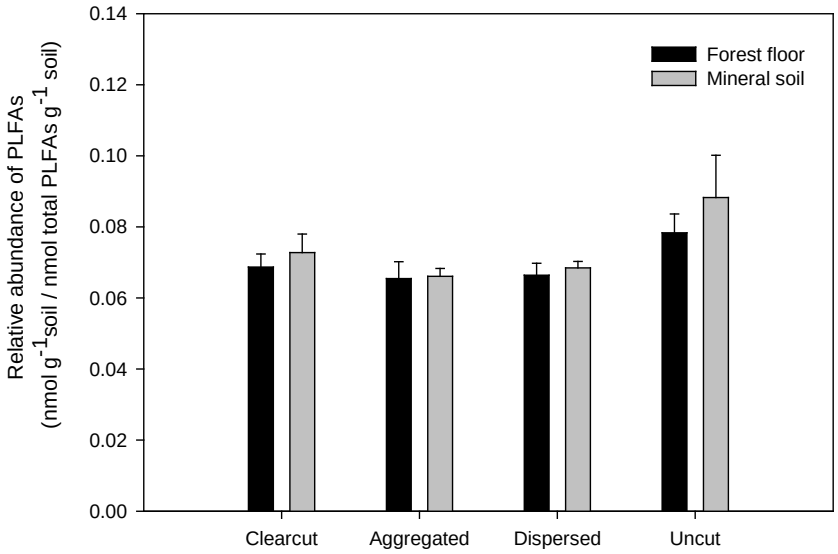

Figure 4.47 Relative abundance of actinomycetes of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

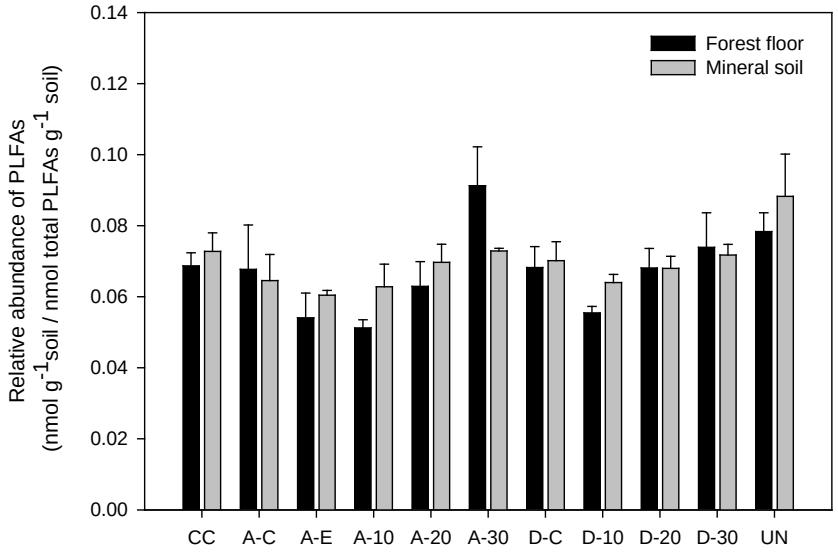

Figure 4.48 Relative abundance of actinomycetes of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between A-30 vs. A-10, A-30 vs. A-E, and A-30 vs. D-10 in the forest floor, but were not significantly different in the mineral soil.

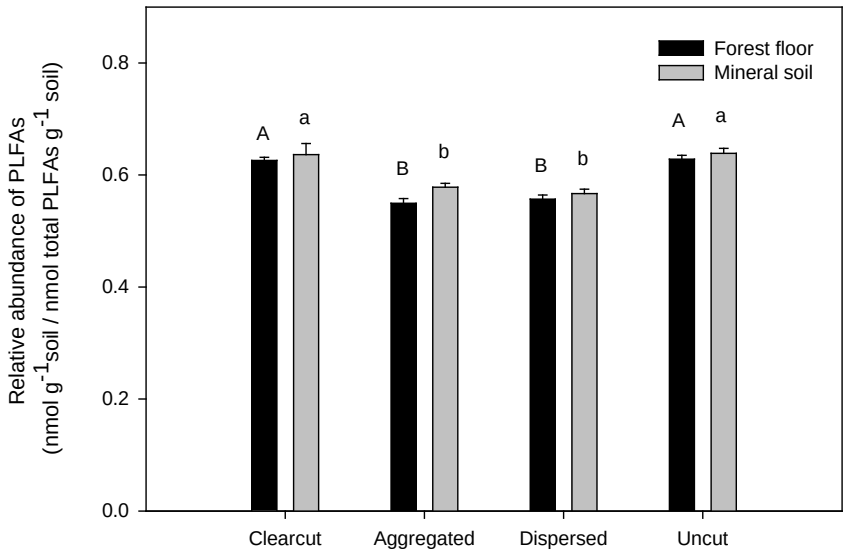

Figure 4.49 Relative abundance of total bacteria of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different (upper case letters for forest floor, lower case letters for mineral soil).

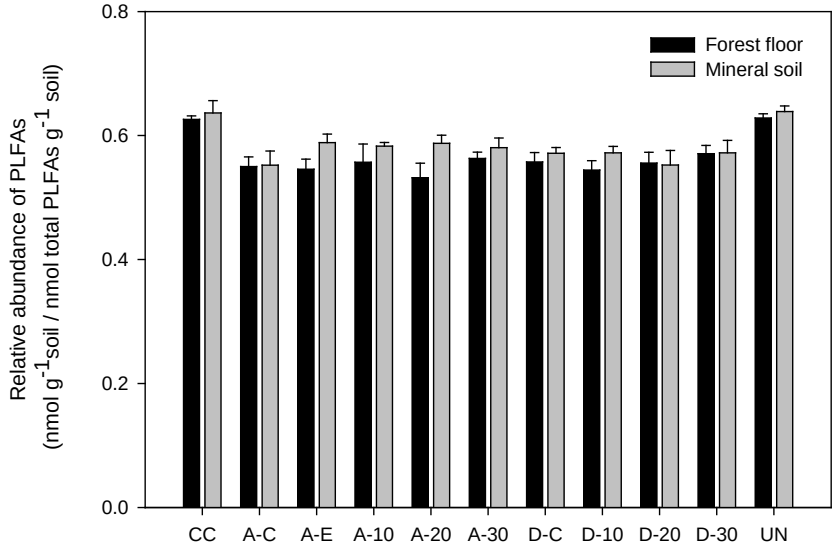

Figure 4.50 Relative abundance of total bacteria of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

4.5.2.3 Fungal PLFAs

In the forest floor, fungi were significantly more abundant in the green-tree retention treatments than in the clearcut and uncut forest (Fig. 4.51). A similar pattern was observed in the mineral soil, although the difference among the four treatments was not statistically significant (Fig. 4.51). Significant differences among sampling locations were only observed between the centre of the aggregate retention patches and the clearcut and uncut forest (Fig. 4.52). However, there was a decreasing trend in fungal abundance in the mineral soil of the aggregated retention treatment from the centre of the patch to 30m from the patch edge (Fig. 4.52). The abundance of arbuscular mycorrhizal (AM) fungi in the forest floor was lowest in the aggregated retention treatment (Fig. 4.53). Higher fluctuations in the abundance of AM fungi were observed among sampling locations in the aggregated retention treatment than in the dispersed retention treatment (Fig. 4.54).

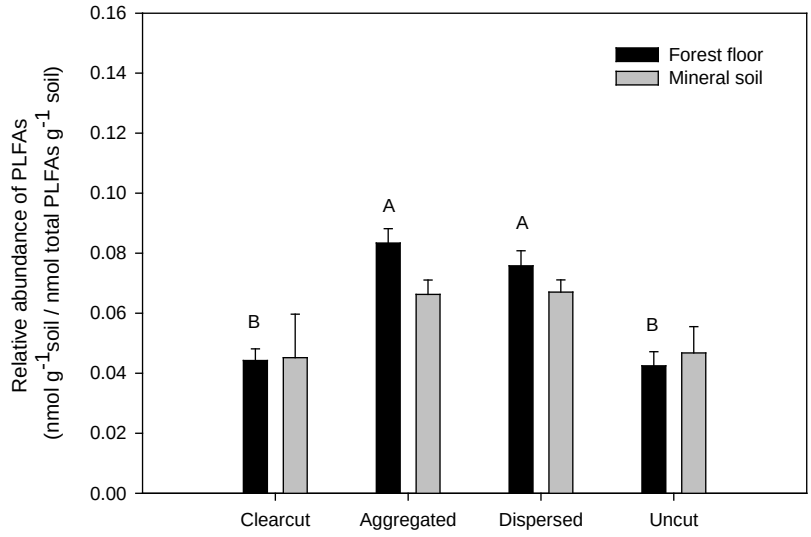

Figure 4.51 Relative abundance of fungi of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means connected with the same letter were not significantly different. Means were not significantly different in the mineral soil.

