SOIL MICROBIAL ENZYME ACTIVITY AND NUTRIENT AVAILABILITY IN RESPONSE TO GREEN TREE RETENTION HARVESTING IN COASTAL BRITISH COLUMBIA

by

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ABSTRACT

Green Tree Retention (GTR) was evaluated for its potential to retain soil microbial activity and nutrient availability after harvesting in the Coastal Western Hemlock biogeoclimatic zone of B.C., Canada. Soil samples were collected from four sizes (5, 10, 20, and 40 m diameter) of GTR patch at the centre, edge, and along a northerly transect to 30 m beyond the groups of live trees prior to and a few months after harvest.

PRS™ Probes were used to determine the availability of nutrients; total nitrogen, nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}), encountered by plant roots before and after harvest. Before harvest, total nitrogen, NO_3^-, and NH_4^+ availability was similar in the organic layer and mineral layers. Phosphate availability was significantly higher in the organic layer than in the mineral layer before harvest. After harvest, nitrogen levels increased in both soil layers with NO_3^- levels significantly elevated in the mineral layer and NH_4^+ levels significantly elevated in the organic layer. There was no significant change in PO_4^{3-} after harvest. Nutrient availabilities after harvest varied little along the transects of the different sizes of retention patches. Increased availability of total nitrogen, NH_4^+, and PO_4^{3-} was more noticeable in the smallest (5 m in diameter) patch size when compared to the larger patch sizes (10 m, 20 m, and 40 m in diameter) after harvest.

The activities of five soil enzymes important in carbon, nitrogen and phosphorus cycling - β-glucosidase, chitinase, phosphatase, phenol oxidase and peroxidase - were measured using colorimetric or fluorimetric substrates and a microplate technique. Before harvest, hydrolytic enzyme activity (β-glucosidase, chitinase, and phosphatase) was higher in the organic layer than in the mineral layer. After harvest, hydrolytic enzyme

activity was still higher in the organic layer than in the mineral layer, although glucosidase activity decreased in the organic layer and increased in mineral soil after harvest, and chitinase activity decreased in the organic layer after harvest. Changes in glucosidase and chitinase activity (decrease in organic soil activity and increase in mineral soil activity) were more noticeable in the smallest (5 m in diameter) patch size when compared to the larger patch sizes (10 m, 20 m, and 40 m in diameter) after harvest. Phosphatase activity was significantly lower in the 5 m patch size after harvest and showed a trend of declining activity with increasing distance from the GTR patches after harvest in the larger retention patches. Before harvest, oxidative enzyme activity (phenol oxidase and peroxidase) was higher in the mineral layer than in the organic layer. After harvest, oxidative enzyme activity was still higher in the mineral layer than in the organic layer, although phenol oxidase activity increased significantly in mineral soil after harvest, and peroxidase activity increased significantly in both organic and mineral soil after harvest. The stimulation of the lignin-degrading oxidative enzymes following harvest may have been caused by lignin-rich woody substrate from slash left on site. The increase in phenol oxidase and peroxidase activity after harvest was more noticeable in the smallest (5 m in diameter) patch size when compared to the larger patch sizes (10 m, 20 m, and 40 m in diameter) after harvest. The change in enzyme activity and nutrient availability in response to harvest was greatest in 5 m retention patches for total nitrogen, NH_4^+, PO_4^{3-}, β-glucosidase, chitinase, phosphatase, phenol oxidase and peroxidase, suggesting that a minimum diameter of 10 m for GTR plots may be useful to retain soil microbial activity and nutrient availability after harvest.

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DEDICATION

This thesis is dedicated in loving memory to my great grandmother Margaret Daradick, who spent many days with me on hands and knees in the garden, revealing the wonderful things that can grow from a healthy soil with wisdom and enthusiasm.

INTRODUCTION

As clearcutting in British Columbia's coastal forests becomes less socially acceptable, forest managers are evaluating the effects of alternative silvicultural regimes with a multitude of objectives such as timber production, economics, soil disturbance, biodiversity, and public perception in mind. Within the Pacific Northwest, two silvicultural experiments are underway that are assessing the ecological, social and economic effects of a variety of silvicultural treatments at an operational scale. The first experiment, set up by the Washington State Department of Natural Resources, is called "Silvicultural Options for Harvesting Douglas-fir Young-Growth Production Forests" in Washington State (Curtis et al. 2004), and the second experiment, set up by the B.C. Ministry of Forests, is called "Silviculture Treatments for Ecosystem Management in the Sayward (STEMS) (de Montigny 2004). The focus of the research outlined in this thesis is the STEMS experiment in the Sayward Landscape Unit north of Campbell River on Vancouver Island, Canada, which is studying seven silvicultural treatments: 1) Uncut Control, 2) Extended Rotation with Commercial Thinning, 3) Dispersed Retention, 4) Aggregate Retention, 5) Group Selection, 6) Modified Patch Cut, and 7) Clearcut (de Montigny 2004). There are three replicates of the STEMS experiment; the first located in the Snowden Demonstration Forest, close to Campbell River, was harvested in 2001. The second replication at Elk Bay (where this study is focused), 75 km N of Campbell River, was harvested by Interfor in 2005. The third site at Gray Lake, in the Loveland Bay Provincial Park, will be harvested by B.C. Timber Sales in 2007. Detailed information about the STEMS research trial is available online at http://www.for.gov.bc.ca/hre/stems. The research in this dissertation used the second replication of the STEMS project,

concentrating specifically on the Aggregated Retention treatment (referred to here as Green Tree Retention). The management objective of the Green Tree Retention harvest pattern set out by the STEMS project is to create an uneven-aged stand that enhances visual quality and preserves biodiversity. The purpose of this thesis is to investigate the potential of Green Tree Retention to retain vital belowground ecosystem functions, namely, extracellular soil enzyme activity and nutrient availability after harvest. Effects of the size of standing patches of living trees in a 25-hectare Green Tree Retention harvest block, on enzyme activity and nutrient availability with distance from the patch were tested in the upper soil layers (LFH plus top 10 cm of mineral horizon) of the research site.

LITERATURE REVIEW

Current timber harvest practices in British Columbia's public forest resource incorporate economic, social, and environmental factors with sustainability in mind. Alternative logging strategies are increasingly being used in place of clearcutting, which replaced small-scale selective logging in the Pacific Northwest along with the advent of steam-powered technology the late 1800's (Rajala 1998). Harvesting patterns that more closely emulate natural disturbance patterns in size and frequency are being employed. Green Tree Retention is a partial-cut harvest pattern where islands of living trees are retained in groups on the landscape. The goals of Green Tree Retention are i) to retain forest structure and function, ii) to enhance visual quality, and iii) to preserve biodiversity in the ecosystem.

Logging disturbs the forest environment. Trees are extracted and the canopy barrier between the atmosphere and soil is eliminated, allowing sunlight to reach the forest floor more directly, and increasing surface temperature and moisture variability (Hasset and Zak 2005). The soil profile is unsettled by harvest activity, mixing organic and mineral soil layers and altering porosity to the possible detriment of soil productivity over time (Powers et al. 1989). Litter quality and quantity is affected, with dead roots beginning to decompose belowground and aboveground leaf fall being replaced by a pulse of woody slash (Covington 1981). The decomposition process of soil organic matter, which is essential for nutrient cycling and sustaining site productivity (Prescott 2005), changes as logging residues above and beneath the soil surface break down (Palviainen et al. 2005). Forest nutrient cycling is disrupted because nutrient uptake into living tree roots is severed (Vitousek and Reiners 1975) and release of exudates from living roots, which contribute to nutrient cycling processes, ceases. Transportation of harvested material off-site also draws nutrients from the ecosystem (Yanai 1998). Maintenance of woody material on the soil surface after harvest is the only method recommended to date by the B.C. Ministry of Forests as a method of preserving soil biodiversity after harvest (Forest Practices Code of British Columbia Act 1995). Nitrogen (N) is an important nutrient that is known to be mobilised in forest soil after harvest; availability spikes after tree removal, but is soon vulnerable to leaching loss as nitrate (Cole 1995). Higher levels of soil nitrogen have been explained by quicker decomposition of organic matter in moist and warm forest gaps (Bormann et al. 1974). Hart et al. (1994) postulated that decreased assimilation of N into microbial biomass may explain elevated soil N after harvest, with the halt of labile carbon inputs from living tree

roots restraining microbial growth, and demand for N. The biological component of the phosphorus (P) cycle can also be affected by a disturbed forest environment, but is less vulnerable to leaching because phosphate ions are easily bound to soil mineral particles (Yanai 1998). In the face of harvest disturbance, essential processes and functions must be retained to meet sustainability goals. This study addresses forest health from a belowground perspective, using enzyme activity and nutrient availability as functional indicators of soil biological community health and stability.

Timber harvest can alter soil characteristics such as pore space, organic layer thickness and integrity, temperature and moisture. The organic soil layer is a distinct living space for many soil organisms such as decomposing fungi (Wallwork 1970) and a variety of micro and macro-fauna that graze on fungal biomass (Price 1975). Pulleman and Tietema (1999) found that drying forest soil resulted in reduced microbial activity, and hypothesised that bacteria were more likely to be vulnerable to desiccation stress than fungi. Thus, Green Tree Retention has the potential to provide refugia for harvest-sensitive microbes that have limited dispersal capacity. Removal of organic matter and soil compaction are common results of harvesting that can alter the biological community in the soil (Marshall 1993). One year after a factorial combination of three levels of organic matter removal treatment, and three levels of compaction were applied at three locations in the SBS installation of LTSP, soil fauna were sampled sorted and identified from the top 10 cm of soil (Battigelli et al. 2004). Soil fauna in the LTSP study were found to respond to harvest in the short-term with reduced densities in mineral soil where the organic layer of soil had been displaced, which Battigelli et al. (2004)

attributed to a reduction in fungal biomass, a food source for soil fauna that inhabit the organic layer. Another study by Siira-Pietakäinen et al. (2001) showed a decrease in soil PLFA fungal:bacterial ratio, measured using PLFA in the organic layer of forest soil after clearcut, due to a reduction in fungal PLFA 18:2 ω6,9 the first growing season after harvest. Varying intensities of forest harvest have resulted in no change to fungal biomass, estimated using PLFA analysis (Hannam et al. 2006), a decrease in fungal biomass (Siira-Pietikäinen et al. 2001), and a significant boost to fungal:bacterial ratio in total microbial biomass (Entry 1986) within 5 years of harvest disturbance. Entry (1986) attributed the increased proportion of fungal biomass after harvest to slash left on soil surface, which provides substrate for saprotrophic fungi.

Green Tree Retention is a timber-harvest pattern that leaves patches of standing live trees within the cutblock, which can result in different site characteristics than clearcut harvest (Lajzerowicz et al. 2004). Green Tree Retention aims to mimic natural disturbance pattern and frequency (B.C. Ministry of Forests and Range 1995). Retaining live trees should present moderate conditions, more like a forest than a clearcut. Standing trees in a harvested area cycle nutrients and water, provide litter and root exudates to the soil, and shelter soil from sun, wind, and rain. Transpiration through leaves from remaining trees in Green Tree Retention patches leaves less water in the soil, reducing the threat of nutrient leaching, flooding and erosion after harvest (Feller 1997). Removing less timber volume may improve the visual quality of forested landscapes (Marc 2003). Retained structural diversity in GTR plots can provide a seed source that facilitates regeneration (Franklin et al. 1997) and shelters a wide variety of forest

organisms from logging disturbance (Matveinen-Huju et al. 2006). Barg and Edmonds (1999) assessed the effects of GTR on key functions of a Douglas-fir forest in the Pacific Northwest USA. Within GTR plots, 15 metres in diameter, there were intermediate air temperatures, soil temperatures, and spring evapotranspiration. However, Barg and Edmonds (1999) did not find that site characteristics such as net ammonification, nitrification, or total N mineralization in GTR plots were more like uncut than clearcut plots. Green Tree Retention has been shown to decrease light transmitted to the forest floor, and maintain lower temperatures, when compared to soil in clearcuts, in Washington State (Heithecker and Halpern 2006). The mineral N flush from partial-cut harvesting was less dramatic than the mineral N flush from clearcutting, compared to intact stands in Quebec's Abitibi region (Lapointe et al. 2005). Other factors contributing to the effects of GTR harvesting on nutrient status and cycling may include season, slope and aspect. Leaving patches of standing trees can alleviate the exposed surface desiccation of forest soil (Rose and Muir 1997). Standing trees adjacent to cut areas have been shown to provide suitable habitat for ectomycorrhizal fungi (Amaranthus and Perry 1994) for distances up to 5 meters from single trees in a dispersed retention pattern (Luoma et al. 2006) and up to 16 metres into a cut block from an intact forest edge (Hagerman et al. 1999). Preserving patches of live trees may also retain the structure and function of the free-living microbial community in soil through the supply of C from root exudation (Butler et al. 2003, Hogberg and Read 2006). To preserve soil microorganisms that are symbiotically root associated, like mycorrhizae, or indirectly dependent on living tree roots to provide readily available carbon, retaining some trees on-site is important.

Current knowledge outlined here suggests that aggregates of living trees, as seen in GTR, may be a more suitable partial-harvest strategy than dispersed retention.

Enzymes are proteins with substrate-specific activity that can quickly and easily be measured in the soil (Burns and Dick 2002). Enzymes naturally originate from plant, animal, and microbial sources, although microbes are the major contributors to soil enzyme activity (Speir and Ross 1978). They may be present in the soil via secretion from living organisms or leakage from dead cells, and may be free to react with substrates, or be bound to soil components (Nannipieri 1994). The activities of extracellular soil enzymes produced by soil microorganisms play an important role in nutrient cycling because they catalyse the breakdown of complex organic matter into nutrients that are available for plant uptake (Sinsabaugh et al. 1994). Enzyme activity is affected by soil physico-chemical characteristics such as temperature, moisture, and substrate availability, as well as microbial biomass and the presence of vegetation (Decker et al. 1999). Timber harvest can alter these factors in the soil environment and potentially affect forest nutrient cycling (Dahlgren and Driscoll 1994). Soil enzyme activity levels directly represent potential degradative ability of the soil community (Moorhead and Sinsabaugh 2000). Enzyme activity has been shown to respond quickly to environmental disturbances (Boerner et al. 2000), and it is easy to measure (Miller et al. 1998). The most important enzymes in the forest soil are those that degrade the most abundant biopolymers, the organic compounds lignin and cellulose (Fioretto et al. 2005), as well as those that cycle P and N (Sinsabaugh and Moorhead 1996). Enzyme-mediated mineralization tends to be substrate-specific, potentially affecting nitrogen and phosphorus differently (McGill and Cole 1981). β-1, 4-glucosidase is an enzyme

produced by bacteria, fungi and protozoa and is involved in cellulose degradation (Burns and Dick 2002). Hasset and Zak (2005) attributed a decline in glucosidase activity under high and low harvest intensities to a decline in soil microbial biomass. Glucosidase activity in the O horizon has been shown to decrease, along with a decrease in soil N and P concentrations, after harvest where slash is chipped and piled into windrows, which is likely due to the redistribution of substrate on site (Waldrop et al. 2003). Glucosidase activity increased in response to the growth of new active roots in herbaceous scrub plants, providing evidence that glucosidase can be used as an indicator for an environment rich in easily available carbon. N-acetyl-β-glucosaminidase (chitinase) is an enzyme that releases nitrogen bound to chitin, a ubiquitous organic polymer (Sinsabaugh et al. 1993). Chitinase is naturally produced by bacteria, fungi and plants (Jollès and Muzzarelli 1999), and chitinase activity is a reliable indicator of fungal biomass in soil (Miller et al. 1998). Acid phosphatase, produced by plant roots and soil microorganisms, catalyses the breakdown of organic P and releases plant-available inorganic P (Ho 1979). Nitrogen and phosphorus are critical nutrients in a forest ecosystem; N and P cycles interact in the process of organic matter decomposition (Wang et al. 2007). Phosphorus is processed biochemically as it is needed and production of phosphatase, a nitrogen-rich enzyme, is dependent on adequate N supply (McGill and Cole 1981). Peroxidase and phenol oxidase are oxidative enzymes produced by white-rot fungi that play an important role in lignin degradation (Ander and Eriksson 1976, Baldrian 2006). Peroxidase and phenol oxidase activity have been shown to respond negatively to increased N (Gallo et al. 2004), which is attributed to direct physiological repression of enzyme expression in soil fungi. Phenol oxidase activity is positively correlated with fungal biomass and

organic matter degradation in soil (Baldrian 2006), which shows that enzyme activity can be an indicator of microbial habitat and activity. The activities of oxidative enzymes have been stimulated in soil with addition of a lignin-rich substrate to the environment (Snajdr and Baldrian 2006). Since forest harvest leaves lignin-rich debris aboveground and belowground, oxidative enzyme activity could be expected to increase after harvest. Green Tree Retention is a partial harvest option that may mitigate this change to soil enzyme activity.