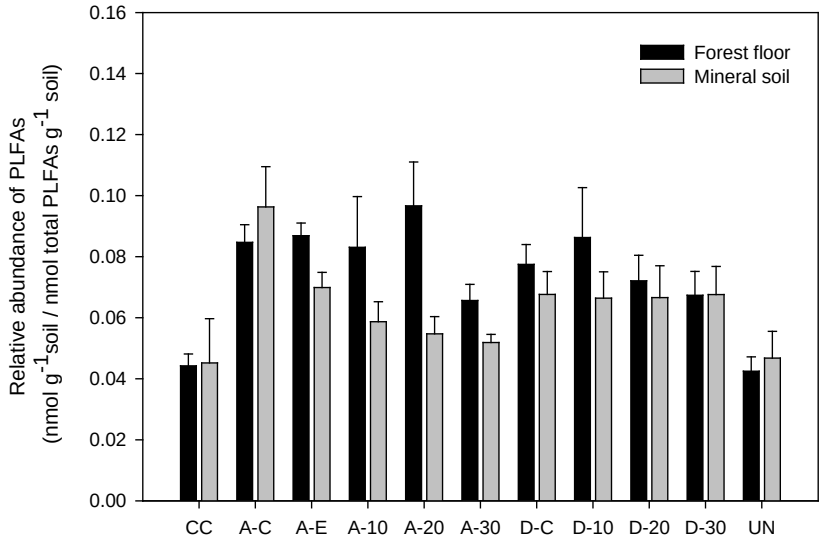

Figure 4.52 Relative abundance of fungi of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between A-20 vs. UN and A-20 vs. CC in the forest floor, but were not significantly different in the mineral soil.

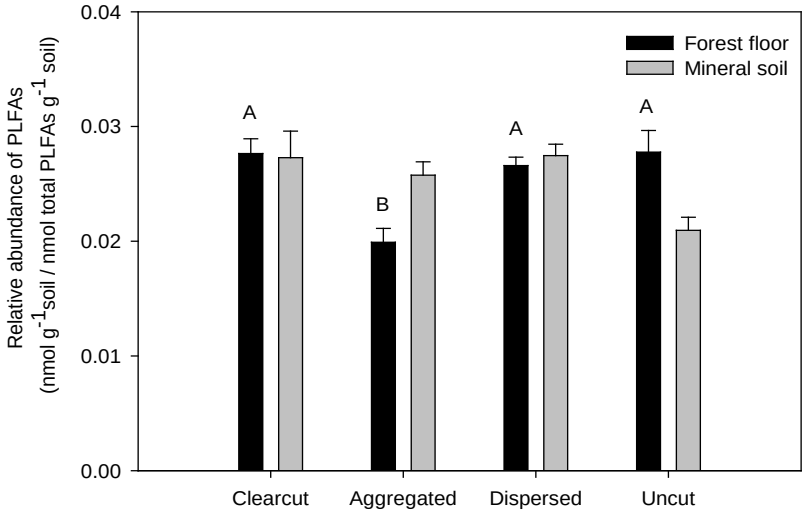

Figure 4.53 Relative abundance of arbuscular mycorrhizal (AM) fungi of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means connected with the same letter are not significantly different. Means were not significantly different in the mineral soil.

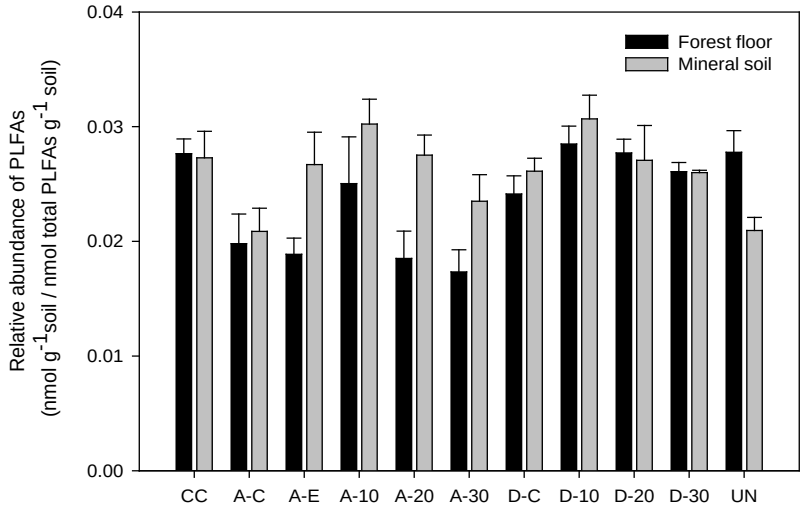

Figure 4.54 Relative abundance of arbuscular mycorrhizal (AM) fungi of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were significantly different between D-10 vs. A-30, UN vs. A-30, D-20 vs. A-30, and CC vs. A-30 in the forest floor and were not significantly different in the mineral soil.

4.5.2.4 Ratios of signature PLFAs

The ratio of fungal to bacterial PLFAs was generally higher in both green-tree retention treatments than in the clearcut or uncut forest (Fig. 4.55). In the mineral soil of the aggregated retention treatment, there was a decreasing trend in the ratio of fungal to bacterial PLFAs with distance from the centre of retention patches (Fig. 4.56). The ratio of Gram-positive to Gram-negative bacteria was not affected by harvesting (Fig. 4.57), but there seemed to be an increasing trend from the edge (in the forest floor) and the centre (in the mineral soil) of retention tree patches into the opening in the aggregated retention treatment (Fig. 4.58).

The ratio of saturated (15:0, 16:0, 17:0, and 18:0) to monounsaturated (16:1ω7c, 16:1ω9c 16:1ω7c, 16:1ω5c, i17:1ω8c, 18:1ω9c, 18:1ω7c, and 18:1ω5c) PLFAs was not significantly different among the four harvest treatments in the forest floor, but in the mineral soil the aggregated retention treatment was significantly higher from the clearcut (Fig. 4.59). There was no significant difference among different sampling locations in the four treatments (Fig. 4.60). The ratio of cyclopropyl PLFAs (cy17:0 and cy19:0) to their monoenoic precursors (16:1ω7c and 18:1ω7c) was highest in the aggregated retention treatment (Fig. 4.61). The ratio fluctuated more in the aggregate retention treatment than in the dispersed retention treatment, although there was no significant difference observed among sampling locations (Fig. 4.62).

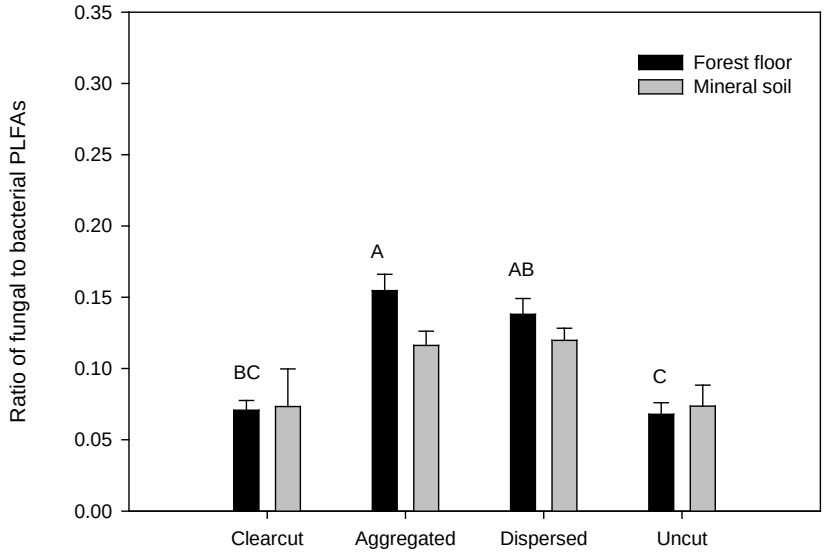

Figure 4.55 Ratio of fungal to bacterial PLFAs of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different. Means were not significantly different in the mineral soil.

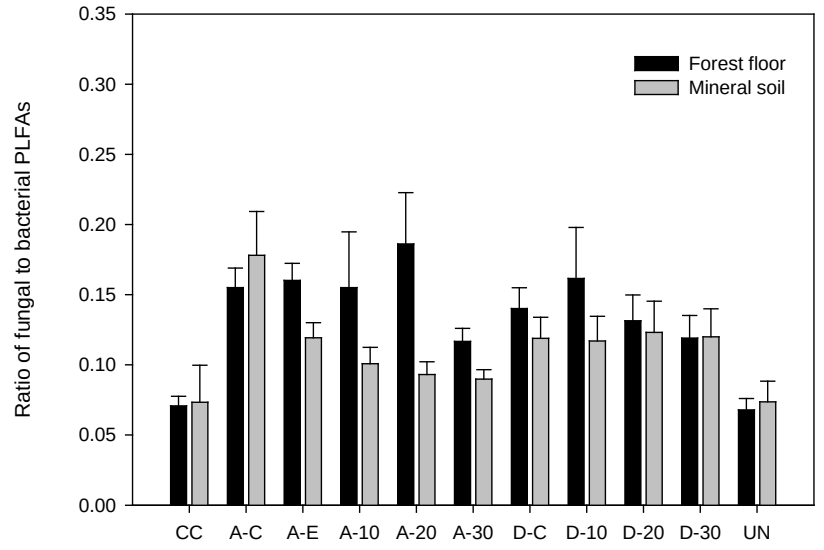

Figure 4.56 Ratio of fungal to bacterial PLFAs of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

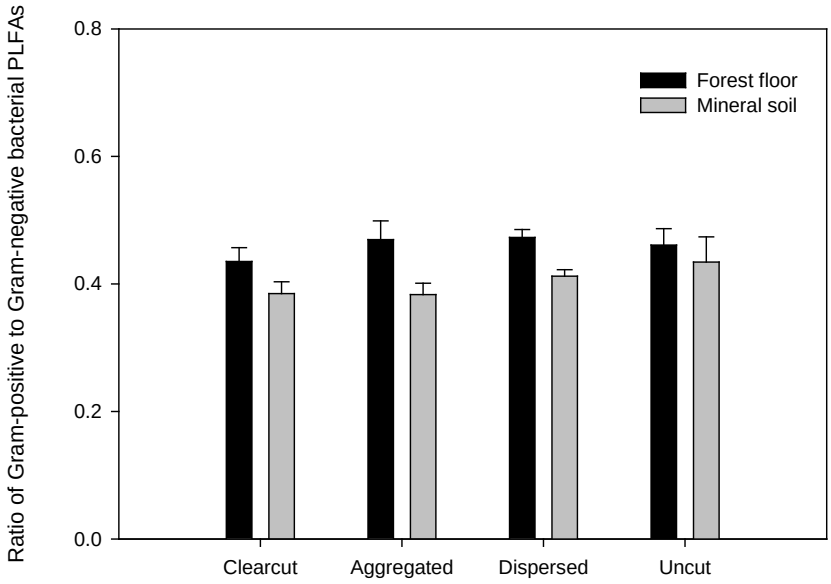

Figure 4.57 Ratio of Gram-positive to Gram-negative bacterial PLFAs of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means were not significantly different in either soil layer.

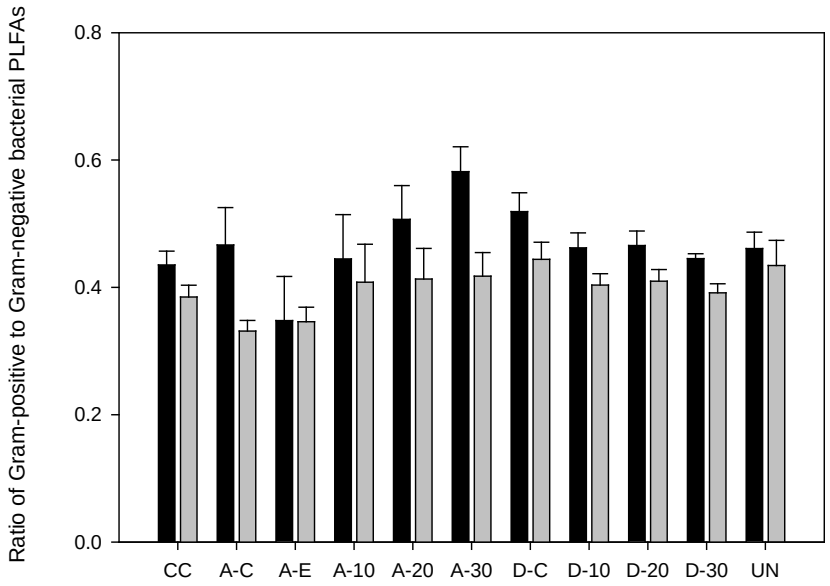

Figure 4.58 Ratio of Gram-positive to Gram-negative bacterial PLFAs of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

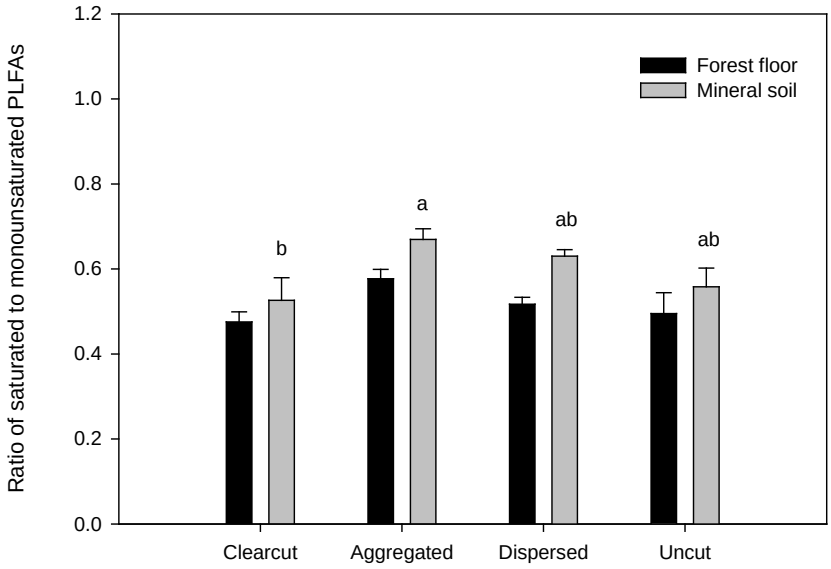

Figure 4.59 Ratio of saturated to monounsaturated PLFAs of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different. Means were not significantly different in the forest floor.

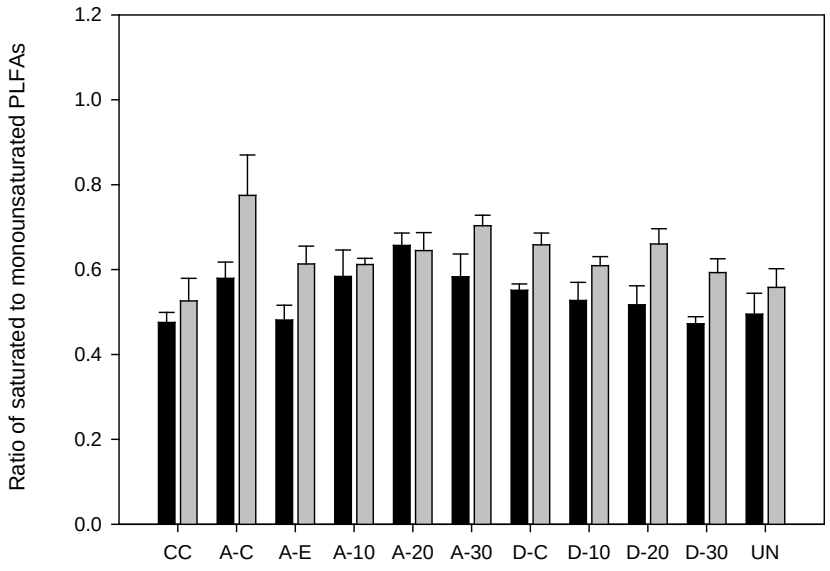

Figure 4.60 Ratio of saturated to monounsaturated PLFAs of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

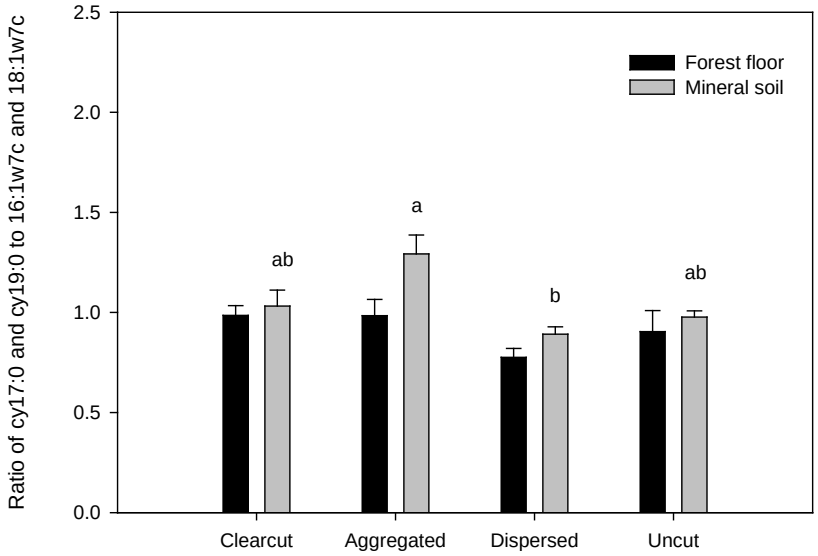

Figure 4.61 Ratio of cyclopropyl PLFAs (cy17:0 and cy19:0) to their monoenoic precursors (16:1ω7c and 18:1ω7c) of the forest floor and mineral soil in the aggregated retention, dispersed retention and clearcut plots and uncut forest. Error bars represent one standard error. Means with the same letter were not significantly different. Means were not significantly different in the forest floor.

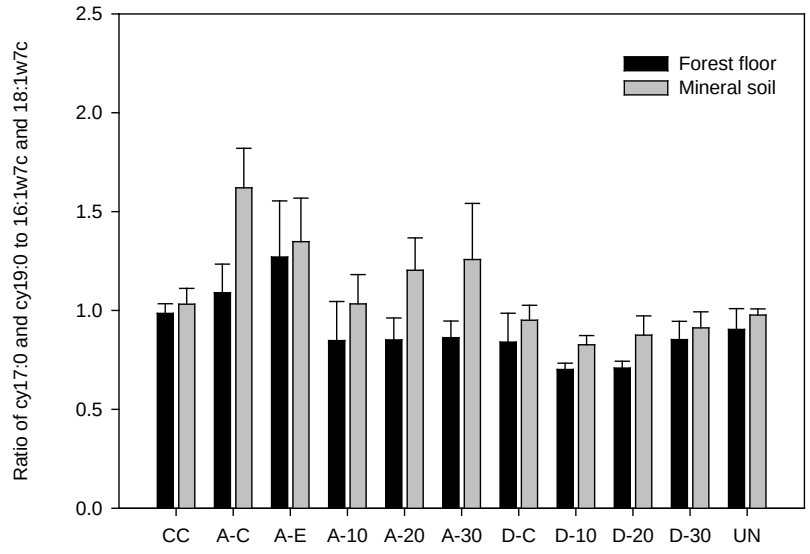

Figure 4.62 Ratio of cyclopropyl PLFAs (cy17:0 and cy19:0) to their monoenoic precursors (16:1ω7c and 18:1ω7c) of the forest floor and mineral soil at each sampling location in the clearcut (CC), aggregated retention (A) and dispersed retention (D) treatments (C-Centre; E-Edge; 10, 20, 30-distance in m from edge), and uncut forest (UN). Error bars represent one standard error. Means were not significantly different in either soil layer.