Nitrogen is a critical forest nutrient, which is available for plant uptake in both nitrate (NO_3^-) and ammonium (NH_4^+) forms (Lutz 1959). Mineralization of N and P in forest soil was originally thought to increase availability of these nutrients after harvest because trees were no longer present to absorb them via root uptake (Wood 1984; Covington 1981; Fisher et al. 2000). The reduction in the soil microbial biomass after harvest (Entry et al. 1986) also increases the availability of N in post-harvest soil because less N is being assimilated into that microbial biomass (Hart et al. 1994). Labile nitrate is vulnerable to leaching from forest ecosystems after harvest, with fewer trees to take up nutrients from the soil (Schulze 2000). Prescott (1997) found that clearcutting increased N availability in forest floor material of a montane forest, significantly more than Green Tree Retention harvest (25 stems left standing per hectare) increased N availability two years after harvest. Prescott (1997) attributed more available N in soil to reduced carbon inputs from root exudates after harvest, limiting microbial activity and ability to immobilize N. However, Barg and Edmonds (1999) found no significant differences in total N mineralization between clearcut and GTR (20-30 stems left standing per hectare)

sites 2-5 years after harvest. The stability of N mineralization rates in surface mineral soil across harvest treatments found by Barg and Edmonds (1999) may be explained by the stability of soil microclimate conditions and microbial biomass measurements before and after harvest. More recently, research in the Montane Alternative Silvicultural Systems (MASS) program on Vancouver Island has shown a lack of response in available N to partial-cut harvesting (Titus et al. 2006). In this study, where patches of varying diameters are retained, larger patches of living trees have the potential to maintain larger functioning root zones within the GTR cutblock which may foster healthy microbial communities in the soil and preserve nutrient cycling and availability.

Phosphorus is a nutrient in high demand in forest ecosystems, available to plants as orthophosphate (PO_4^{3-}), and $H_2PO_4^-$ in acidic soil solution (Bohn et al 2001). Macrae et al. (2005) found that partial harvest and intact forest plots did not significantly differ in water-extractable soil P concentrations. Simard et al. (2001) found clearcuts had higher mean extractable P in organic soil layer 2 years after harvest, and this pulse of nutrients carried through to the mineral soil layer. The pulse of available phosphorus was not seen 14 and 21 years after harvest in concurrent studies. Clearcut harvesting has been shown to increase amounts of labile P in the organic horizon but not in mineral subsoil (Piirainen et al. 2004), which they attributed to increased mineralization of organic P in the O-horizon, and efficient adsorption of PO_4^{3-} ions by aluminum and iron oxides in mineral soil. Phosphate ions are more likely to be bound to mineral particles in the A horizon than they are to be lost in streamflow (Macrae et al. 2005). Current literature shows that phosphorus is less vulnerable to leaching and erosion losses than nitrogen,

even after clearcut harvest. Green Tree Retention has the potential to sustain soil P levels, a component of ecosystem function.

Nutrient availability can be measured in a number of ways: including conventional extractions that measure total and organic nutrients available at one point in time, and Plant Root Simulator (PRS^{TM}) probes that measure bioavailable nutrients over a period of time. PRS^{TM} probes present an economical and uncomplicated way to quantify nutrient supply rates in the field, causing minimal disturbance to the study environment. PRS^{TM} probes contain either a cation or an anion exchange resin encased in plastic (see Figure 2, Materials and Methods section) that is inserted directly into soil in the field, where the resin is presented with the environmental conditions affecting nutrient availability. Nutrient supply rate as measured by PRS^{TM} probes is expressed as: weight of nutrient adsorbed per surface area of ion exchange resin over burial period (i.e., μg nutrient·10 cm^{-2} · 62 $days^{-1}$). PRS^{TM} probe technology relies on the principle of Donnan potential, seen in plant root attraction and absorption of nutrient ions from the soil (Barber 1995). PRS^{TM} probes can absorb nutrients throughout the burial period until soil buffering capacity is reached.

Thesis theme and objectives

Based on the current literature, we expect timber harvest to have a measurable effect on soil biochemical characteristics. The objectives of this study are to determine: i) whether soil nutrient (total N, NO_3^-, NH_4^+, and PO_4^{3-}) availability, and the activities of

β-1, 4-glucosidase, N-acetyl-β-glucosaminidase, phosphatase, phenol oxidase and peroxidase measured in GTR plots one growing season after harvest in a coastal second-growth Douglas-fir stand in BC are more similar to pre-harvest levels than data collected in the surrounding cut area. ii) will these parameters measured in larger patches be more similar to pre-harvest levels than data collected in smaller patches? and iii) will the influence of GTR patch diminish as sampling extends into the clear-cut area. To address these objectives we studied nutrient availability and enzyme activity at the centre, edge and along a transect from the edge going out 30 m into the clear-cut, in four replicates of four aggregated-retention plot sizes (5 m, 10 m, 20 m, and 40 m diameter). We addressed the hypothesis that enzyme activity and nutrient availability will remain closer to pre-harvest levels at soil sampling locations within, and at the edge of GTR plots, than at pre-determined sampling locations in the harvested area. We also addressed the hypothesis that enzyme activity and nutrient availability will remain closer to pre-harvest levels at soil sampling locations within larger GTR plots. Furthermore, we addressed the hypothesis that the influence of GTR patch will decrease with distance away from remaining live trees. This research will assist with recommendations of the Forest Practices Code which at the moment, only recommend coarse woody debris retention to maintain soil biota after harvest.

INTRODUCTION TO THE STUDY

I studied the effects of Green Tree Retention (GTR) harvesting on forest floor microbial communities in a coniferous temperate rainforest on the eastern coast of Vancouver Island, British Columbia. The aim is to determine the effectiveness of GTR in

providing shelter for soil microorganisms, and sustaining their function in forest nutrient cycling. I evaluate this by measuring soil enzyme activity and nutrient availability. I also explore how the size of GTR patch affects soil processes, in patches with diameters ranging from 5 m to 40 m.

MATERIALS AND METHODS

Study Site

The site was located at Elk Bay (50°20'N, 125°28'W) within the Sayward Landscape Unit on the east coast of Vancouver Island, British Columbia, in the very dry maritime subzone of the Coastal Western Hemlock (CWHxm) biogeoclimatic zone (Green and Klinka 1994). The climate is characterized by warm, dry summers and moist winters. Mean annual temperature is 8.2°C, and mean annual precipitation is 1505 mm which falls mostly as rain in the winter months. The low elevation terrain (37-160 meters above sea level) has gently rolling topography with numerous lakes and streams. The soil type is Orthic Humo-Ferric Podzol with moder humus forms. The organic soil layers range from 10 to 30 cm deep and the well-drained mineral layers are strongly podzolized with distinct Ae and Ai horizons. The soil supports mostly western hemlock (89%), western redcedar (5%), and Douglas-fir (6%) trees with a sparse understorey containing sword fern. The forest is second-growth, the site was previously harvested and burned in the 1940s. The study site has a north/northeast aspect which faces Discovery Passage, a frequently traveled cruise ship route with high landscape visibility (Figure 1).

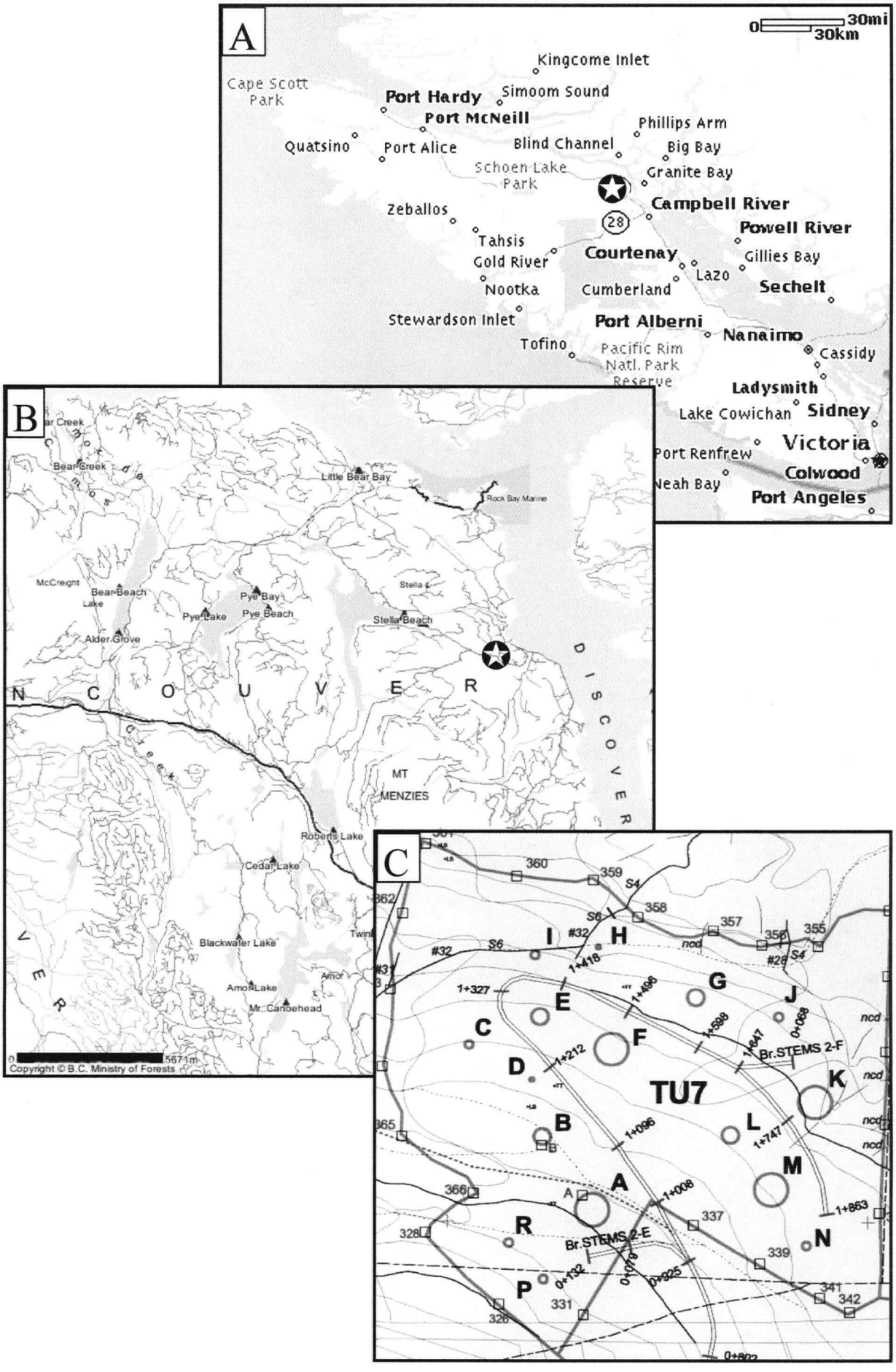

Figure 1. A: Map of Vancouver Island with a star showing Sayward Valley
B: Close-up map of Vancouver Island with a star showing Elk Bay, location of study site.
C: Green Tree Retention treatment layout, showing circular plots.

The 25-hectare study site was established in February 2004, one year before harvest was to take place. Permanent GTR plot locations were determined, marked at their centre with a 1-meter length of rebar driven into the ground, and mapped so that samples could be collected from the same location before and after harvest. Sample locations extending towards magnetic north from the centre of each GTR plot were predetermined and marked with PVC piping painted orange and labeled with metal tags at the center, edge, 5 m, 10 m, 15 m, 20 m and 30 m beyond the edge. Four replicates of each GTR plot size (5 m, 10 m, 20 m, and 40 m in diameter) were situated randomly within the 25-hectare site.

Sampling

For enzyme activity analysis, four soil cores of the entire forest floor layer, 10 cm deep into the mineral soil and 5 cm in diameter, were collected at predetermined sampling locations before and after harvest. Soil cores were divided into organic and mineral components. Each sampling location was represented by a single bulked organic-soil sample and a single mineral-soil sample from the four sample cores collected. Pre-harvest samples were collected at the centre, edge and 30 m beyond each GTR plot in October 2004. Harvest occurred from April to July 2005. Post-harvest samples were collected at the center, edge, 5 m, 10 m, 15 m, 20 m and 30 m beyond the edge of each GTR plot in October 2005. A stainless-steel corer was used to extract samples from the soil, and they were placed into labeled plastic bags for transportation in a cool box with ice. Samples were mixed, sieved (<8 mm) and stored at -20 ° C prior to analysis.

Enzyme Assays

Methylumbelliferone (MUB) linked substrates were used to determine the rate of enzyme activity in organic and mineral soil at pre-determined locations before variable-retention harvest. We evaluated the activities of the following enzymes:

1. β-1,4-glucosidase (Glucosidase)
   (EC 3.2.1.21)
   A hydrolytic enzyme that breaks down cellulose
2. N-acetyl-β-glucosaminidase (Chitinase)
   (EC 3.2.1.52)
   A hydrolytic enzyme that degrades chitobiose sugars (chitin polymer subunits)
3. Acid Phosphatase (Phosphatase)
   (EC 3.1.3.2)
   A hydrolytic enzyme that releases phosphate ions
4. Phenol oxidase
   (EC 1.10.3.2)
   An oxidative enzyme that degrades phenolic compounds
5. Peroxidase
   (EC 1.11.1.7)
   An oxidative enzyme that degrades phenolic compounds

Soil slurries containing 0.02 g of soil from each composite sample suspended in 100 mL of 50 mM sodium acetate buffer (pH 5.0) were homogenized (Kinematica Polytron benchtop homogenizer PT 10-35; Polytron Devices Inc., Paterson, NJ) for one minute at speed level 5. Eight replicates of each enzyme assay were performed on individual 96-well black microplates with a 300-μL capacity in each well (Costar microplate 3904; Corning Life Sciences, Acton, MA). Each plate also contained eight replicates of a standard positive control, a substrate negative control, a quench standard, a soil background, and a buffer negative control (Table 1). Plates were kept at 25°C, allowing enzymes present in soil samples to cleave MUB-linked substrates for an

appropriate incubation time (Table 3). Reactions were terminated with the addition of 25 µL of 0.5N NaOH to each well. Fluorescence of MUB cleaved from enzyme substrate was measured using an f-max fluorimeter (CytoFluor II Fluorescence Multi-Well Plate Reader; Applied Biosystems, Foster City, CA) with excitation energy set at 360 nm and emission energy set at 460 nm. Enzyme activity is reported as nanomol of 4-MUB linked substrate (4-MUB- ß -D-glucoside, 4 MUB-N-acetyl- ß -glucosaminide, or 4 MUB-phosphate) converted per hour per gram of dry soil (nmol $\cdot g^{-1} \cdot h^{-1}$).