4.6 Correlations among measured soil environmental and biological variables

Of all measured soil environmental variables (moisture, pH, and nutrient availability), soil moisture was found to show strong correlations with many of the biological variables, i.e. SIR of chitin and carboxymethyl cellulose (CMC), all five enzyme activities, total microbial biomass, and Gram-positive bacteria (Table 4.9). Soil pH was strongly negatively correlated with the abundances of Gram-positive bacteria, actinomycetes, and total bacteria, and positively correlated with the abundance of fungi (Table 4.9). Strong correlations between nutrient availability and biological variables were only observed between Fe, S, and Al availabilities and peroxidase activity (Table 4.9). No strong correlations were found between enzyme activities and the availability of nutrients they release, i.e. phosphatase and PO_4^{3-} and N-acetyl-glucosaminidase and NO_3^- or NH_4^+.

Table 4.9 Spearman's rank correlations between measured biological variables (SIR rates, enzyme activities, and PLFA signatures) and environmental variables. Numbers in bold indicate strong correlations (ρ > 0.4).

	SIR rate					Enzyme activity					PLFA signature						
	Gluc.	Chitin	CMC	Mal. acid	Ser.	Glu.	NAG.	Pho.	Phe.	Per.	Tot. mic. bio.	G+ bac.	G- bac.	Actin.	Tot. bac.	Fung.	AM fungi
Soil moisture	-0.21	**-0.52**	**-0.44**	-0.18	-0.38	**0.61**	**0.59**	**0.81**	**-0.56**	**-0.67**	**0.80**	**0.48**	-0.17	0.03	-0.03	0.10	-0.04
pH	0.07	0.27	0.14	0.03	-0.04	-0.08	-0.06	-0.35	0.08	0.12	-0.15	**-0.41**	-0.29	**-0.50**	**-0.45**	0.38	0.11
NO_3^-	-0.17	-0.24	0.05	-0.07	0.11	0.07	0.16	0.24	-0.02	-0.10	0.13	0.20	0.09	0.18	0.23	-0.22	0.20
NH_4^+	-0.04	-0.06	0.15	0.08	0.22	-0.03	0.01	0.21	0.14	0.08	0.06	0.18	0.05	0.12	0.18	-0.16	0.24
Ca	-0.07	-0.09	0.15	0.05	0.28	0.02	-0.05	0.08	0.14	0.22	-0.07	0.10	0.27	0.20	0.32	-0.31	0.06
Mg	-0.04	-0.08	0.20	0.19	0.33	0.01	-0.07	0.06	0.17	0.24	-0.05	0.09	0.24	0.30	0.31	-0.31	0.02
K	0.05	0.14	0.36	0.13	0.08	-0.02	0.00	-0.12	0.15	0.10	-0.07	-0.21	-0.05	-0.03	-0.12	0.04	0.15
P	0.02	-0.01	-0.07	-0.19	-0.20	0.24	0.28	0.15	-0.07	-0.14	0.08	-0.22	-0.05	-0.21	-0.18	0.20	0.22
Fe	-0.21	0.27	0.27	0.07	0.15	-0.17	-0.16	-0.28	0.18	**0.42**	-0.30	-0.10	-0.16	0.08	-0.07	0.00	0.04
Mn	-0.19	-0.14	0.06	-0.05	-0.18	0.07	0.24	0.03	-0.11	0.15	-0.02	-0.13	0.18	0.07	0.07	0.05	-0.18
Cu	-0.13	0.11	0.21	-0.04	-0.04	-0.11	-0.04	-0.24	0.16	0.19	-0.25	-0.19	-0.04	-0.03	-0.09	0.09	-0.11
Zn	-0.09	-0.06	0.25	0.16	0.19	-0.03	0.07	-0.10	0.19	0.35	-0.15	-0.08	0.21	0.23	0.19	-0.13	-0.02
B	-0.03	0.07	-0.07	-0.25	-0.26	0.16	0.22	0.11	-0.13	-0.07	0.13	-0.04	-0.29	-0.17	-0.30	0.27	-0.02
S	-0.06	0.16	0.19	-0.02	-0.04	-0.13	-0.16	-0.32	0.05	**0.40**	-0.31	-0.20	-0.08	0.12	-0.09	0.09	-0.25
Pb	-0.02	-0.05	-0.05	0.02	-0.23	0.27	0.24	0.08	-0.11	0.03	0.09	-0.16	0.01	-0.16	-0.13	0.26	-0.38
Al	-0.06	0.20	0.39	0.14	0.29	-0.21	-0.10	-0.28	0.16	**0.52**	-0.32	-0.05	0.09	0.26	0.16	-0.20	-0.11

SIR rates: Gluc. = glucose, CMC = carboxymethyl cellulose, Mal. acid = malonic acid, Ser. = serine; **Enzyme activities**: Glu. = β-1,4-glucosidase (glucosidase), NAG. = β-1,4-N-acetylglucosaminidase (NAGase), Pho. = acid phosphatase (phosphatase), Phe. = phenol oxidase, Per. = peroxidase; **PLFA signatures**: Tot. mic. bio. = total microbial biomass, G+ bac. = Gram-positive bacteria, G- bac. = Gram-negative bacteria, Actin. = actinomycetes, Tot. bac. = total bacteria, Fung. = fungi, AM fungi = arbuscular mycorrhizal fungi.

Many of the measured soil microbial community structural and functional data correlated with each other. SIR rates of chitin, CMC, and serine were strongly correlated with at least one of the five measured enzyme activities (Table 4.10). Chitin- and CMC-induced respiration rates were also strongly negatively correlated with total microbial biomass (Table 4.11). The total microbial biomass was strongly positively correlated with hydrolytic enzyme (β-glucosidase, NAGase, and phosphatase) enzyme activities and strongly negatively correlated with oxidative enzyme (phenol oxidase and peroxidase) activities, whilst the abundance of Gram-positive bacteria was strongly negatively correlated with peroxidase activity (Table 4.12). Strong positive correlations were observed between pairs of hydrolytic enzyme activities and between both oxidative enzyme activities, whilst negative correlations were observed between the activities of hydrolytic and oxidative enzymes (Table 4.13).

Table 4.10 Spearman's rank correlations between SIR rates and enzyme activities. Numbers in bold indicate strong correlations (ρ > 0.4).

	Glucose	Chitin	CMC	Malonic acid	Serine
β-Glucosidase	-0.05	**-0.54**	-0.34	-0.14	**-0.43**
NAGase	-0.20	**-0.43**	-0.18	-0.12	**-0.43**
Phosphatase	-0.18	**-0.54**	-0.31	-0.15	-0.38
Phenol oxidase	0.39	**0.40**	0.33	0.31	**0.46**
Peroxidase	0.16	**0.49**	**0.47**	0.18	0.32

Table 4.11 Spearman's rank correlations between SIR rates and PLFA signatures. Numbers in bold indicate strong correlations (ρ > 0.4).

	Glucose	Chitin	CMC	Malonic acid	Serine
Total microbial biomass	-0.18	**-0.61**	**-0.42**	-0.16	**-0.40**
Gram-positive bacteria	-0.18	-0.32	-0.18	-0.08	-0.01
Gram-negative bacteria	0.07	0.09	0.20	0.01	0.09
Actinomycetes	0.03	0.00	0.05	0.17	0.26
Total bacteria	0.00	0.01	0.21	0.04	0.18
Fungi	0.06	-0.09	-0.23	-0.14	-0.34
Arbuscular mycorrhizal fungi	0.13	0.21	0.24	0.09	0.28

Table 4.12 Spearman's rank correlations between enzyme activities and PLFA signatures. Numbers in bold indicate strong correlations (ρ > 0.4).

	β-glucosidase	NAGase	Phosphatase	Phenol oxidase	Peroxidase
Total microbial biomass	**0.68**	**0.61**	**0.77**	**-0.55**	**-0.67**
Gram-positive bacteria	0.13	-0.01	0.32	-0.24	**-0.42**
Gram-negative bacteria	-0.25	-0.07	-0.02	0.19	0.24
Actinomycetes	-0.12	-0.29	-0.09	-0.01	-0.04
Total bacteria	-0.25	-0.17	0.03	0.14	0.09
Fungi	0.29	0.32	0.10	-0.11	-0.01
Arbuscular mycorrhizal fungi	-0.24	-0.07	-0.02	0.21	-0.01

Table 4.13 Spearman's rank correlations between individual enzyme activities. Numbers in bold indicate strong correlations (ρ > 0.4).

	β-glucosidase	NAGase	Phosphatase	Phenol oxidase	Peroxidase
β-glucosidase	1.00	**0.71**	**0.70**	**-0.41**	**-0.47**
NAGase		1.00	**0.76**	**-0.47**	-0.34
Phosphatase			1.00	**-0.45**	**-0.54**
Phenol oxidase				1.00	**0.45**
Peroxidase					1.00

5 DISCUSSION

5.1 Comparisons of harvested treatments and uncut forest

The first objective of this study was to determine if retention of living, green-trees was better than clearcutting to maintain nutrient availability and the structure and function of the soil microbial community in a Douglas-fir, western hemlock forest in the Coastal Western Hemlock (CWH) biogeoclimatic ecosystem classification (BEC) zone five years after harvest. Using an adjacent uncut forest to provide a baseline comparison, the soil microbial community structure and function as well as *in situ* nutrient availability were found to respond differently to the three different harvesting treatments. The general expectation that green-tree retention would be more favourable than clearcutting in maintaining nutrient availability and soil microbial community structure and function was not supported by most of the findings from this study.