Table 1. Setup of reagents for hydrolytic enzyme assays in black Costar microplates

	Sodium Acetate Buffer	200 µM Substrate	10mM 4-MUB Standard	Soil Suspension
Soil sample		50 µL		200 µL
Standard positive control	200 µL		50 µL	
Substrate negative control	200 µL	50 µL		
Quench standard			50 µL	200 µL
Soil background	50 µL			200 µL
Buffer negative control	250 µL			

Activities of the lignin-degrading enzymes phenol oxidase and peroxidase were determined using colorimetric assays. In a procedure similar to the assay described above, enzyme activity was assessed by the oxidation of *L*-3, 4-dihydroxyphenylalanine (*L*-DOPA), a reaction that causes a quantitative colour change. Soil slurries containing 0.25 g of soil from each composite sample suspended in 100 mL of 50 mM sodium acetate buffer (pH 5.0) were homogenized. Eight replicates of each enzyme assay were performed on individual 96-well clear microplates (Costar microplate 3370; Corning Life

Sciences, Acton, MA). Each plate also contained eight replicates of a buffer negative control, a soil background, and a substrate negative control (Table 2). Hydrogen peroxide (10 μL 0.3% H_2O_2) was added to each well in the peroxidase assay plates. Plates were kept at 25°C, allowing enzymes present in soil samples to react with *L*-DOPA substrate for an appropriate incubation time (Table 3). Absorbance was detected by a colorimetric plate reader (SpectraMax 340 Microplate Spectrophotometer; Molecular Devices Corporation, Sunnyvale, CA) at 460 nm. Enzyme activity is reported as nanomol *L*-DOPA converted per hour per gram of dry soil (nmol $\cdot g^{-1} \cdot h^{-1}$).

Table 2. Setup of reagents for oxidative enzyme assays in clear Costar microplates. * Hydrogen peroxide was added to peroxidase assay plates only.

	Sodium Acetate Buffer	*L*-DOPA substrate	Hydrogen Peroxide 0.3%	Soil Suspension
Soil sample		50 μL	10 μL*	200 μL
Substrate negative control	250 μL	50 μL	10 μL*	
Soil background	50 μL		10 μL*	200 μL
Buffer negative control	250 μL		10 μL*	

Table 3. Enzyme assays performed and incubation time at 25°C

Enzyme Reaction	Incubation time
β-1,4-glucosidase	3 hours
Phosphatase	2 hours
Chitinase	3 hours
Phenol oxidase	18 hours
Peroxidase	5 hours

Nutrient Availability

Plant Root Simulator (PRS^{TM}) probes (Western Ag Innovations Inc. Saskatoon, Canada) were used to determine the availability of total nitrogen, NO_3^-, NH_4^+ and PO_4^{3-} before and after harvest. PRS^{TM} probes were placed vertically in the soil at the same predetermined sampling locations used for soil sampling for each GTR plot before and after harvest. Four cation-exchange and four anion-exchange PRS^{TM} probes (Figure 2) were inserted into organic and mineral soil layers at each sampling location, and incubated in the field for 62 days. After incubation PRS^{TM} probes were cleaned gently with a brush to remove visible soil, and rinsed with deionised water. Analyses of nutrients adsorbed to probe membranes through the burial period were performed by Western Ag Innovations Inc. Saskatoon, Canada. There, the probes were eluted using a weak acid or salt solution. The eluate was analyzed for (NH_4^+) and (NO_3^-) levels using automated colourimetry. Total N was calculated by adding NH_4^+ and NO_3^-. Phosphorus levels in the eluate were determined using inductively-coupled plasma (ICP) spectrophotometry. Nutrient supply rates determined using PRS^{TM} probes are reported as the amount of nutrient adsorbed per amount of adsorbing surface area per time of burial in soil (i.e., μg nutrient·10 cm^{-2} · 62 $days^{-1}$), and are a measure nutrients available to plant roots in the soil (Qian and Schoenau 2002).

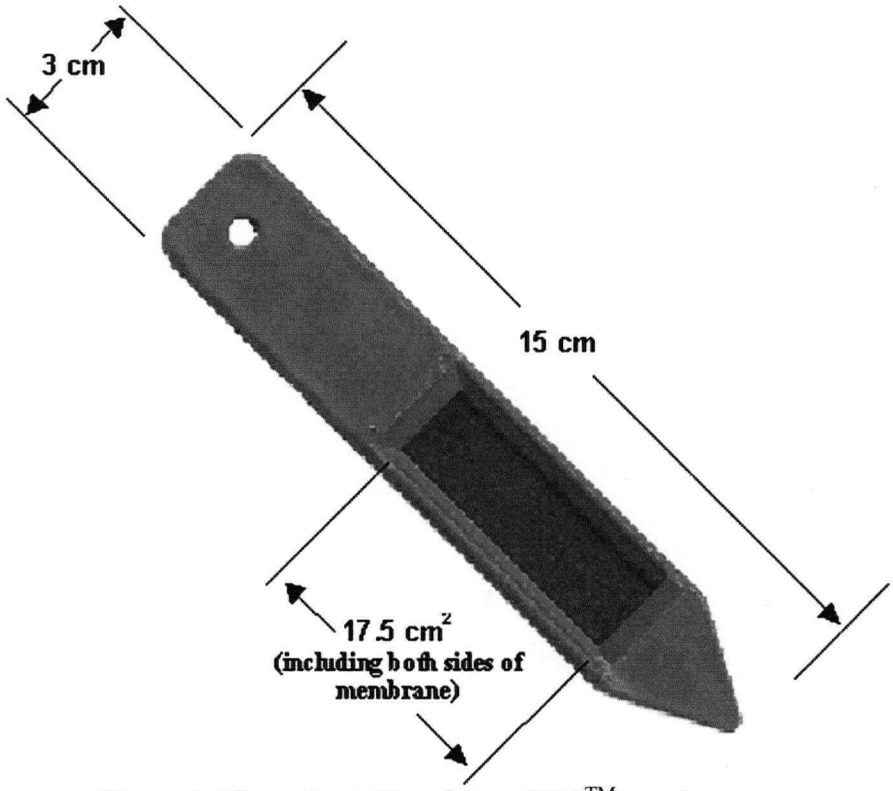

Figure 2. Plant Root Simulator (PRS™) probe

Statistical Analysis

Before harvest, the experimental design was a split-plot in a completely randomised design in mineral and organic soil layers. Sampling locations within the unreplicated GTR experimental unit are the same before and after harvest. Data were analysed using JMP IN Academic Version 4.0.3 (SAS Institute Inc., Cary, NC). After harvest, the experimental design was a completely randomised design arranged in a factorial treatment arrangement. The correct testing of each factor was decided from the components of variance for split-plot model (pre-harvest), or factorial treatment model (post-harvest).

Table 4. Analysis of variance table for split-plot model, showing the proper F-tests.

Source of variation	Degrees of freedom	F-test	$F_{critical\ value}$ degrees of freedom
Plot size (M)	3	MS_M/MS_{E1}	(3, 12)
Error 1 (E1)	12		
Sampling Location (S)	6	MS_S/MS_{E2}	(6, 72)
Plot size x Sampling Location (MxS)	18	MS_{MxS}/MS_{E2}	(18, 72)
Error 2 (E2)	72		
Total	111		

Table 5. Analysis of variance table for factorial model, showing the proper F-tests.

Source	Degrees of freedom	F-test	$F_{critical\ value}$ degrees of freedom
Plot size (F1)	3	$MS_{(F1)}/MS_E$	(3, 84)
Sampling location (F2)	6	$MS_{(F2)}/MS_E$	(6, 84)
Plot size X Sampling location	18	MS_{F1xF2}/MS_E	(18, 84)
Error	84		
Total	111		

To determine if Green Tree Retention harvest had an effect on soil biochemical characteristics, treatment means were tested for significant differences using a one-way analysis of variance (ANOVA). Non-parametric Kruskal-Wallis ANOVA (one-way ANOVA on ranks) was used when the assumptions of parametric tests were not met. Post-hoc pairwise comparisons were made with the Bonferroni test ($P < 0.05$).

RESULTS

Soil biochemical characteristics before harvest

Availability of total N, NO_3^--N, and NH_4^+ -N in the organic soil layer was similar to the availability of that nutrient in the mineral soil layer before Green Tree Retention harvest (Table 6). Phosphate (PO_4^{3-} -P) levels were significantly higher in the organic than the mineral soil layer before harvest (Table 6). There was very little variation in the availability of these nutrients at predetermined sampling locations in varying sizes of future patches before harvest (Tables 7, and 8). Nitrate (NO_3^--N) did show some pre-harvest variability between different future patch sizes in the mineral soil layer, with a higher mean value of 4.700 $\mu g \cdot 10\ cm^{-2} \cdot 62\ days^{-1}$ in the future 40-m patch, and a lower mean value of 1.633 $\mu g \cdot 10\ cm^{-2} \cdot 62\ days^{-1}$ in the other patch sizes (Table 8).

Before harvest, activity of extracellular soil enzymes had low variability in organic and mineral soil layers when measured at predetermined locations throughout the 25-hectare cutblock (Table 9, Table 10). The hydrolytic enzymes (glucosidase, chitinase, and phosphatase) were significantly more active in the organic layer than in the mineral layer before harvest (Table 6). The oxidative enzymes (phenol oxidase and peroxidase) were significantly more active in the mineral layer than in the organic layer before harvest (Table 6).

Soil biochemical characteristics after harvest

Four months after harvest was completed, overall availabilities of all nutrients increased (Table 6), and a significant difference was seen between soil layers for PO_4^{3-} -P and NH_4^+ -N which had significantly higher levels in the organic layer, as well as NO_3^--N which had significantly higher availability in the mineral layer (Table 6). There was no significant difference in availability of total N, NO_3^--N, NH_4^+ -N nor PO_4^{3-} -P between soil samples collected from differing patch sizes in either soil layer after harvest (Figures 3, 4, 5, and 6). Within GTR plots, there were no significant differences in nutrient availability at the centre, edge, and 5 m, 10 m, 15 m, 20 m, and 30 m beyond plot edges in any patch size, although nutrient availabilities tended to increase as sampling occurred further from patch edge, up to maximum levels between 10 m and 15 m away in both soil layers after harvest (Appendices 1, 2, 3, and 4). Five-meter retention patches were least likely to maintain soil nutrient availability near pre-harvest levels (Figures 3, 4, 5, and 6).

Four months after harvest was completed, there was still a significant difference in enzyme activity expressed in the organic layer than in the mineral soil for all the enzymes measured, as there was prior to harvest (Table 6). Enzyme activities in organic and mineral soil changed very little, or trended towards median values between pre-harvest highs and lows for all except peroxidase, which was significantly more active after harvest in both soil layers (Table 6). There was no significant response to patch size (Figures 7, 8, 9, 10, and 11) or sampling location (Appendices 5, 6, 7, 8, and 9) after harvest for any enzyme that could be attributed to treatment effect. However, similar to

nutrient availability after harvest, enzyme activities tended to increase with distance from the patch edge to 10-15 m into the harvested area, with the exception of phosphatase (Appendices 5, 6, 7, 8, and 9). Phosphatase activity was not significantly stimulated or inhibited in organic or mineral soil four months after harvest was completed (Figure 9), although there was an indication of higher phosphatase activity from organic soil samples collected in the centre or at the edge of the largest patch size (40 m) when compared to samples collected up to 30 m beyond the GTR plot, but due to large variation in activity this was not significant (Appendix 7). Phosphatase activity was significantly lower in 5-m plots after harvest, but since phosphatase activity was also significantly lower in "future" 5-m plots before harvest we cannot conclude that this was related to patch size treatment. Glucosidase activity was reduced in organic soil after harvest. Glucosidase activity decreased the most in the smallest patch size (5 m) (Figure 7). Chitinase activity was significantly reduced in the organic layer, and elevated in the mineral layer after harvest (Table 6). The greatest decrease in organic soil chitinase activity occurred in the smallest plots, and the greatest increase in mineral soil chitinase activity also occurred in the smallest (5 m) plots following harvest (Figure 8). Post-harvest peroxidase activity increased significantly in both soil layers (Table 6); most noticeably in 5-m GTR plots (Figure 10). This increase in peroxidase activity was consistent between all patch sizes, at all sampling locations after harvest (Appendix 8). Phenol-oxidase activity was stimulated after harvest in the organic soil layer (Table 6), most noticeably in 5-m GTR plots (Figure 11).

Table 6. Enzyme activity and nutrient availability in organic and mineral soil layers assessed before and after Green Tree Retention harvest.

Enzyme	Time	Organic	Mineral
		nmol $\cdot g^{-1} \cdot h^{-1}$	
Glucosidase	before	716b (70.2) B	60a (6.2) A
	after	505b (34.2) A	138a (8.7) B
Chitinase	before	551b (82.7) B	95a (16.6) A
	after	319b (25.8) A	122a (5.9) A
Phosphatase	before	1073b (94.5) A	560a (66.8) A
	after	948b (71.6) A	476a (33.4) A
Phenol	before	677a (77.7) A	1508b (116.8) A
Oxidase	after	921a (116.7) B	1503b (75.3) A
Peroxidase	before	2522a (328.5) A	6428b (483.0) A
	after	5727a (367.5) B	11000b (543.6) B
Nutrient	Time	Organic	Mineral
		$\mu g \cdot 10\ cm^{-2} \cdot 62\ days^{-1}$	
Total N	before	9.2a (0.5) A	10.1a (0.5) A
	after	79.2a (6.5) B	70.2a (6.0) B
NO_3^--N	before	1.7a (0.3) A	2.4a (0.3) A
	after	11.9a (1.0) B	23.0b (2.7) B
NH_4^+-N	before	7.1a (0.5) A	7.4a (0.5) A
	after	67.3b (0.5) B	47.3a (5.7) B
PO_4^{3-}-P	before	1.7b (0.2) A	1.1a (0.2) A
	after	2.9b (0.4) A	1.5a (0.6) A

Note: Values are means of all samples at all locations with SE given in parentheses. Sample size (n=112). Values with different lower case letters significantly different (comparison is between organic and mineral soil) ($p < 0.05$). Values with different upper case letters significantly different (comparison is between pre- and post-harvest) ($p < 0.05$).

Table 7. Nutrient availability assessed in organic (O) and mineral (M) soil layers by PRS™ ion exchange probes at centre, edge, and 30 m beyond the edge of future Green Tree Retention patches before harvest.

Nutrient	Soil Layer	Centre	Edge	30m
		μg 10 cm^{-2} 62 days^{-1}		
Total N	O	9.4a (0.8)	9.4a (1.2)	8.8a (0.8)
	M	9.7a (0.7)	10.4a (0.9)	10.2a (1.1)
NO_3^--N	O	1.716a (0.559)	1.844 a (0.549)	1.563a (0.304)
	M	2.217a (0.554)	2.428a (0.525)	2.553a (0.619)
NH_4^+ -N	O	7.26a (0.75)	7.07a (0.87)	6.91a (0.84)
	M	7.25a (0.64)	7.53a (0.96)	7.41a (0.93)
PO_4^{3-} -P	O	1.2a (0.2)	1.7a (0.3)	2.0a (0.5)
	M	1.1a (0.3)	1.0a (0.2)	1.3a (0.3)

Note: Values are means for all patch sizes with SE given in parentheses. Sample size (n=16). Values with different letters are significantly different (comparison is between sampling locations) ($p < 0.05$).

Table 8. Nutrient availability assessed in organic (O) and mineral (M) soil layers by PRS™ ion exchange probes in future 5 m, 10 m, 20 m, and 40 m diameter Green Tree Retention patches before harvest.

Nutrient	Soil Layer	5 m	10 m	20 m	40 m
		μg 10 cm^{-2} 62 days^{-1}			
Total N	O	7.9a (0.6)	12.0a (1.6)	7.9a (0.7)	9.1a (0.8)
	M	9.1a (0.8)	10.4a (1.4)	9.4a (0.9)	11.4a (1.0)
NO_3^--N	O	1.658a (0.634)	2.796a (0.681)	0.792a (0.284)	1.563a (0.304)
	M	1.556a (0.439)	1.850a (0.319)	1.492a (0.469)	4.700b (0.775)
NH_4^+ -N	O	5.75a (0.37)	8.82a (1.35)	6.54a (0.69)	7.22a (0.87)
	M	7.25a (0.82)	8.29a (1.33)	7.46a (1.05)	6.58a (0.58)
PO_4^{3-} -P	O	1.16a (0.1)	2.4a (0.5)	1.8a (0.5)	1.3a (0.2)
	M	0.9a (0.2)	1.8a (0.5)	0.9a (0.2)	1.0a (0.3)

Note: Values are means for centre, edge, and 30 m locations with SE given in parentheses. Sample size (n=12). Values with different letters are significantly different (comparison is between patch sizes) ($p < 0.05$).

Table 9. Extracellular soil enzyme activity in organic (O) and mineral (M) soil layers at centre, edge, and 30 m beyond the edge of future Green Tree Retention patches before harvest.

Enzyme	Soil Layer	Centre	Edge	30m
		nmol $\cdot g^{-1} \cdot h^{-1}$		
Glucosidase	O	791a (180.2)	761a (86.8)	596a (69.7)
	M	47a (5.2)	61a (9.9)	72a (14.7)
Chitinase	O	491a (70.5)	482a (59.8)	680a (232.5)
	M	69a (6.8)	84a (15.3)	131a (46.8)
Phosphatase	O	1067a (166.4)	1084a (173.0)	1067a (164.6)
	M	476a (95.5)	564a (126.4)	639a (126.2)
Phenol	O	658a (107.2)	633a (148.7)	739a (150.7)
Oxidase	M	1525a (215.1)	1360a (131.7)	1637a (248.7)
Peroxidase	O	2420a (604.8)	3159a (578.4)	1986a (517.2)
	M	7235a (1040.2)	5647.5a (837.0)	6401a (570.8)

Note: Values are means for all patch sizes with SE given in parentheses. Sample size (n=16). Values with different letters are significantly different (comparison is between sampling locations) ($p < 0.05$).

Table 10. Extracellular soil enzyme activity in organic (O) and mineral (M) soil layers in future 5 m, 10 m, 20 m, and 40 m diameter Green Tree Retention patches before harvest.

Enzyme	Soil Layer	5 m	10 m	20 m	40 m
		nmol $\cdot g^{-1} \cdot h^{-1}$			
Glucosidase	O	749a (113.1)	774a (67.3)	773a (238.6)	569a (85.7)
	M	58a (5.8)	69a (19.9)	50a (11.9)	64a (8.5)
Chitinase	O	800a (303.6)	556a (93.4)	411a (58.6)	438a (63.0)
	M	80a (8.9)	127a (64.0)	88a (18.5)	85a (9.1)
Phosphatase	O	691a (157.3)	1273a (191.2)	1139.3a (131.6)	1188a (238.7)
	M	446a (142.1)	477a (85.3)	746a (159.0)	571a (136.0)
Phenol	O	638a (167.6)	619a (138.4)	776a (149.0)	677a (179.9)
Oxidase	M	1210a (272.1)	1309a (170.0)	1809a (230.0)	1704a (231.8)
Peroxidase	O	1867a (490.0)	1880a (629.4)	3249a (149.0)	3092a (640.0)
	M	6680a (783.2)	5099a (669.5)	6277a (1350.4)	7657a (891.9)

Note: Values are means for centre, edge, and 30 m locations with SE given in parentheses. Sample size (n=12). Values with different letters are significantly different (comparison is between patch sizes) ($p < 0.05$).

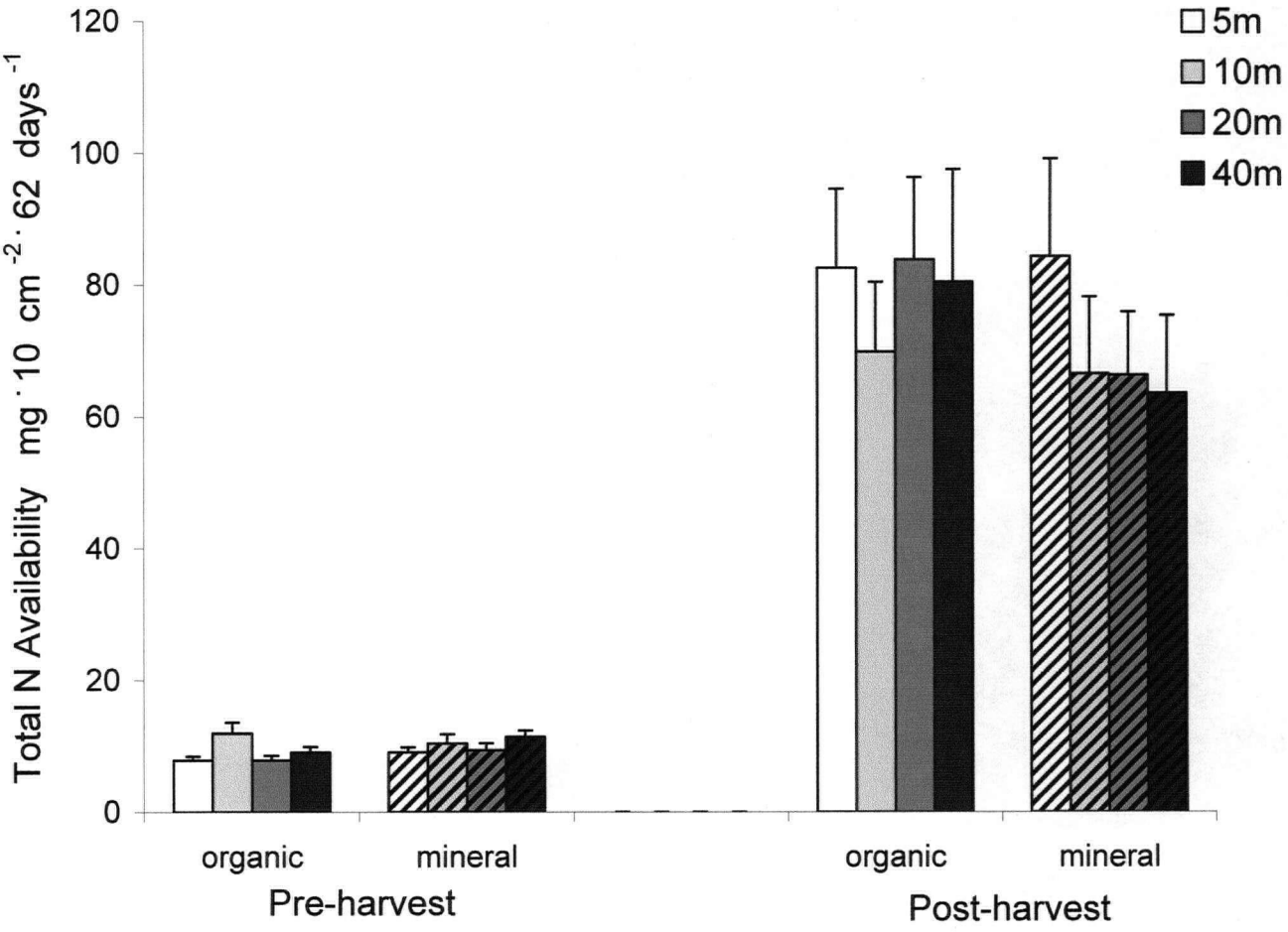

Figure 3. Total nitrogen availability pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

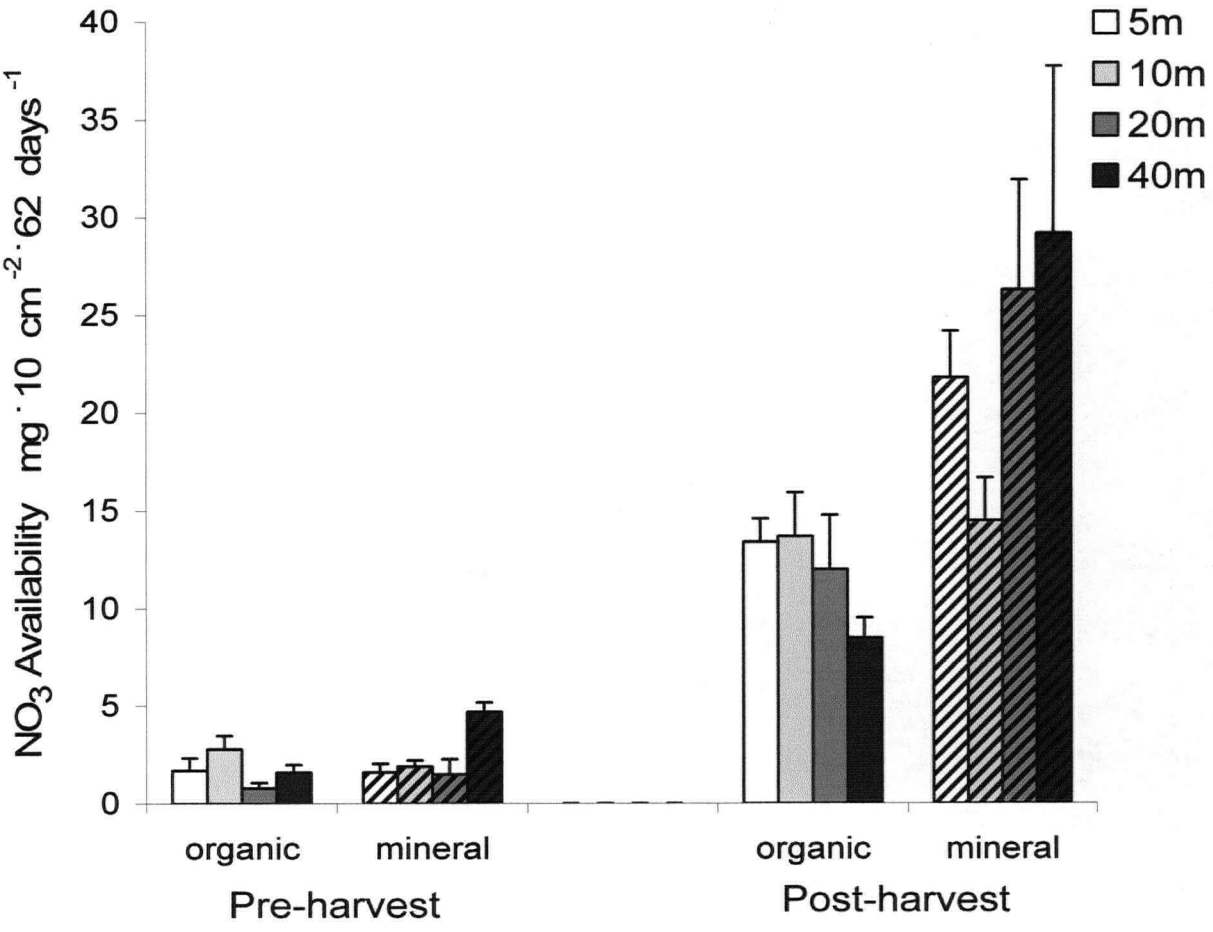

Figure 4. Nitrate availability pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

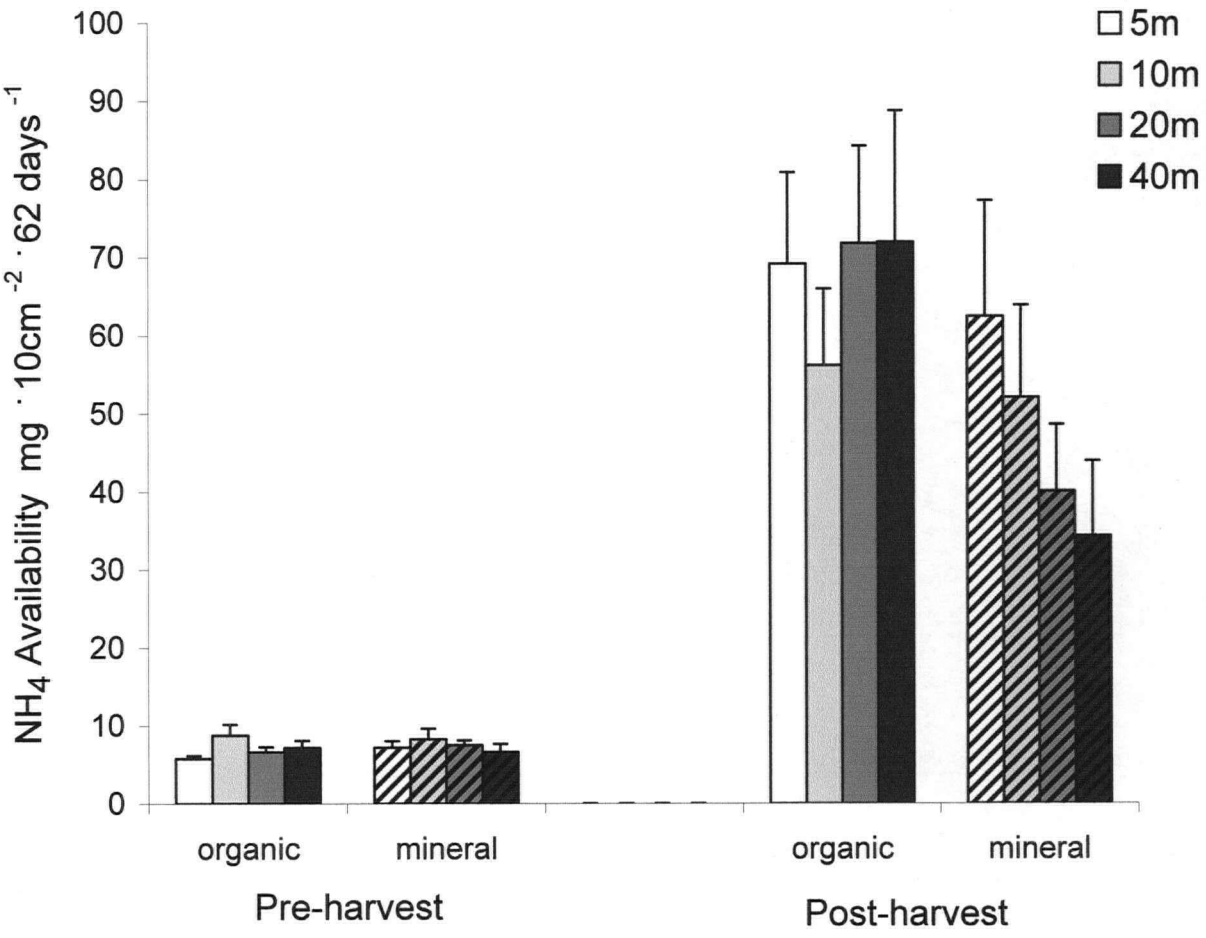

Figure 5. Ammonium availability pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