Hypothesis 1: Clearcutting will increase N availability, but will less affect P availability.

Five years after harvest, forest floor and mineral soil nitrate (NO_3^-) availability was slightly elevated in the clearcut relative to the uncut forest, although the difference was not statistically significant. Forest floor ammonium (NH_4^+) availability, on the other hand, was found to be significantly lower in the clearcut as well as in both green-tree retention treatments, than in the uncut forest. Interestingly, about 6.4 to 9.6 fold increases in both NO_3^- and NH_4^+ availabilities were observed four months after aggregated retention harvesting at the second replicate of the STEMS trial (STEMS 2) located within the same BEC zone (i.e. CWHxm), about 27 km northeast of STEMS 1 on Vancouver Island (Daradick 2007). Increased soil N concentration following harvesting has often been attributed to several factors, such as reduced uptake by plant roots, increased N mineralisation and nitrification (i.e. NO_3^- production from NH_4^+) rates due to elevated temperature and moisture, and lower N immobilisation by a smaller microbial biomass as a result of reduced C availability (Prescott 1997, Covington 1981, Titus et al. 2006). Post-harvest N

increase, however, has been shown to be, in most cases, temporary and is likely to decline with time after harvest. If the effects of harvesting on soil N at STEMS 1 and STEMS 2 had been similar, the flush of N after harvesting would have been brief and no longer observable after five years. However, it should be noted that the soils from the clearcut and uncut forest at STEMS 1 belonged to different site series that differ in soil nutrient regime (SNR), i.e. rich to very rich SNR (site series 05) in the clearcut vs. very poor to medium SNR (site series 01) in the uncut forest. Soils from the aggregated and dispersed retention treatments also belonged to site series 01. Therefore, the possibility exists that there is an inherent difference in N concentrations irrespective of harvest treatment; this could not be confirmed without the availability of pre-harvest data. A decrease in NH_4^+ in the clearcut and dispersed retention treatments was accompanied by a slight increase in NO_3^-. This might be attributed to reduced plant uptake of NO_3^- or lower nitrification in the clearcut and dispersed retention treatments than in the aggregated retention and uncut forest. Low availability of NH_4^+ in all three harvested treatments could also be due to reductions in decomposition and N mineralisation, which were not measured in this study. Alternatively, decreased NH_4^+ in all three harvested treatments may be related to a probable decrease in the population of soil fauna, which excrete excess N as ammonium (Marshall 2000).

Clearcutting and green-tree retention harvesting did not significantly affect the availability of orthophosphate (PO_4^{3-}), although there was a slight increase of this ion in the harvested treatments relative to uncut forest. This lack of difference in PO_4^{3-} is not unexpected because P is much less mobile than N due to its tendency of being tightly bound to soil mineral particles (Wood et al. 1984, Macrae et al. 2005). Yanai (1998) found no significant loss of P after harvest, despite reduced plant uptake of P within the first 2 years after clearcutting. Similar to the present finding, no significant differences in pre- and post-harvest PO_4^{3-} availability were observed four months after aggregated retention harvesting at STEMS 2 (Daradick 2007) and during the 5-year period after clearcutting and partial-cutting in second-growth grand fir, Douglas-fir, and western red cedar forests in northern Idaho (Gravelle et al. 2009). A decline in uptake of PO_4^{3-} due to reduced

root mass may be the reason for the slightly higher availability of PO_4^{3-} in the harvested treatments than in the uncut control.

Hypothesis 2: Soil N and P availabilities in the green-tree retention treatments will be intermediate between a clearcut and uncut forest.

Relative to the uncut forest, all three methods of harvesting had no significant effect on soil NO_3^- and PO_4^{3-} availabilities, but significantly reduced soil NH_4^+ availability. Neither of the green-tree retention treatments was superior to clearcutting in maintaining soil NH_4^+ availability. The expectation that green-tree retention treatments would have less effect on changes in nutrient availability than clearcutting was not supported by the current finding. As mentioned previously, this observation may have been confounded due to underlying site differences in soil nutrient regime. Site-level differences could also be the reason for a significant difference in NO_3^- availability between the aggregated and dispersed retention treatments. Bengtson et al. (2007) found that nutrient availability was spatially auto-correlated, i.e. locations close to each other are more similar than locations farther away, at the scale of tens to hundreds of metres at STEMS 2 prior to harvesting. Whilst partial-cutting has been reported to have intermediate effects on soil N availability compared to clearcutting (Lapointe et al. 2005, Lindo and Visser 2003), it is not uncommon to find no significant difference in soil N after clearcutting and partial-cutting (Kranabetter and Coates 2004, Bradley et al. 2001, Titus et al. 2006, Jerabkova 2006).

In the present study, NO_3^- dominated the mineral N pool in all three harvested treatments, whilst NH_4^+ was the more dominant mineral N form in the uncut forest. Plant uptake of NO_3^- in the uncut forest is most likely higher than in the harvested treatments due to relatively higher amounts of root biomass. Comparing the availabilities of NO_3^- and NH_4^+ in the forest floor and mineral soil, the clearcut appeared to have the largest increase of these ions down the soil profile. Although NH_4^+ is less mobile than NO_3^-, this observation may indicate that these ions drain away more readily in the clearcut than in the other treatments, and therefore green-tree retention might be better than clearcutting to reduce nutrient leaching.

Hypothesis 3: Enzyme activities will be lower in the clearcut than uncut forest, but will be retained in the green-tree retention treatments.

Five years after harvest there were no differences in enzyme activities among treatments, except for a significant decrease in phosphatase activity in the forest floor of both green-tree retention treatments as compared to the uncut forest. Despite the lack of statistical significance, there was a trend of decreased forest floor enzyme activities in all of the harvested treatments relative to the uncut forest, but green-tree retention treatments appeared to retain forest floor β-glucosidase, N-acetyl-glucosaminidase (NAGase), phenol oxidase, and peroxidase activities to levels intermediate between the clearcut and uncut forest. Decreases in soil enzyme activities following harvesting have also been observed by other studies (e.g. Hassett and Zak 2005, Waldrop et al. 2003), and this has often been linked to lower soil moisture, changes in the quantity and biochemical composition of litter, and declines in soil microbial biomass. In this study, reductions in soil moisture and total microbial biomass were most likely responsible for the decreased enzyme activities in the harvested treatments, as indicated by strong correlations between these variables and each of the five enzyme activities. It is particularly interesting to find no significant decrease in enzyme activities (except for phosphatase activity) in the harvested treatments in the present study because Daradick (2007) found a significant post-harvest decrease in β-glucosidase and NAGase activities, and a significant increase in phenol oxidase and peroxidase activities, whilst phosphatase activity was not significantly different after aggregated retention harvesting in the same forest type at STEMS 2. However, Daradick's study was undertaken only four months after harvest, in comparison to the five-years post-harvest sampling in this study. In addition, the relatively higher forest floor disturbance with harvesting at STEMS 2 compared to STEMS 1 (Grayston, pers. comm.) could have also contributed to the contrasting effect of harvesting on soil enzyme activities between the two studies. Forest floor removal and soil compaction, which commonly occur during harvesting, have been demonstrated to decrease enzyme activities (Tan et al. 2008, Hassett and Zak 2005). Further, there was no vegetation other

than the retention trees in Daradick's study, whereas replanted seedlings had been in the ground for about 4.5 years when soil samples were taken in this study. The presence of young seedlings in this study could have contributed to soil enzyme activities as plants also exude enzymes (Ladd 1978). The increased phosphatase activity in the uncut forest relative to the harvested treatments could be attributed to relatively low availability of PO_4^{3-} as a result of higher uptake by mature tree roots. Low availability of inorganic P in soil has been known to stimulate the production of phosphatase (Stevenson and Cole 1999).

Hypothesis 4: Harvesting will change microbial utilisation of carbon substrates, for example it will decrease chitin utilisation, an indicator of fungal biomass.

Soil microbial communities in the four treatments did not differ in their catabolic diversity profiles measured collectively based on the utilisation of five C sources - glucose, chitin, CMC, malonic acid, and serine. The similarity in substrate-induced respiration (SIR) rates across treatments may indicate that harvesting did not affect the ability of the soil microbial community to use a variety of C substrates commonly found in litter and root exudates, or that the metabolic activities of the soil microbial community have recovered to levels comparable to an uncut forest within five years after harvest. Decreased ability in the microbial biomass to utilise a wide range of substrates is often seen as an indication of reduced soil health (Chapman et al. 2007). It is interesting that despite shifts in soil microbial community structure (i.e. low fungal-to-bacterial ratios in the clearcut and uncut forest vs. high fungal-to-bacterial ratios in both spatial patterns of green-tree retention), microbial utilisation of a variety of C sources was similar in all treatments. This could indicate that the respiration of these substrates was not limited to a particular taxonomic group of microorganisms. It is also surprising that the utilisation of chitin, which is an indicator of fungal biomass (Kjøller and Struwe 2002), was not significantly lower in the clearcut and uncut forest where fungal biomass was low. Microbial respiration of serine was significantly lower in the dispersed retention treatment than the aggregated retention treatment. This may have been due to a higher serine concentration in the soil of the aggregated retention treatment due to higher

root biomass, which then selected for microorganisms capable of serine utilisation. Serine is a known tree root exudate (Grayston et al. 1997) and the selective effect of exudates on the soil microbial community has been observed previously (Grayston et al. 1998, Treonis et al. 2005).