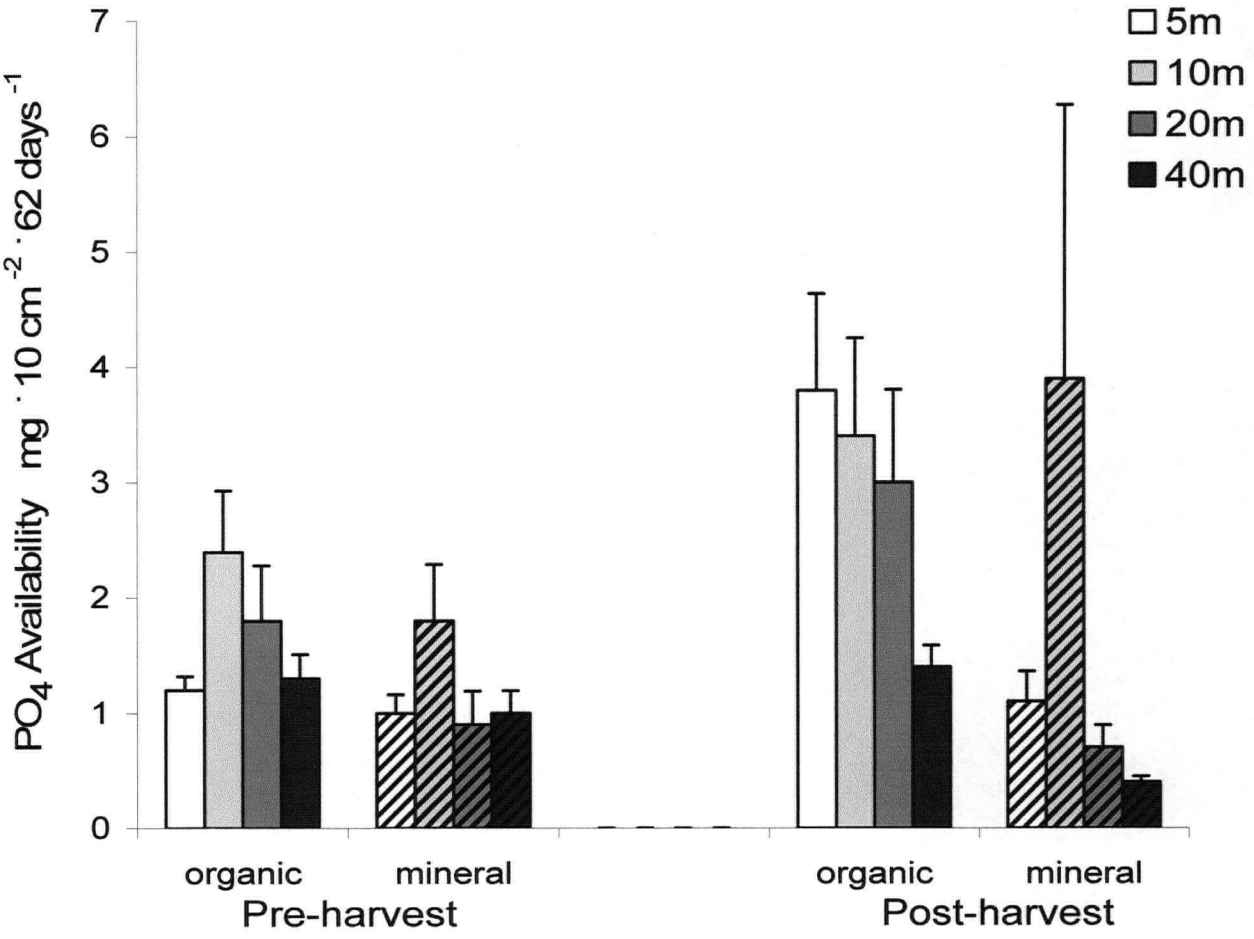

Figure 6. Phosphate availability pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

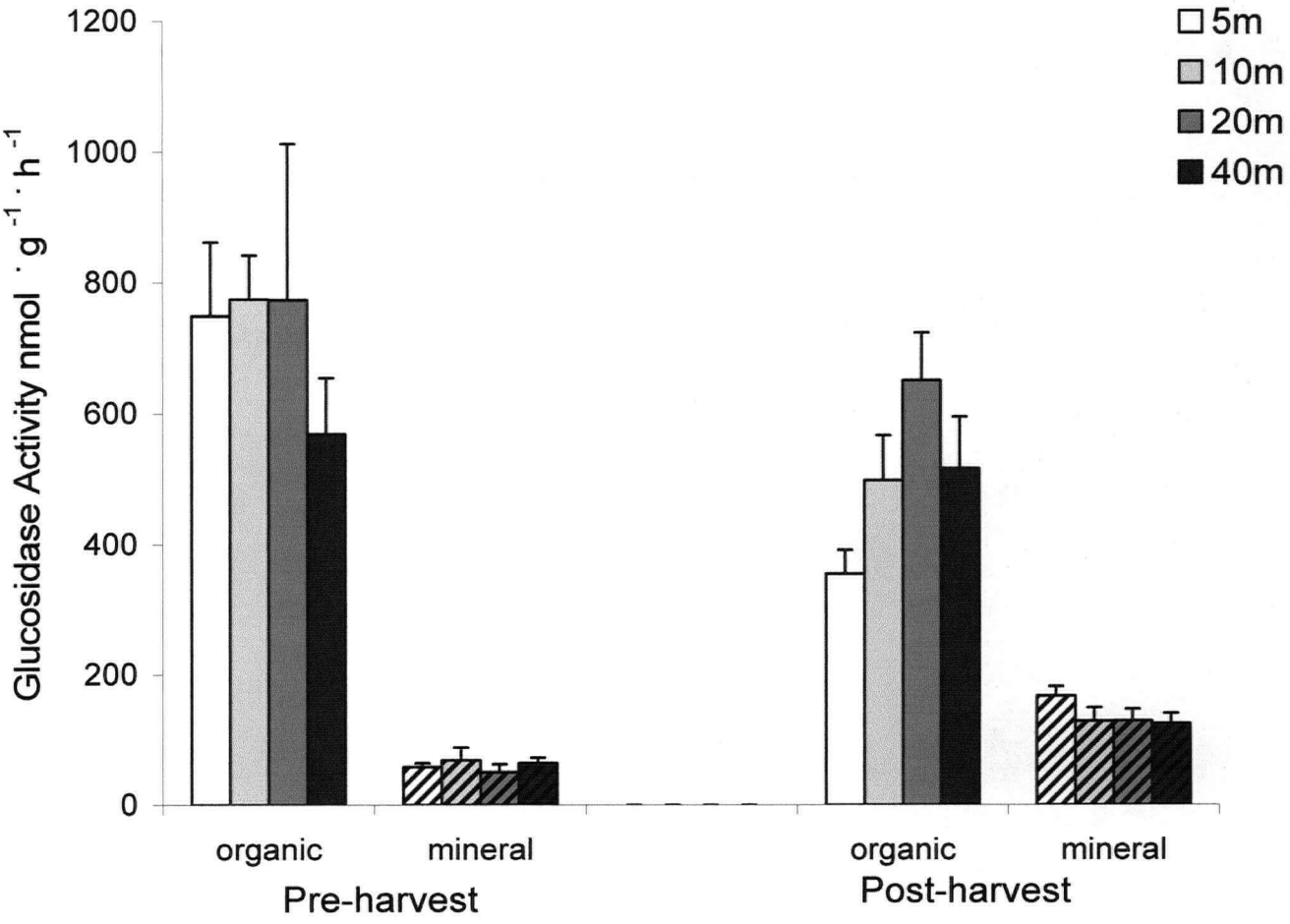

Figure 7. Glucosidase activity pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

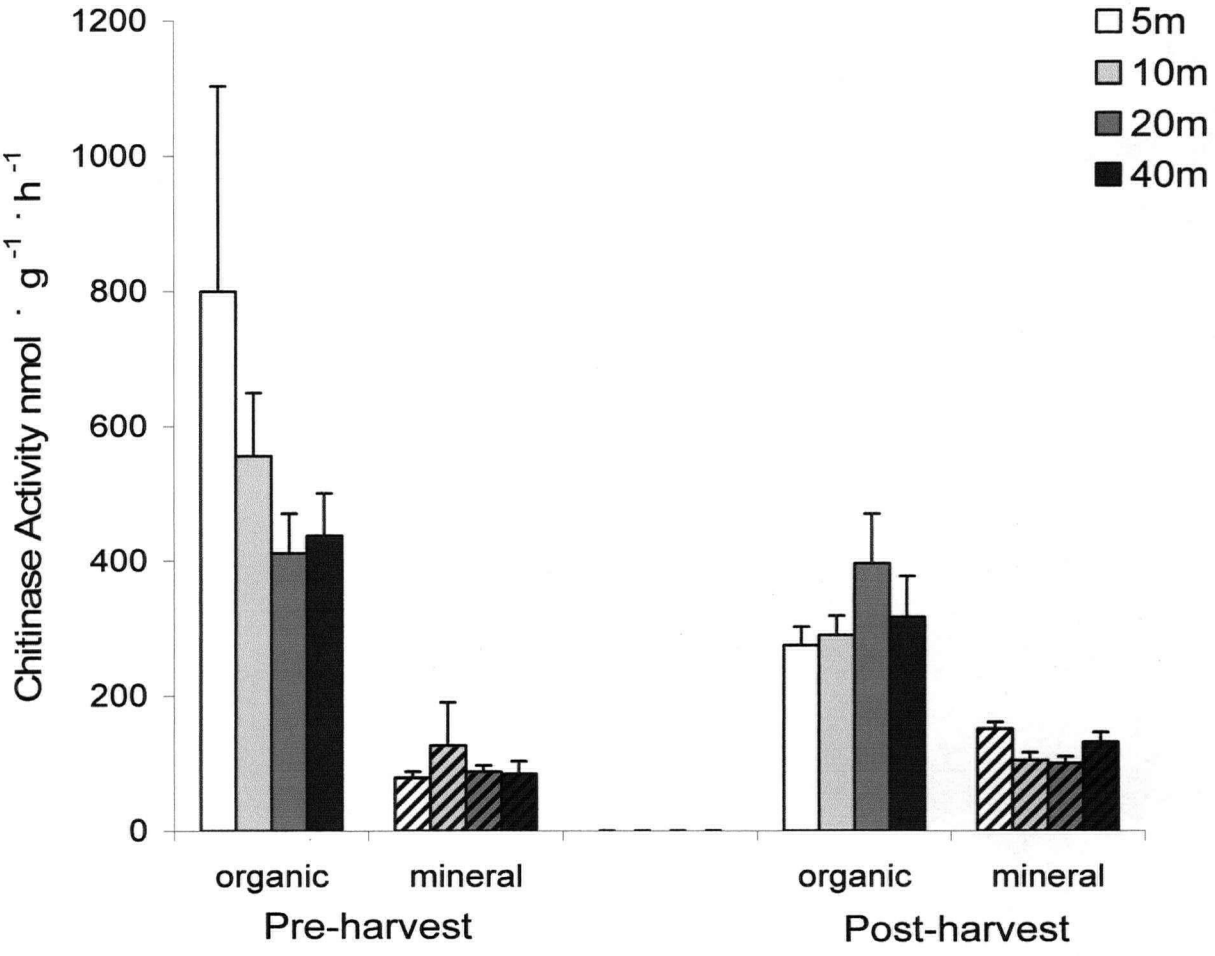

Figure 8. Chitinase activity pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

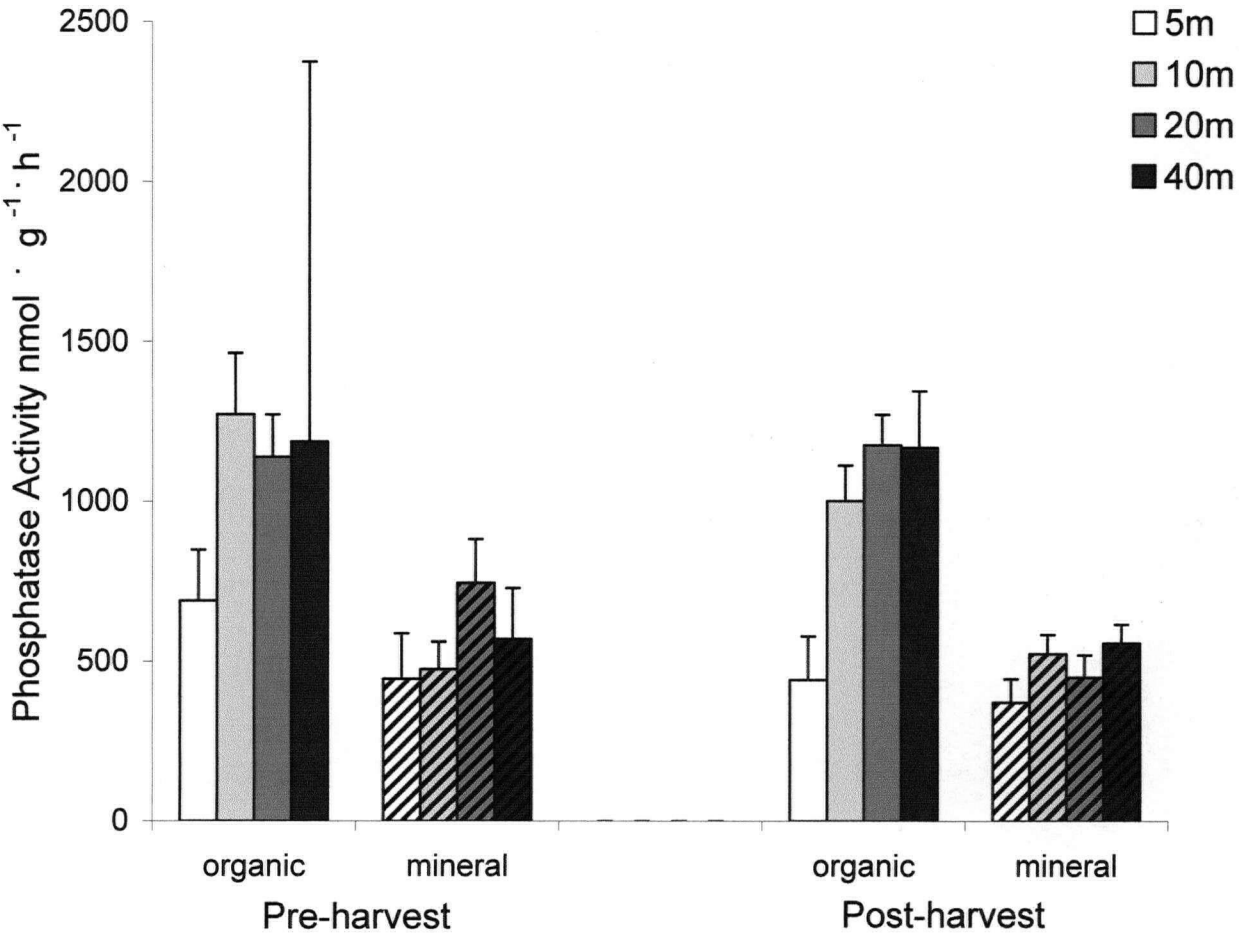

Figure 9. Phosphatase activity pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

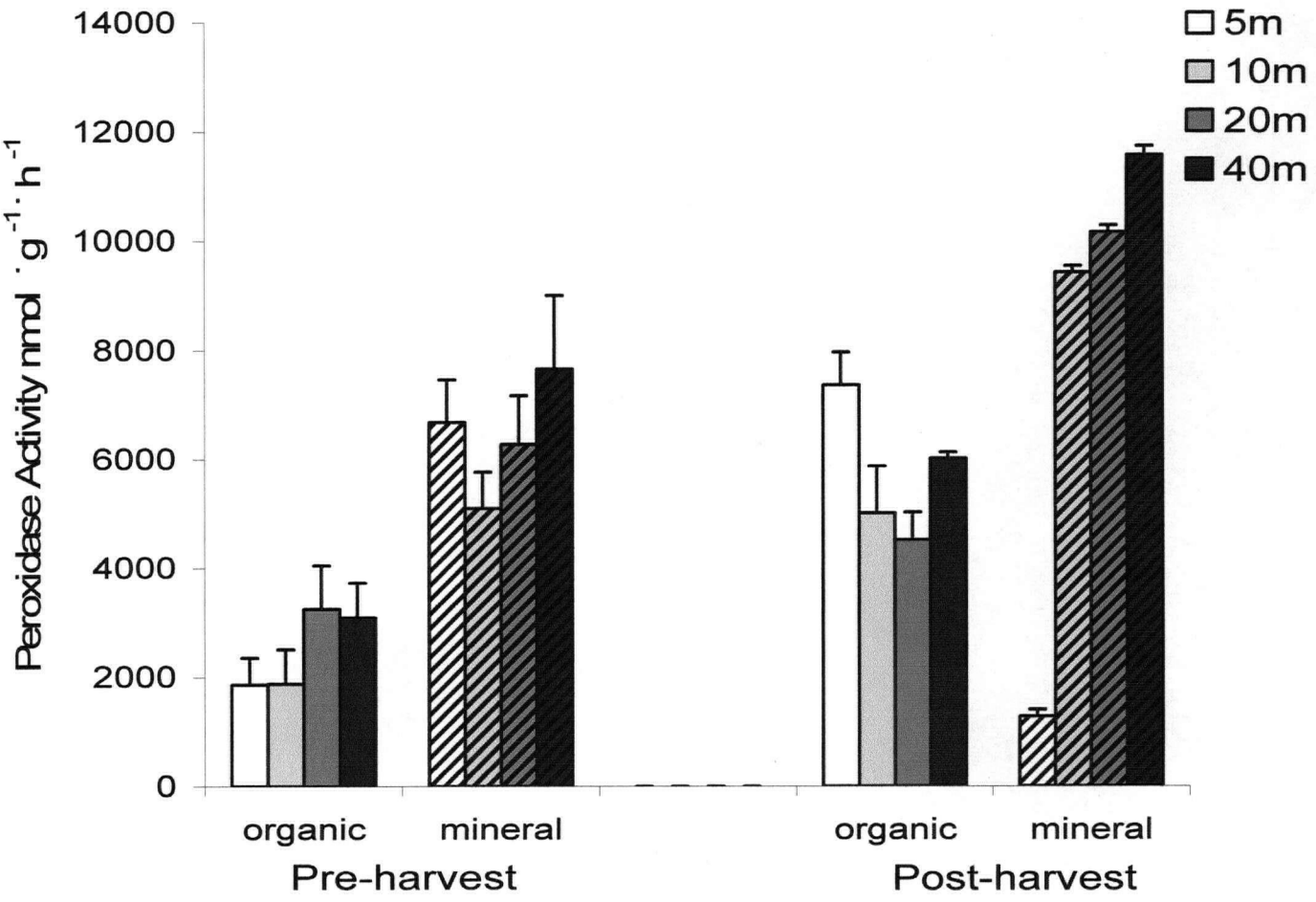

Figure 10. Peroxidase activity pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

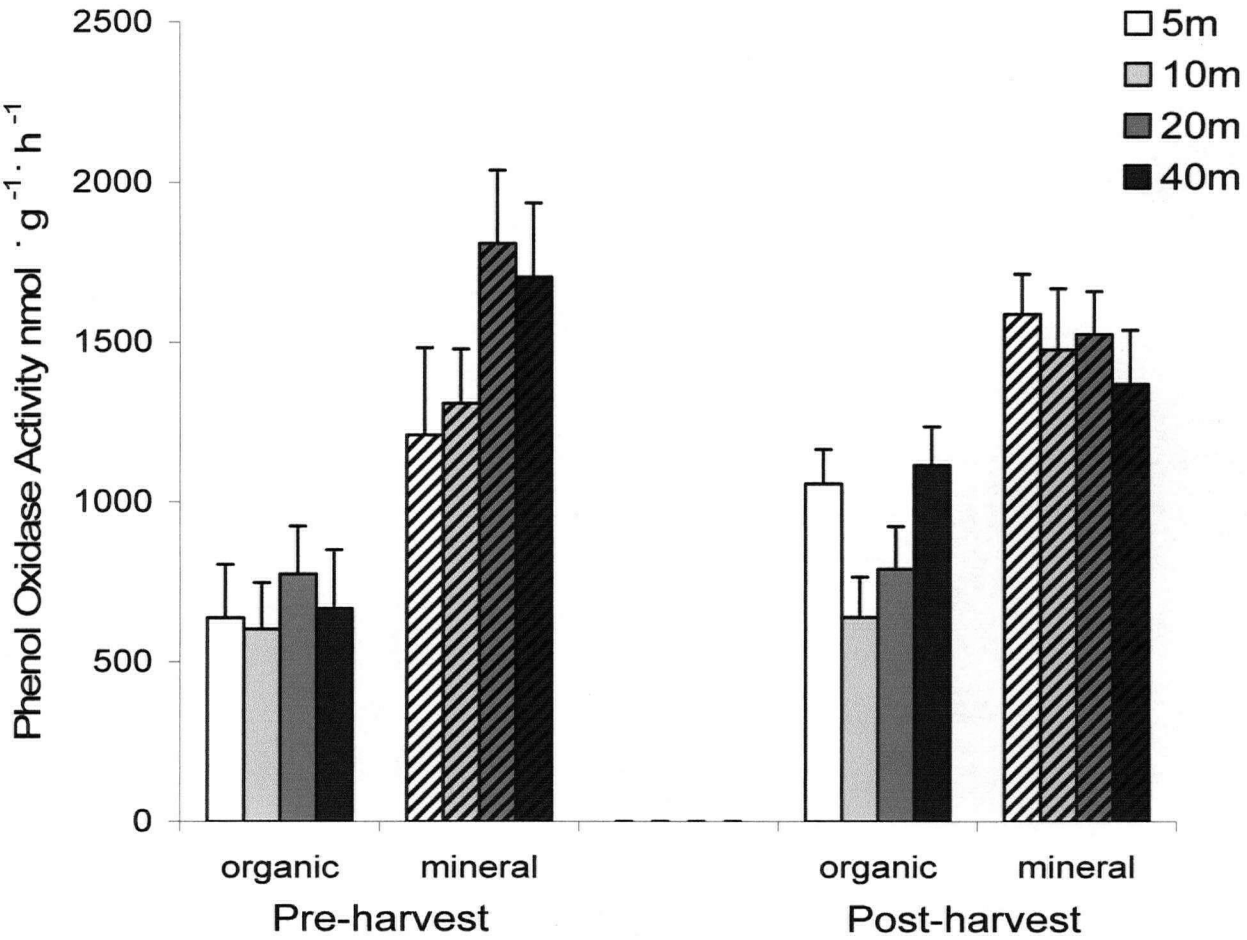

Figure 11. Phenol oxidase activity pre-harvest and post-harvest, in organic and mineral soil layers, measured in Green Tree Retention plots 5 m, 10 m, 20 m, and 40 m in diameter. Each value is the mean and standard error from all sampling locations.

DISCUSSION

The first objective of this study was to determine whether soil nutrient availability and soil enzyme activity measured within GTR plots were more similar to pre-harvest levels than data collected in the surrounding cut area. To address this objective, I examined soil nutrient availability, and soil enzyme activity at the centre, edge, and up to 30 m beyond the edge of GTR plots within a 25-hectare cutblock. Overwhelmingly, data examined from these sampling locations within and beyond plots did not differ significantly from each other at four months after harvest was completed. Glucosidase and chitinase activity decreased in organic soil layers after harvest when data from all sampling locations was pooled (Figure 7 and 8). Upon closer examination of individual sampling locations, there was a tendency of glucosidase and chitinase activity to increase at 10 m and 15 m beyond plot edges in larger retention plots (Appendices 5 and 6). This inhibition of enzyme activity, although not significant, may be evidence of a disturbance effect (physical disruption of the soil surface from harvest activities) that has been shown to hinder the activity of extracellular enzymes at soil surface (Curci et al. 1997). Phosphatase activity remained close to pre-harvest levels at four months after harvest was complete. The difference seen between these three hydrolytic enzymes in their response to harvest in the short term supports the findings of McGill and Cole (1981), who found that enzyme activity tends to be substrate-specific, potentially affecting nitrogen and phosphorus differently. Glucosidase, which is produced by bacteria, fungi and (Burns and Dick 2002), has been shown to be inhibited after harvest, which was attributed to a decline in soil microbial biomass (Hasset and Zak 2005). Chitinase is naturally produced

by bacteria, fungi and plants (Jollès and Muzzarelli 1999), and chitinase activity is a reliable indicator of fungal biomass in soil (Miller et al. 1998). The disruption of the activities of glucosidase and chitinase at the soil surface may be explained in this study by reduced fungal biomass after harvest. Phosphatase, produced by plant roots and soil microorganisms, was not inhibited in the short term after harvest in this study. Explanations for this may include stability of phosphatase enzymes in the soil, and the continued production of phosphatases by living tree roots in GTR plots (Zahir et al. 2001).

The second objective of this study was to determine whether these parameters measured in larger patches were more similar to pre-harvest levels than data collected in smaller patches? To address this objective, I compared data collected four months after harvest from GTR plots 5 m, 10 m, 20 m, and 40 m in diameter. I did not find a statistically significant difference between soil nutrient availability or enzyme activity measured in different sizes of GTR plot, but I did see a trend for post-harvest changes to be greater in the smallest plot size. Total N, nitrate, ammonium, and phosphate availability increased more in 5-m plots after harvest than they did in the larger plot sizes. The activities of glucosidase and chitinase decreased in organic soil and increased in mineral soil, with the greatest magnitude of change occurring in the 5-m plots. The activities of peroxidase and phenol oxidase were stimulated in both soil layers after harvest, most strongly in the 5-m plots. The greater post-harvest changes to response variables seen in the smallest plots lends support to the premise of GTR as a harvest pattern because the amount of trees in the smallest plots was only 1-2 trees per patch. A

scattering of 1-2 trees across a harvested landscape is more akin to dispersed retention harvesting. Soil characteristics in the proximity of 1-2 trees were less likely to retain pre-harvest levels of soil nutrient availability and enzyme activity.

The third objective of this study was to determine whether the influence of GTR patch diminished as sampling extended into the clear-cut area. To address this objective, I examined soil nutrient availability, and soil enzyme activity at the centre, edge, and up to 30 m beyond the edge of GTR plots within a 25-hectare cutblock. Significant differences between sampling locations within and beyond GTR plots were not found for any measured response variable, although phosphatase activity did display a trend of declining activity with increasing distance from GTR plots after harvest in 10-m, 20-m, and 40-m plots (Appendix 7). This pattern may reflect the fact that tree roots produce phosphatase and suggest roots may be the major source of phosphatase in this forest. The fact that this trend was not seen in 5-m plots, due to the fact that phosphatase activity was lower at all locations in the 5-m plots, supports the idea that larger aggregates of live trees may be better suited to preserve nutrient availability and enzyme activity in soil than dispersed retention of single trees across a harvested landscape.

Comparing soil sample characteristics before and after harvest at predetermined locations, overall nutrient availabilities have significantly increased after harvest for total N, NO_3^--N, and NH_4^+ -N, but not PO_4^{3-} -P. Partial cuts have been shown to preserve nitrogen levels after harvest (Prescott 1997, Barg and Edmonds 1999), although elevated nitrate availability has been found in harvest gaps larger than 0.1 ha (Prescott et al. 2003).

Bradley et al. (2000) demonstrated that lower microbial immobilization, due to reduced nutrient rhizodeposition and through-fall, may explain a post-harvest flush of available nitrogen. These quick reactions to harvest disturbance support the findings of Boerner et al. (2000), who also showed that the activities of extracellular soil enzymes were vulnerable to change in the face of prescribed burning disturbance. In accordance with the findings of Olander and Vitousek (2000), who showed that soil phosphatase activity was regulated by phosphorus availability, it is possible that the unresponsiveness of phosphatase 4 months after harvest reflects the amount of phosphorus available in the soil after harvest, which also remained unchanged. Phosphate ions in and beyond GTR patches, as measured by PRS^{TM} probes, were not significantly different after harvest disturbance. The tendency of phosphorus ions to become bound to soil mineral particles after harvest has been demonstrated in podzolic soils (Zhang and Mitchell 1995; Yanai 1998), and may explain why we did not find a significant change in P-ion availability a few months after GTR harvest. Neither phosphatase activity nor PO_4^{3-} -P were significantly altered in the 25-hectare treatment area, but all other enzymes and nutrients did respond to harvest. Attempts to correlate phosphatase activity with PO_4^{3-} -P levels were unsuccessful.

The differences in the activities of extracellular enzymes between the organic soil horizon and the mineral soil horizon seen before harvest were still noticeable after harvest. The hydrolytic enzymes had higher relative activity in the organic horizon before harvest, possibly because the organic substances that these enzymes are responsible for breaking down are plentiful in an intact forest floor (Fisher and Binkley 2000). After

harvest, hydrolytic enzyme activity declined in the organic layer. This is possibly due to a decline in microbial biomass, which was shown to correspond with decreased enzyme activity under partial and clearcut harvest treatments (Hasset and Zak 2005). The decrease in hydrolytic enzyme activity may also be explained by mixing of mineral soil particles, with lower hydrolytic enzyme activity, into the organic soil layer from harvest disturbance (Powers et al. 1989; Yanai et al. 2003). The oxidative enzymes phenol oxidase and peroxidase, which are produced by white-rot fungi (Lang et al. 1997), had higher relative activity in the mineral horizon before harvest, which greatly increased after harvest (especially peroxidase). This increase in oxidative enzyme activity is interesting, considering NO_3^- and NH_4^+ availability also increased in both soil layers after harvest. Oxidative enzyme activity has been shown to be repressed by fertilization of at least 20 kg $N \cdot ha^{-1} \cdot yr^{-1}$ to soil (Carreiro et al. 2002; Gallo et al. 2004). The pulse of nitrogen seen in the GTR site after harvest was not large enough to suppress oxidative enzyme activity. Phenol oxidase and peroxidase levels were significantly higher post-harvest, which I attribute to the large increase in lignin-rich woody substrate that was found in organic soil samples and mineral soil samples processed after harvest. Snajdr and Baldrian (2006) found that the production of phenol oxidase and peroxidase by ligninolytic saprotrophs was significantly boosted with the addition of a lignin-rich substrate (wheat straw). The higher activity of oxidative enzymes in organic and in mineral soil at our study site may also be attributed to lignin-rich substrate additions in and on the soil, as harvest activities have left inputs of woody debris above ground, and coarse and fine dead roots belowground. In the face of increased N and increased lignin-rich substrate in post-harvest soil, the stimulation of oxidative enzyme activity by

increased substrate availabilities offset any inhibition that may have occurred due to increased N concentrations.

The methods of determining nutrient availability and enzyme activity were practical and reliable for our needs. Plant root simulator ™ probes, although convenient, have limitations to their usefulness and accuracy. PRS™ probes cannot be buried indefinitely; they are useful for measurement of nutrient supply rate up to the point of buffering capacity in the soil. Contact is essential between the soil and the ion exchange resin, which can be a problem if they are displaced due to weather or wildlife activity in a forest setting. Probe plastic casing is vulnerable to breakage in rocky or heavy dry soils. Expression of nutrient supply rates from PRS™ probe data is limited; these values cannot be converted to a nutrient available per volume of soil basis. Plant roots offer a stronger sink to soil ions than ion exchange membranes. In the case of extended burial periods, if roots grow against ion-exchange membranes, this competing nutrient sink should be taken into consideration.

Enzyme assays are a good indicator of soil quality because they are involved in essential nutrient cycling (Dick et al. 1996). Enzymes in soils are not only from microbes, but also from animals, and plants. The activities of nutrient cycling enzymes in the environment respond promptly to disturbance, and are quick and easy to measure. We used different assay methods to determine hydrolytic enzyme activity and oxidative enzyme activity. This simple test does not tackle the complexities of soil quality and fertility assessment on its own, but it provides data that can be compared at different

times and locations. Assays of enzyme activity from bulk soil in the laboratory include enzymes excreted from biota, as well as stabilized enzymes present in the soil matrix (Nannipieri et al., 2002). Data collected represent potential activities, which may differ from realized rates in field conditions (Dilly and Nannipieri 1998; Tate 2000; Nannipieri et al. 2002). In this study I measured the activity of five enzymes involved in the release of carbon, nitrogen, and phosphorus; numerous other enzymes involved in the decomposition process have not been measured here.

CONCLUSIONS

This evaluation of soil nutrient availability and extracellular soil enzyme activity under Green Tree Retention harvest in the short term has found that there is a tendency for smaller plots to exhibit greater changes than larger plots. Nitrogen availability increased after harvest, but not phosphorus. Changes seen in hydrolytic enzyme activity were more likely due to mixing of soil layers during timber extraction than a response to changes in substrate availability or biological activity. Oxidative enzyme activity, on the other hand, was markedly boosted after harvest, which I attribute to increases in woody substrate content of soil after harvest. Woody debris inputs from aboveground, and severed root remnants belowground, present more resources in the soil to be accessed by saprophytic fungi known to produce peroxidase and phenol oxidase. My aim to find appropriate sizes of retention patch to preserve soil nutrient availability and nutrient cycling potential, and to determine how far the effect of Green Tree Retention patches reached into logged areas was met with limited success. Five-meter retention patches were least likely to maintain soil nutrient availability and enzyme activity near pre-harvest levels. There are an average of 1.5 trees in the 5 m plots, 6.5 trees in the 10 m plots, 14 trees in the 20 m plots, and 47 trees in the 40 m plots. The results suggest aggregated patches of live trees of at least 10-m diameter are better suited to retain soil microbial activity and nutrient availability than the one or two trees present in the 5-m patches, which are more akin to dispersed retention.