The fact that only a limited number of C sources were used in this study could have contributed to the lack of discrimination among treatments. Although increasing the number of C sources will not necessarily increase discriminating power of the SIR method due to potentially strong correlations among these compounds (Campbell et al. 1997), using more substrates may help to uncover differences among treatments. Subjecting soils to a wider variety of C sources would provide better understanding of the microbial metabolic activity (Campbell et al. 1997). It was suggested that a soil microbial community with higher functional diversity would likely catabolise more unusual C sources (Campbell et al. 1997). Direct comparison of SIR findings from this study to others was difficult because very few studies of the effects of harvesting on soil microbial community function used SIR with more than one C source; the SIR method has often been used to estimate the amount of microbial biomass through the respiration of glucose. However, similar to the findings of the present study, Hannam et al. (2006) did not find significant effects of four harvest intensities (10, 20, 50, and 100% retention) on soil microbial catabolic diversity using MicroResp™ with 21 C sources in three forest stands, dominated by white spruce, trembling aspen, or both species, in Alberta.

The consistently higher rates of microbial respiration in the mineral soil than forest floor is unexpected, because soil respiration has been reported to decrease with soil depth as microbial biomass and soil C pools decrease (Jorgensen and Well 1973, Borken et al. 2002, Fang and Moncrieff 2005). Reductions in potential utilisation of C sources with forest floor depth was also observed by Grayston and Prescott (2005) using Biolog® plates. Higher rates of microbial utilisation of C sources have been linked to more readily available C sources in soils, which in turn may have a selective influence on microbial community structure (Grayston et al. 2004). One

possible reason for the higher mineral soil respiration rates might be the differences in the contribution of respiration rates from the fungal and bacterial component of the microbial biomass in the forest floor and mineral soil. Fungi have lower respiration rates per unit biomass than bacteria (Anderson and Domsch 1975), therefore, differences in SIR rates could be due to the higher fungal biomass in the forest floor than the mineral soil. Although PLFA analysis (calculated using relative abundance) did not indicate that fungal biomass was reduced in the mineral soil, it is likely that the absolute abundance of fungi was greater in the forest floor than the mineral soil, as both fungi and bacteria have been reported to decrease with soil depth as the amount of organic matter decreases (Agnelli et al. 2004, Ekelund et al. 2001).

Hypothesis 5: Clearcutting will decrease fungal biomass and increase bacterial biomass.

The abundances of both fungal and bacterial biomass were similar in the clearcut and uncut forest, although there was a slight increase in the abundance of bacterial biomass in the clearcut. Thus, the hypothesis above was not supported by the current findings. The results indicated that the composition of the soil microbial community showed a distinct separation into two clusters: (i) clearcut and uncut forest and (ii) aggregated and dispersed retention treatments. The clearcut and uncut forest had a significantly higher bacterial biomass and lower fungal biomass (or lower fungal-to-bacterial PLFA ratios) than either of the green-tree retention treatments. Changes in soil microbial community structure in the present study may be related to differences in soil moisture and pH among the treatments as soils in the clearcut and the uncut forest had lower pH and higher moisture than either of the green-tree retention treatments. Due to the lack of pre-harvest data, it is not possible to determine whether the shift in soil microbial community structure in the green-tree retention treatments was the result of harvesting or due to pre-existing differences in microbial community structure. Soil moisture has been reported as one of the main factors affecting the soil microbial community. For example, soil moisture explained for variations in microbial community structure and function across seven BEC zones in western Canada

(Brockett 2008). Moore-Kucera and Dick (2008) also found that changes in soil microbial community structure across a chronosequence of clearcut forests were mainly attributed to soil moisture. Soil pH has also been attributed to changes in soil microbial community structure, mainly in the ratios of fungi to bacteria (Frostegård et al. 1993, Bååth and Anderson 2003). Pre-harvest vegetation data indicated that tree species composition at STEMS 1 also followed the same trend observed for soil microbial community structure, i.e. the clearcut (87% Douglas-fir (Fd), 10% western hemlock (Hw), 0% western redcedar (Ch)) was similar to uncut forest (88% Fd, 10% Hw, 0% Ch), whereas the aggregated retention (77% Fd, 14% Hw, 9% Ch) was similar to the dispersed retention (79% Fd, 16% Hw, 4% Ch) (deMontigny 2004). The presence of cedar prior to harvest in both green-tree retention treatments could have contributed to higher soil pH in these two treatments compared to the uncut and clearcut. Western red cedar forests are characterised by high soil pH and base saturation as a result of high Ca content in cedar litter (Grayston and Prescott 2005, Berch et al. 2001). Other than increased soil pH, higher moisture in cedar forests was proposed as the reason for lower fungal-to-bacterial ratios in cedar forest floors than in Douglas-fir, western hemlock, and spruce forest floors (Grayston and Prescott 2005). Soils with high pH commonly have low fungal-to-bacterial ratios (Frostegård and Bååth 1996). Although it is intriguing that treatments with higher soil pH had high, instead of low, fungal-to-bacterial ratios, soil moisture could be the stronger factor influencing this ratio than soil pH. Gram-negative bacteria are known to be more sensitive than Gram-positive bacteria and fungi to low water availability (Harris 1981). In this study, treatments with lower soil moisture, i.e. both green-tree retention treatments, also had lower abundance of Gram-positive and Gram-negative bacteria and higher abundance of fungi than treatments with higher soil moisture. The fungal-to-bacterial biomass ratio has been shown to relate to N availability, with higher ratios in nutrient-limited environment and lower ratios in nutrient-rich environment (Pennanen et al. 2001). However, there was no indication that N availability was the reason for the differences in fungal-to-bacterial ratios

in the present study because the availabilities of NO_3^- and NH_4^+ in the clearcut were not significantly different from either the aggregated or the dispersed retention treatment.

Despite the similarity in microbial community composition between the clearcut and uncut forest, forest floor enzyme activities tended to be lower in the clearcut than uncut forest. This is interesting because the opposite observation, i.e. differences in microbial community composition not resulting in differences in microbial community function, has been more commonly reported, and has often been attributed to the functional redundancy of the soil microbial community (Marschner et al. 2003). It should be noted that PLFA analysis is not capable of detecting subtle shifts in microbial community structure, i.e. species-level, which may have been responsible for the functional differences in the clearcut and uncut forest. Not all members of the soil microbial community are capable of producing all soil enzymes. For example, phenol oxidase and peroxidase are known to be mainly produced by the white-rot fungi (Ander and Eriksson 1976). PLFA analysis would not be able to detect changes in the species composition of the soil fungal community because the PLFA biomarker 18:2ω6,9 represents total fungal biomass.

The ratio of cyclopropyl PLFAs to their monoenoic precursors was higher in the aggregated retention treatment than other treatments. This ratio is often used as an indicator of microbial stress in soil (Fierer et al. 2003). The monoenoic PLFAs are converted to cycolpropyl PLFAs as Gram-negative bacteria move from a log to a stationary phase of growth (Smith et al. 2000). Harvest disturbances, such as compaction and forest floor removal, have been previously shown to increase the ratio of cyclopropyl PLFAs to their monoenoic precursors, indicating that the growth conditions in the disturbed sites were less favourable than in undisturbed sites and that the turnover rate of Gram-negative bacteria was higher in the disturbed sites (Ponder and Tadros 2002).

5.2 Comparisons of spatial patterns of green-tree retention

The second objective of this study was to determine which spatial pattern of green-tree retention is more suitable to maintain nutrient availabilities and soil microbial community structure and function of an uncut forest by comparing these properties at different locations relative to retention trees in both green-tree retention treatments. Ammonium (NH_4^+) concentrations, β-glucosidase, N-acetyl-glucosaminidase (NAGase), phosphatase, and peroxidase activities, and the abundance of PLFA biomarkers characteristic of Gram-positive bacteria, actinomycetes, fungi, and arbuscular mycorrhizal (AM) fungi were all significantly affected by sampling location in the four harvest treatments.

Hypothesis 6: Nutrient availabilities and soil microbial community structure and function in the centre and at the edge of aggregated retention patches and beside the dispersed residual trees will be more similar to those found in the uncut forest than sampling locations in the cut area away from the retained trees.

The results showed no indication that nutrient availability and soil microbial community structure and function within the aggregated retention patches and beside dispersed residual trees were more similar to the uncut forest than were sampling locations away from retention trees, with the exception of forest floor phosphatase and mineral soil peroxidase enzyme activities in the aggregated retention treatment. The activities of these enzymes in the cut area of the aggregated retention treatment were significantly lower than in the uncut forest, but no significant differences were observed between the area within the retention patches and the uncut forest. The decrease in enzyme activities with distance into the opening in the aggregated retention treatment appears to be related to decreasing soil moisture. Jones et al. (2008) found that in the centre of aggregated retention patches of varying sizes (5- to 40-m diameter) a similar ectomycorrhizal (ECM) fungal community to uncut forest was retained, whilst there was a marked decrease in diversity of the ECM fungal community in the harvested areas at STEMS 2. In their study, soil samples were collected 4-6 months after harvesting, therefore there is a possibility that the

richness of the soil ECM fungal community within the retention patches would decline with time (Jones et al. 2008). In the present study, the differences between other measurements of microbial community structure and function in the centre of the aggregated patches and next to the dispersed retention trees and the uncut forest may reflect the fact that the uncut forest was spatially more distant from the retention treatments. Spatial variability in soil microbial communities is known to be vast (Deschesne et al. 2007, Martiny et al. 2006). This highlights the importance of using comparisons of factors measured before harvest and after in the exact locations.