In addition to measuring soil biochemical characteristics, it would be useful to evaluate soil biota before and after harvest to determine if any correlations exist between the structure of soil microbial communities and the ecosystem functions measured in this

dissertation within and beyond Green Tree Retention patches. This may also help to explain differences in enzyme activities and nutrient availability between soil layers and sample locations. Techniques used to measure soil biological structure such as phospholipid fatty acid (PLFA) analysis, and PCR-DGGE could generate a broad-scale, and a fine-scale community profile respectively to shed more light on how this partial harvest option affects the belowground ecosystem.

As this analysis was only conducted a few months after harvest, data collection should continue in the future to follow the levels of soil nutrient availability and extracellular enzyme activity at all sizes of Green Tree Retention Patches, and at all locations within and beyond those patches over time as the harvested area develops. Sampling over the long term may capture the magnitude and duration of post-harvest effects on measured parameters; harvest effect may be more significant as time goes on.

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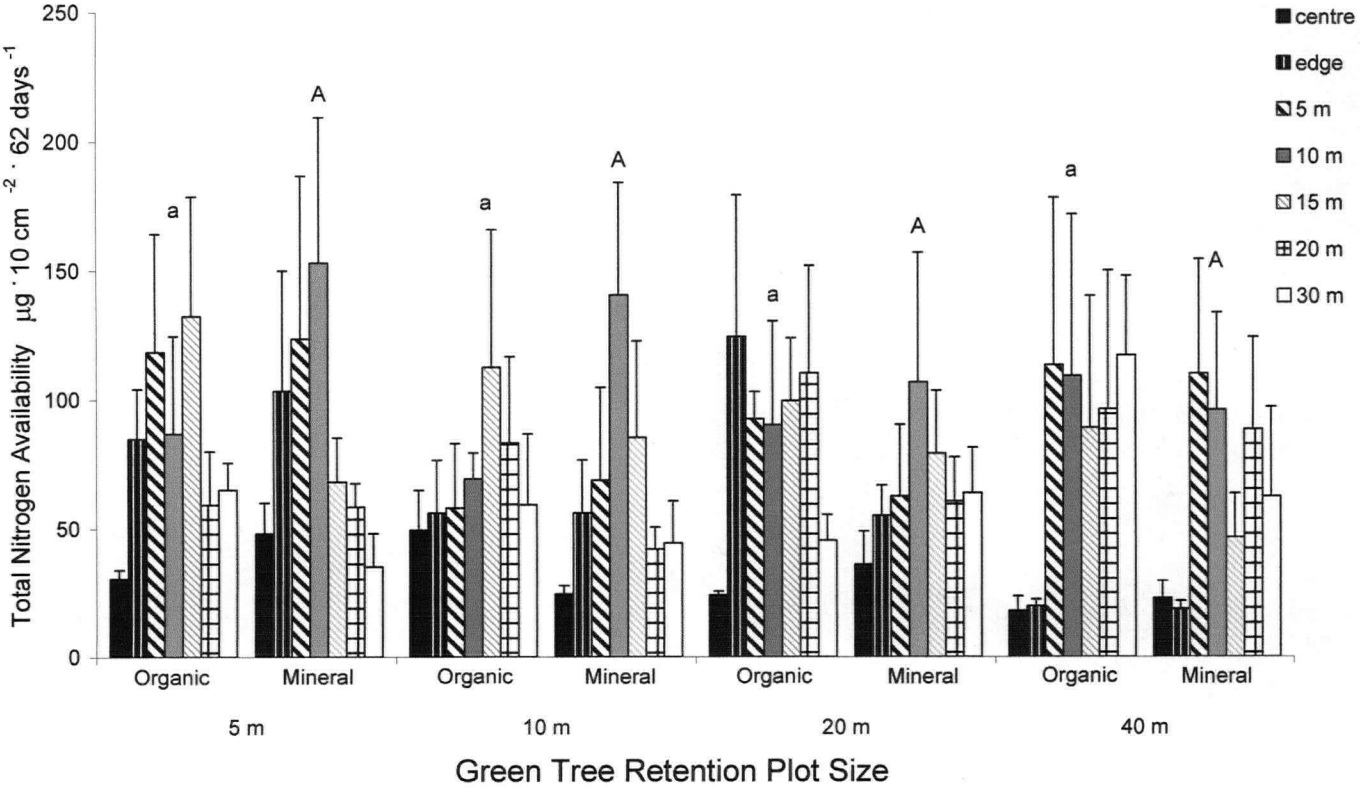
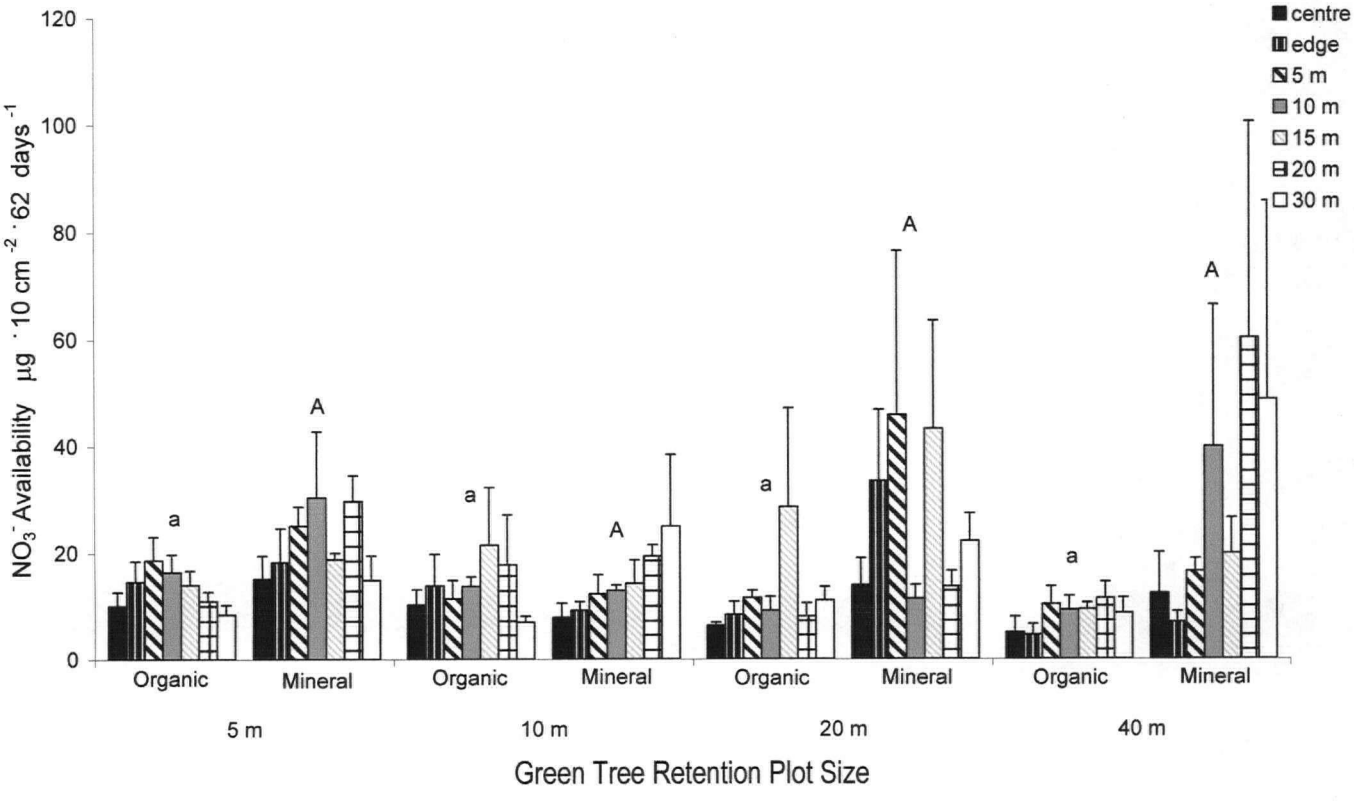
APPENDICES

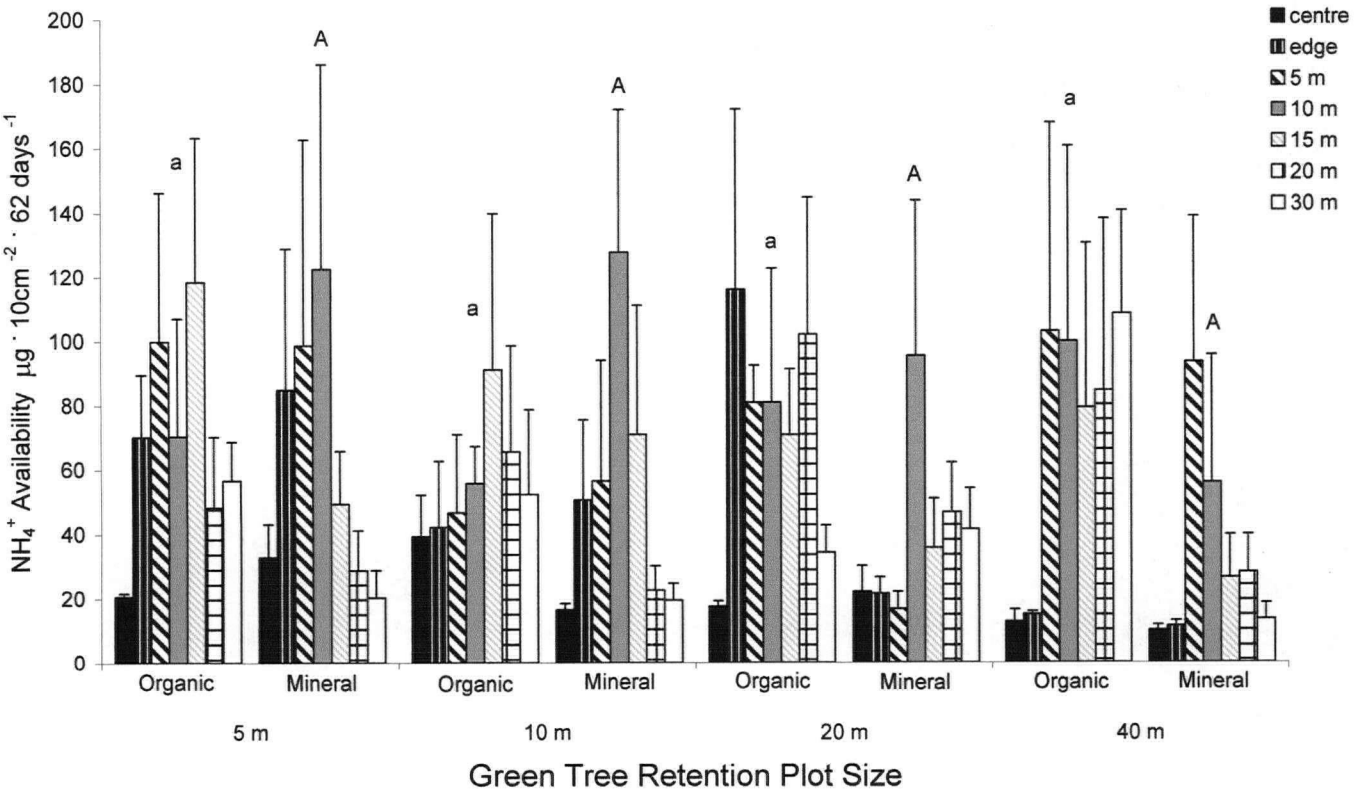
Appendix 1. Post-harvest total N availability in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on nutrient availability.



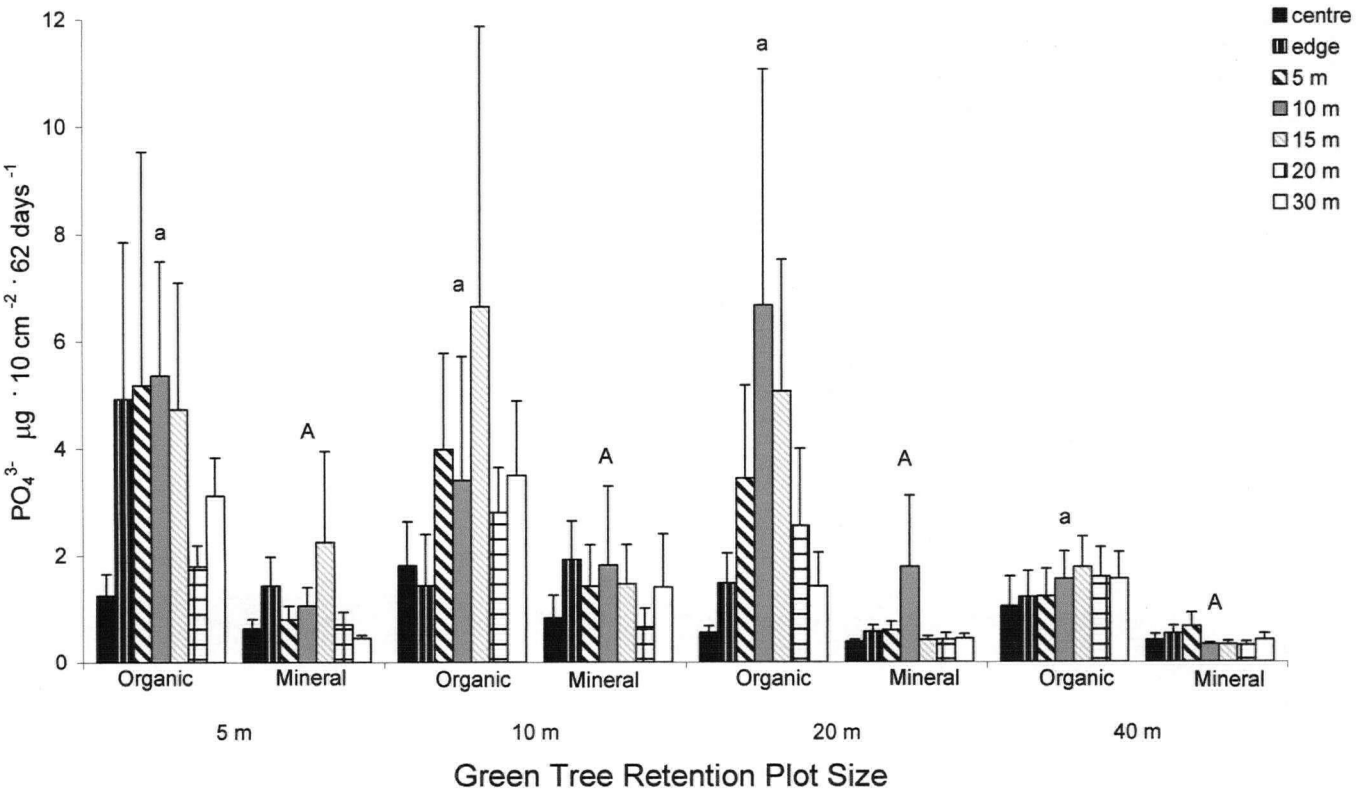
Appendix 2. Post-harvest NO_3^- availability in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on nutrient availability.