Hypothesis 7: Nutrient availabilities and soil microbial community structure and function will be maintained up to 10 m from the edge of the aggregated retention patches and from dispersed residual trees.

This hypothesis was supported in part, as nutrient availability and soil microbial community structure and function were maintained well beyond 10 m from retention trees into the harvested areas by both patterns of green-tree retention. However, the activities of β-glucosidase, NAGase, and phosphatase showed a decreasing trend from the edge of the aggregated retention patches to 20-30 m out into the cut area, and peroxidase and phenol oxidase activities showed a decrease from the patch edge up to 10 m out into the cut area. β-glucosidase catalyses the release of glucose from cellulose and is produced primarily by fungi and bacteria (Hayano and Tubaki 1985). NAGase catalyses the breakdown of chitin and is mainly produced by white-rot fungi and actinomycetes (Miller et al. 1998). Phosphatase catalyses the release of inorganic PO_4^{3-} from organic P sources and is produced by bacteria, fungi, and plants (Speir and Ross 1978). Therefore, it is not surprising that phosphatase activity decreased with increasing distance from the aggregated retention patches, due to the direct decrease in root biomass with increasing distance from the retention patch (Welke et al. 2003) and the indirect effect of root biomass reduction on the enzyme-producing soil microbial communities reliant on root exudates for their activities (Vaughan et al. 1994). NAGase activity may have decreased in the cut area due to lower

abundance of soil fauna - a source of exoskeleton-derived chitin - as they rely on litter and microbes for their food source (Lindo and Visser 2003). In the aggregated retention treatment, total microbial biomass in the forest floor and the abundance of fungi in the mineral soil also showed a decreasing trend from the patch edge to 20 m and 30 m into the cut area, respectively. Much of the microbial community is reliant on exudates or litter material from plants for growth (Clarholm and Rosswall 1980), and therefore it is not surprising that microbial biomass decreased with increasing distance from the retention patches. Further, mycorrhizal fungi need the fine roots of trees to form their symbiotic associations (Harvey et al. 1980) and this may explain the decrease in fungal biomass with increasing distance in the aggregated retention treatment. A decline in ectomycorrhizal (ECM) fungal diversity and abundance with distance into the cut areas has been reported by a number of studies, e.g. Hagerman et al. 1999b, Kranabetter and Wylie 1998, Durall et al. 1999, Cline et al. 2005. Unfortunately, PLFA analysis is not able to differentiate the ECM fungal component of the total fungal biomass.

Hypothesis 8: Nutrient availabilities and soil microbial community structure and function will be retained to a greater distance into the cut area by aggregated retention than dispersed retention.

This hypothesis was not supported by the present results as there was a lack of significant differences in nutrient availabilities and soil microbial community structure and function among sampling locations in each retention treatment. In this sense, the greater root density and higher amount of litter fall concentrated within the aggregated retention patches did not seem to provide stronger influence than the roots and litter fall of individual residual trees in maintaining nutrient availability and soil microbial community structure and function. The similarity in microbial community structure and function across the whole dispersed retention treatment and lack of spatial variation may perhaps be attributed to the inter-tree distance of about 15-20 m (deMontigny pers. comm.). This could have practically subjected each 10-m interval sampling location to the ‘shadow’ from at least two residual trees adjacent to each other. These results

suggest that the root system and litter material of one single tree is sufficient to provide nutrients to support the growth and functioning of the soil microbial community. The present finding is in agreement with the results of Daradick (2007) and Jones et al. (2008), who found that a large aggregated retention patch (40-m diameter) was not necessarily better than smaller patches (5-, 10-, 20-m diameter) to retain nutrient availability, enzyme activities, and ECM fungal community in the cut areas.

Hypothesis 9: Nutrient availabilities and soil microbial community structure and function will be more similar among sampling locations in dispersed retention than in aggregated retention treatment.

This hypothesis was supported, as soil enzyme activities and soil microbial community structure appeared to be maintained more evenly across the cutblock in the dispersed retention treatment than in the aggregated retention treatment. As mentioned previously, this is most likely due to the influence of forest canopy from the retained trees that was more evenly-spaced throughout the cutblock than the forest canopy from aggregated patches. Other than being a nutrient sink, retention trees also influence the cycling of nutrients as they are the main source of leaf litter (Prescott 2002). In the aggregated retention treatment, most of the cut areas were not under the influence of mature residual trees, which may explain higher variability in this treatment. Luoma et al. (2006) found that dispersed retention of trees (15% basal area, spaced 25-30 m apart) could only retain ECM fungal diversity up to 4-5 m from tree bole 1-2 years after harvest. Comparing the aggregated and dispersed retention, Luoma et al. (2004) found no effect of spatial patterns at three levels of retention (15, 40, 75%) on ECM fungal sporocarp production, although the dispersed retention treatment appeared to maintain higher levels of sporocarp production at 15 and 40% retention. There was no indication if proximity to retention trees or patches had an effect on fungal sporocarp production.

In contrast to the trend observed from enzyme activities and the microbial community structure, nutrient availability appeared to fluctuate less with distance into the harvested areas in the aggregated retention treatment than dispersed retention treatment, despite the lack of statistical significance. There is a possibility that higher variability in nutrient availability in dispersed retention treatment might be attributed to the likelihood of a presence of mature trees adjacent to sampling points within the 30-m transect. Roots and litter from single residual trees separated 15-20 m away may result in 'hot spots' of microbial activity.

5.3 Relationships between microbial community structure and function and environmental variables

Hypothesis 10: There will be strong correlations between components of the measured biological variables (microbial community structure and function) and soil variables (moisture, pH, and nutrient availability).

In general, harvesting reduced soil moisture and increased soil pH. Mean soil pH was 0.36 to 0.89 units higher in the harvested plots than in the uncut forest. An increase in soil pH was also observed 3-4 years following clearcutting in a Korean red pine forest (Kim 2008). Kim (2008) suggested that the increase could have resulted from a reduction in root respiration and cation uptake by trees. However, pH in green-tree retention treatments was not intermediate between the clearcut and uncut forest. In the present study, soil moisture appeared to be a stronger influence on the function and, to a lesser extent, the structure of the soil microbial community than other measured soil physicochemical properties, i.e. soil pH and nutrient availabilities. This finding is not unexpected as soil moisture has been previously demonstrated as the factor mainly responsible for the rates of litter decomposition and changes in soil microbial community composition in BC forests (Brockett 2008, Prescott et al. 2005). Soil pH, on the other hand, appeared to mainly affect the structure of the soil microbial community. Soil pH was found to positively correlate with soil fungal biomass and negatively correlate with components of the bacterial biomass. This finding was contradictory to the expectation that fungi would be more

abundant than bacteria at lower pH. The results also showed that bacteria made up a greater proportion of the soil microbial community than fungi in this ecosystem, as indicated by the low fungal-to-bacterial PLFA ratios. Brockett (2008), who studied forest ecosystems at seven BEC zones in western Canada, also observed low fungal-to-bacterial PLFA ratios and low pH only in the CWH BEC zone. She attributed the dominance of bacteria to significantly higher N availability and lower C:N ratio in the CWH zone, relative to the other forest ecosystems in study, that would be favourable for the growth of bacteria. This is in agreement with the findings of Grayston and Prescott (2005) and Högberg et al. (2007), who observed lower soil fungal biomass with increased N availability.

Strong relationships between enzyme activities and nutrient availabilities were only observed between peroxidase activity and Fe, S, and Al availabilities, both of which were higher in the mineral soil than the forest floor. No strong correlations were observed between soil enzymes and the nutrients associated with their activities, e.g. phosphatase with PO_4^{3-} availability and NAGase with N availability. Although PO_4^{3-} availability was not found to strongly correlate with phosphatase activity, harvest treatments with higher PO_4^{3-} availability tended to have lower phosphatase activity. This is not unexpected because phosphatase activity is repressed by high P concentrations (Nannipieri et al. 2002).

The rates of chitin and serine utilisation were negatively correlated with total biomass and hydrolytic enzyme (β-glucosidase, NAGase, and phosphatase) activities and positively correlated with oxidative enzyme (phenol oxidase and peroxidase) activities. The positive correlation between oxidative enzyme activities and chitin utilisation potential might be due to the contribution from fungi, which are known to produce phenol oxidase and peroxidase enzymes to degrade chitin (Kjøller and Struwe 2002). Because total biomass consisted of a larger proportion of bacterial than fungal PLFAs, the negative relationship between chitin utilisation potential and the hydrolytic enzyme activities was not unexpected. Less is known about why serine also exhibited

similar relationships as the ones observed for chitin. A possible reason might relate to the ability of a number of filamentous fungal species, such as *Aspergillus spp*. and *Penicilluum sp*., in producing serine dehydratase - a serine-degrading enzyme (Elzainy et al. 2006).