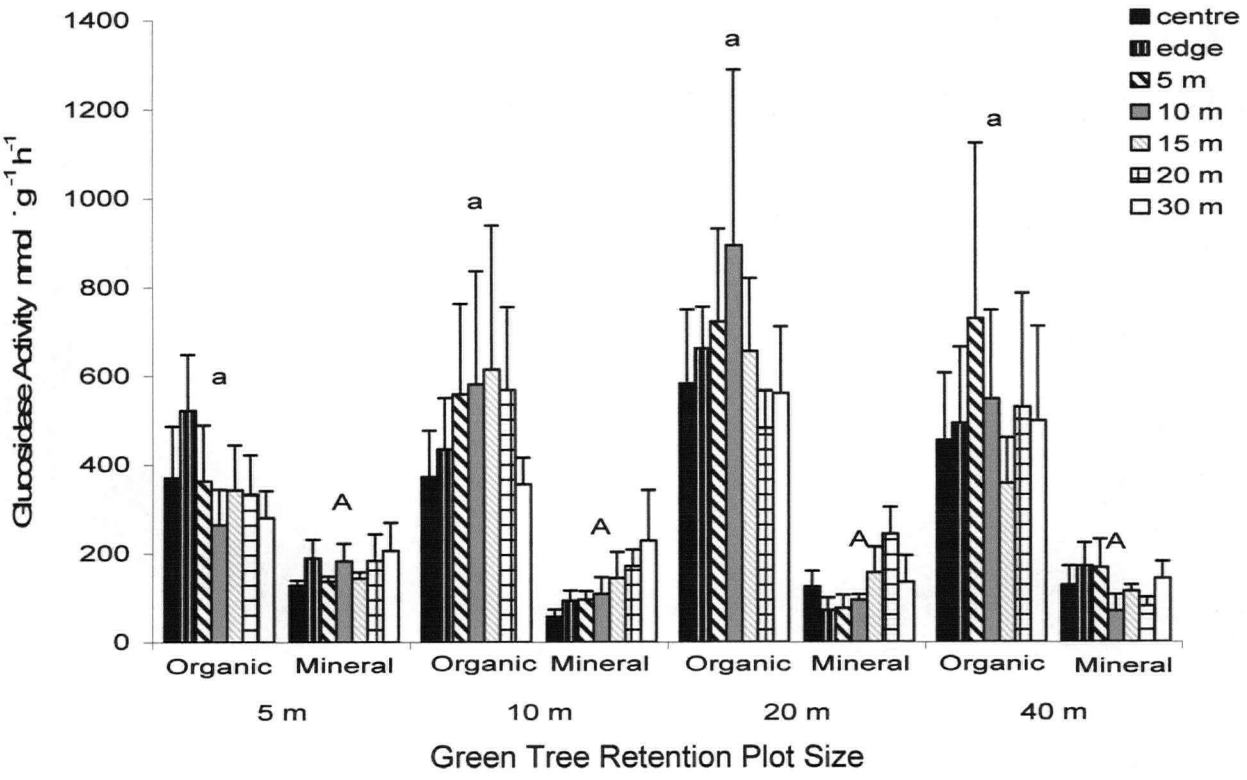
Appendix 3. Post-harvest NH_4^+ availability in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on nutrient availability.



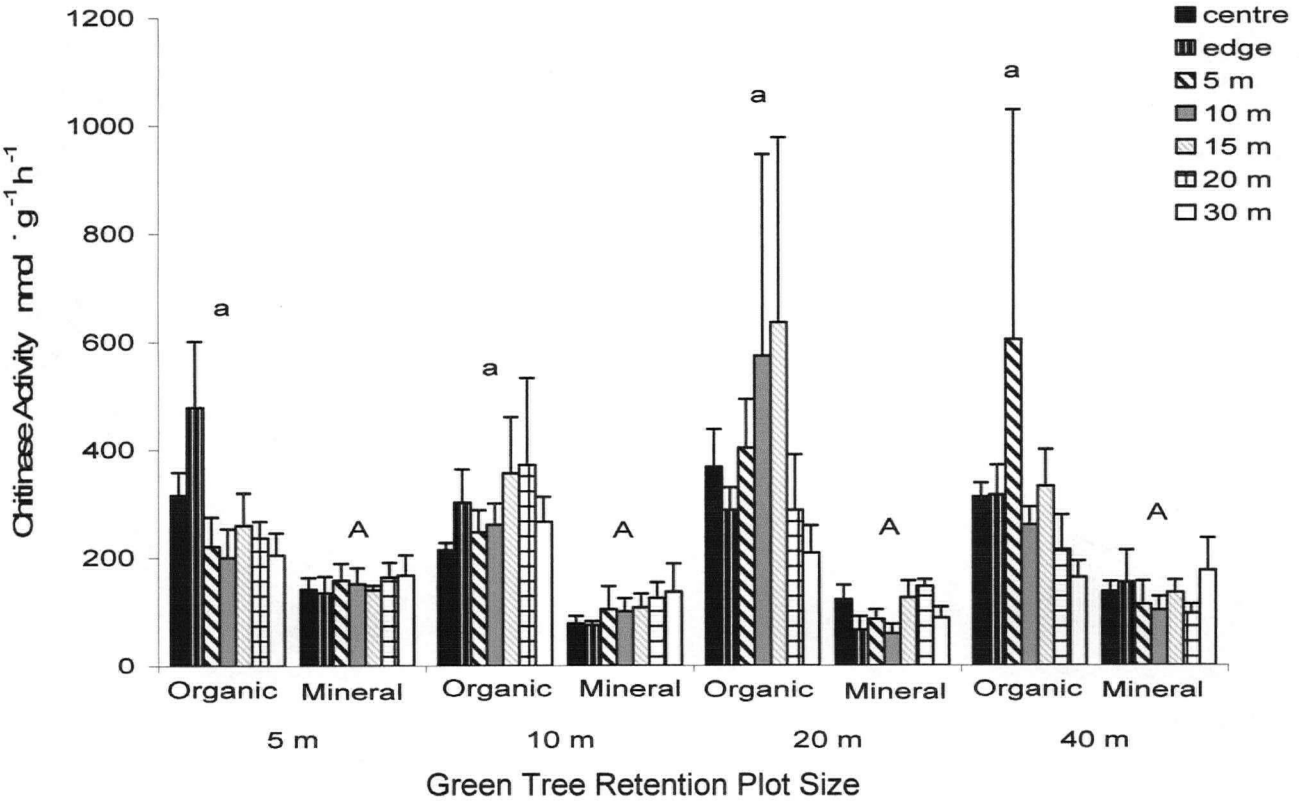
Appendix 4. Post-harvest PO_4^{3-} availability in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on nutrient availability.



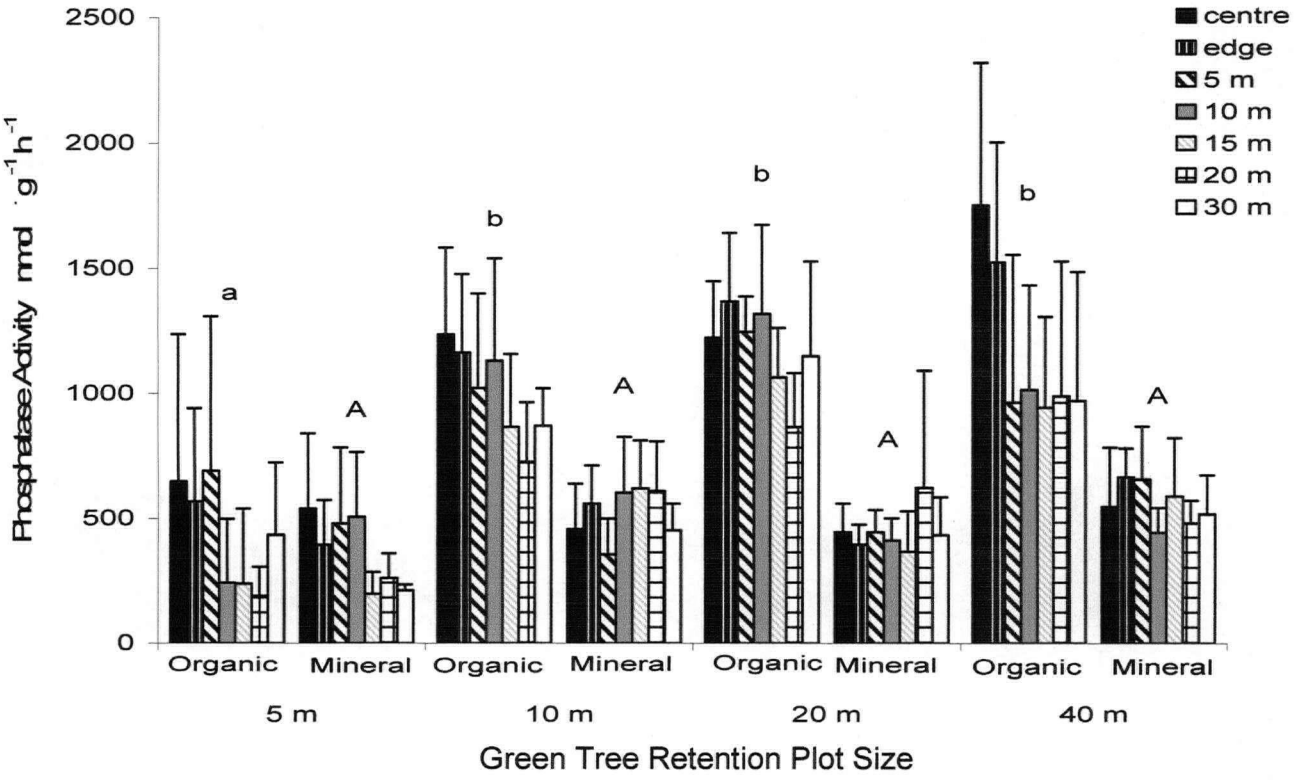
Appendix 5. Post-harvest glucosidase activity in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on enzyme activity.



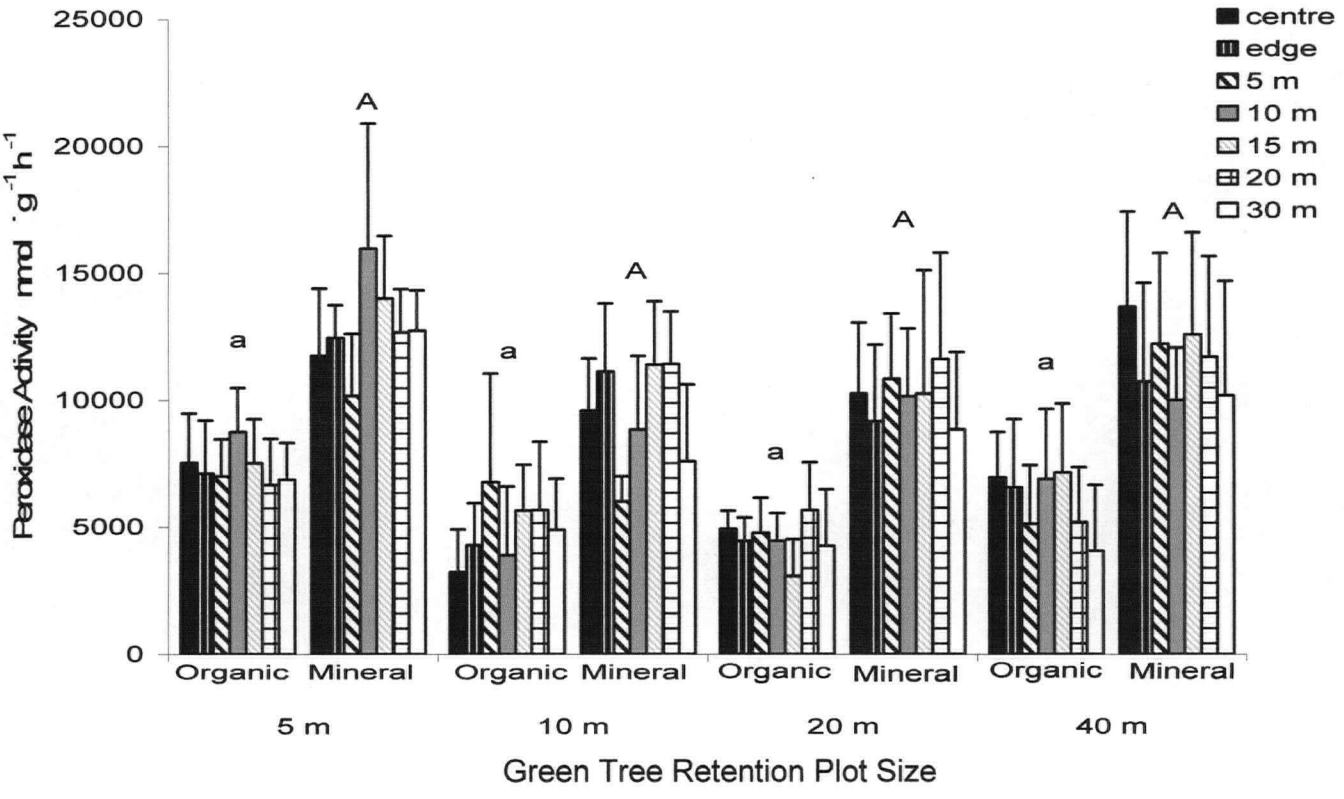
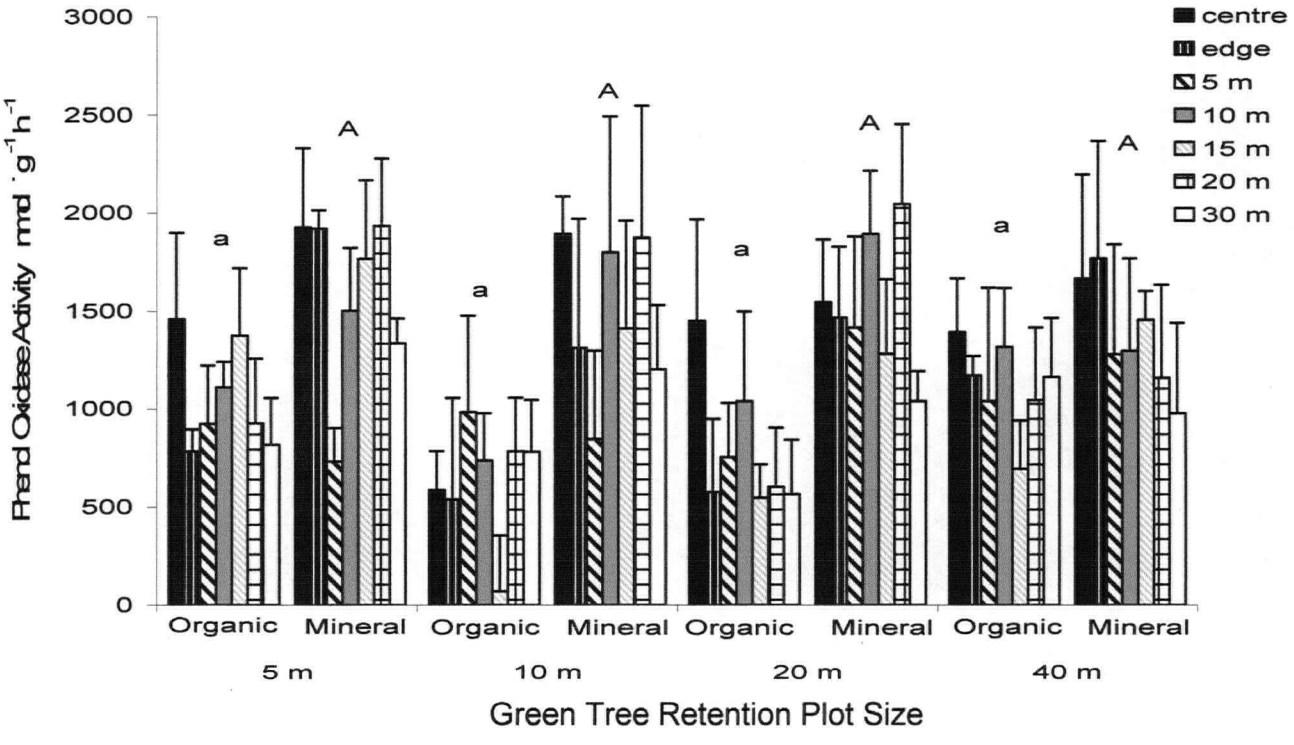
Appendix 6. Post-harvest chitinase activity in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on enzyme activity.



Appendix 7. Post-harvest phosphatase activity in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on enzyme activity.



Appendix 8. Post-harvest peroxidase activity in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on enzyme activity.



Appendix 9. Post-harvest phenol oxidase activity in organic and mineral soil layers from 5 m, 10 m, 20 m and 40 m patch sizes, by sampling position (centre, edge, 5 m 10 m, 15 m, 20 m, and 30 m). Each value is the mean and standard error from four plots. Different letters (lower case for organic, upper case for mineral) indicate a significant difference between patch sizes according to ANOVA or Kruskal–Wallis nonparametric tests ($p < 0.05$). Sampling position had no significant effect on enzyme activity.