The total microbial biomass was strongly positively correlated with all hydrolytic enzyme activities and strongly negatively correlated with both oxidative enzyme activities. In addition, peroxidase activity was negatively correlated with Gram-negative bacteria. The strong relationships between enzyme activities and total microbial biomass suggest that these enzymes are closely tied to the size of the living microbial biomass in soil and that microorganisms are the primary source of these extracellular enzymes in these soils. Hassett and Zak (2005) also attributed a decrease in enzyme activities to lower microbial biomass. The negative correlation between oxidative enzyme activities and total biomass may be explained by higher activities of these enzymes in the mineral soil where total biomass was low. The opposite behaviour of hydrolytic and oxidative enzyme activities down the soil profile (i.e. hydrolytic enzyme activities decreased whilst oxidative enzyme activities increased with soil depth) has been reported previously in the soils of two CWH forests (Daradick 2007, Brockett 2008). Daradick (2007) suggested that the higher activities of phenol oxidase and peroxidase activities in mineral soil could be due to recalcitrant lignocellulolytic materials that may be present in greater amounts in the mineral soil than forest floor as a result of leaching of phenolics from the forest floor which then bind to the mineral soil. The activities of β-glucosidase and NAGase were also found to somewhat positively correlate with soil fungi, indicating the importance of fungi in the cycling of nutrients. The activity of NAGase, which degrade chitin, has been suggested as a quantitative indicator for soil fungal biomass as fungi have been identified as the primary producer of this enzyme (Miller et al. 1998, Rodriguez-Kabana et al. 1983). Fungi have also been suggested as the primary source of β-glucosidase enzyme (Hayano and Tubaki 1985).

Activities of all five enzymes were found to strongly correlate with each other, positively between pairs of hydrolytic enzymes and between both oxidative enzymes, and negatively between the hydrolytic and oxidative enzymes. The reason for the varying correlations in enzyme activity might be the different behaviour of the two groups of enzymes and the availability of substrates down the soil profile, as mentioned previously. Hydrolytic enzymes degrade simpler, more labile C substrates, whereas oxidative enzymes degrade more recalcitrant compounds (Ladd 1978). Thus, it is likely that the two groups of enzymes were secreted under different environmental conditions.

5.4 General limitations in methodology

Although the methods used in this study offer adequate practicality and reliability to achieve the objectives of this study, they are not without limitations that may affect accuracy and reproducibility. In this study, soil nutrient supply rates measured using PRS™-probes tended to show a high variability. Whilst spatial heterogeneity of forest soils could have been a contributing factor to the observed variability, it could also be due to the limitations of the probes. PRS™-probes buried adjacent to plant roots may adsorb less amount of ions due to competition with plant roots, which are stronger sinks for soil ions than the probes. Incomplete contact between the probes and soil is another potential problem often encountered during long-term burial. The PRS™-probes have also been found to be sensitive to soil moisture (Johnson et al. 2005). Thus, differences in soil moisture could have confounded nutrient availabilities in the present study.

The MicroResp™ method was also a practical and quick method to measure substrate utilisation potential of soil microbial community. However, there is a concern of prolonged cold storage of soil samples between collection and analysis that is likely to occur during the MicroResp™ procedure. As carbon substrate availability becomes more limiting during storage, the composition of the microbial community may change (Goberna et al. 2005). Noticeable shifts in substrate-utilisation profiles have been observed after 3-24 days (Gonzalez-Quiñones et al. 2009) and after one month (Goberna et al. 2005) of storage at 4°C. In this study, soils were stored at

4°C for more than three months prior to SIR analysis, and this prolonged storage could have resulted in utilisation profile that is different from the profile obtained had freshly collected soils been used. Since the application of MicroResp™ in this study, the protocol has undergone a few changes to improve accuracy and data handling, such as decreasing the wavelength from 590 nm to 570 nm, using Purified Agar instead of Noble Agar, and modifying the formula used to convert absorbance reading to percent CO_2.

In this study, enzyme activity assays were able to detect changes in soil microbial community function better than substrate-induced respiration. However, the method only measures potential activity under laboratory conditions with optimum pH, temperature, and substrate concentration, and thereby would likely to differ from *in situ* activity. Enzyme activities may also not only represent the functioning of soil microbial community because enzymes are also known to be secreted by plants and soil fauna (Tabatabai and Dick 2002). Further, enzyme activities are substrate-specific, and a suite of only five enzymes tested in this study would not be able to represent the overall soil microbial activity, which also includes a much wider range of enzymatic reactions (Nannipieri et al. 2002). The oxidation of L-DOPA in the oxidative enzyme assay can also be non-enzymatic, and this becomes a problem especially in soils with low Mn availability (Hendel et al. 2005).

PLFA analysis successfully detected shifts in microbial community composition in the present study. It is nonetheless a fairly broad-scale method for the determination of soil microbial community composition. Only a few fatty acids are truly characteristic of certain groups of microorganisms, and many are unspecific. Therefore, actual differences in microbial community structure may be masked by these unspecific fatty acids (Marschner 2007). The PLFA 18:2ω6,9 is a known marker molecule for fungi, but it is also common in the eukaryotes, particularly plants (Zelles 1997), and soil fauna (Ruess et al. 2002). Thus, a proportion of the PLFA 18:2ω6,9 could have derived from very small fragments of plant roots that passed through the sieve during

sample preparation. The proportion, however, should have been relatively small because plant roots have lower surface area to volume ratio than fungal hyphae (Klamer and Bååth 2004). Sample storage is another important factor to be considered when performing PLFA extractions. Prolonged storage (about one year) of freeze-dried soil has been shown to reduce total PLFA concentrations by more than 30% (Wu et al. 2009).

5.5 Recommendations and future directions

1. The implementation of the green-tree retention system is likely to increase in the future, not only in the coastal forests of BC but also beyond. Thus, longer-term research on green-tree retention harvesting should be undertaken to provide forest managers and policy-makers with sound information from the belowground perspective.
2. In this study, only 11% dispersed retention and 18% aggregated retention treatments were evaluated. To help determine the most effective harvesting treatment, it may be necessary to include several other commonly applied levels of retention.
3. The combination of both spatial patterns of green-tree retention has been recommended to maintain essential soil processes across a cutblock (Luoma et al. 2004). It would be useful to include the combined patterns for future research to determine its effectiveness as compared to single patterns of retention.
4. This study was based on one-time sampling taken from a single site, i.e. STEMS 1. Ideally, changes in nutrient availability and the structure and function of the soil microbial community should be monitored before and immediately after harvest, and again periodically after harvest as stands age. The findings from this study should be integrated with the findings from the other two site replicates (STEMS 2 and STEMS 3) to better understand how harvesting affects the CWHxm ecosystem.
5. Site-level differences in soil properties and microbial community structure and function among the four harvest treatments should be explored further. The methods used in this study might have not detected subtle differences that could have been picked up by the

use of other methods. A combination of high and low resolution approaches is recommended.

6. To obtain a complete picture of the effects of harvesting on key soil processes, it would be useful to include the contributions from soil fauna and archaeal community. Soil-inhabiting members of the Archaea have been suggested to potentially play a role in nitrogen cycling, such as nitrification (Leininger et al. 2006, Cavicchioli et al. 2007).

6 CONCLUSIONS

In the ecosystem being studied, five years after harvest, green-tree retention was not substantially better than clearcutting in retaining nutrient availability and soil microbial community structure and function similar to levels found in the uncut forest. The availability of NH_4^+ was significantly reduced in all three harvested treatments compared to the uncut forest, whereas a significant decrease in forest floor phosphatase activity and a shift in microbial community composition were only observed in both green-tree retention treatments. Green-tree retention harvesting appeared to be more beneficial than clearcutting only for the maintenance of the activities of β-glucosidase, N-acetyl-glucosaminidase and peroxidase enzymes in the forest floor. However, it should be noted that this study was undertaken five years after harvest, which may mean that any initial differences in response of the soil microbial community to the various harvesting methods may have become masked with time. In the study by Daradick (2007) in the same forest type, four months after aggregated retention harvesting, she found increased availabilities of NO_3^- and NH_4^+ and activities of phenol oxidase and peroxidase and decreased activities of β-glucosidase and N-acetyl-glucosaminidase, although none of these variables was affected by sampling locations relative to the aggregated retention patches. In this current study tree seedlings were planted five to eight months after harvest. Therefore, five years after harvest, with the growth of these new trees, it might be expected that the response of the soil microbial community to harvesting may not be as severe as after four months. The overall resilience of the soil microbial community to harvesting could have been due to the maintenance of coarse woody debris on harvested sites and minimisation of disturbances on the forest floor during harvesting, although underlying pre-harvest differences among the four treatment sites may also have contributed to some of the findings in this study. One recommendation is that future studies should include pre-harvest samples for comparison to post-harvest samples, rather than comparison to an adjacent uncut forest as a control.

The present results also indicate that the structural characteristics of the soil microbial community did not respond to harvesting in the same way as the functional characteristics. For instance, soil microbial community structure in both green-tree retention treatments was clearly separated from that in the clearcut and uncut forest despite similarity in substrate utilisation potential and a general reduction of enzyme activities in the harvested treatments. This suggests that there is a functional redundancy within the soil microbial community in this ecosystem. Enzyme activities might represent narrow-niche functions of the soil microbial community and are therefore a more sensitive measurement for evaluating changes in the functioning of the soil microbial community than the substrate-induced respiration method.

Despite the aggregated retention patches, which were approximately 20 m in diameter with approximately 20-30 trees, resulting in a greater density of root biomass extending into the cut area, this treatment did not exert a larger forest influence that extended farther into the harvested area than single residual trees. On the contrary, the dispersed retention treatment appeared to be more suitable for the purpose of maintaining the structure and function of soil microbial community more evenly across a cutblock. This suggests that the root system of and litter from a single tree at the spacing of the dispersed retention treatment (15-20 m) was sufficient to maintain a functioning microbial community. Dispersed retention, however, is not without its limitations, which is especially pronounced in terms of windthrow loss. Thus, the application of both patterns of green-tree retention within the same area, or smaller but more numerous patches of the aggregated retention is recommended.

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