CHANGES IN SELECTED SOIL QUALITY INDICATORS IN FORESTED SOILS FOLLOWING SAWLOG HARVEST

A Thesis

Presented to

The Faculty of the Graduate School

At the University of Missouri

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

 $\mathbf{B}\mathbf{y}$

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May 2014

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FOLLOWING SAWLOG HARVEST

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ACKNOWLEDGEMENTS

I would like to take this opportunity to first thank my advisor Dr. Keith Goyne for his meticulous guidance and tireless encouragement. I am truly grateful for the opportunity to be a part of MOFEP soil research as well as Dr. Goyne's soil chemistry lab. The amazing students and professors involved with the soil chemistry lab have thoroughly enriched my educational experience. I would like to thank Dr. Bob Kremer for his advice and wisdom before, during, and after my graduate school experience. Additionally, I would like to thank Dr. John Kabrick for his knowledge and aid in statistical analysis as well as his friendly conversation skills to and from the ASA, CSSA, & SSSA meeting.

I would also like to thank several students for their assistance in field and laboratory work. Bennett Wickenhauser, Satchel Gaddie, Christa Spivy, Gurbir Singh, and Rebecca Nordenholt all provided invaluable aid and support in this project.

Most importantly, I would like to thank my mother for providing inspiration and encouragement to pursue my aspirations and instilling in me the value of hard work. I am also perpetually grateful for the support and encouragement provided by my brother and father.

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Abstract

Our country's needs for wood products, the rising interest in biofuels, and a global interest in soils as a carbon sink place high demands on forest soils. The highly weathered and nutrient-poor soils of the Missouri Ozarks are vulnerable to degradation, thus necessitating improved understanding of forest harvest impacts on soil quality. The objective of this study was to investigate changes in selected soil quality indicators following sawlog harvests. The research was conducted at the Missouri Ozark Forest Ecosystem Project (MOFEP) sites, a long-term experimental study in mixed hardwood forests of southeast Missouri. Pre-harvest and post-harvest soil samples were collected at depths of 0-10 cm and 10-20 cm from sites harvested using clearcutting (CC) and singletree selection (STS) and from no harvest (NH) management sites. Samples were collected from low (<20 % base saturation in diagnostic subsoil horizon) and medium (20-50 % base saturation in diagnostic subsoil horizon) soil nutrient status (SNS) soils. The chemical soil quality indicators examined included total organic carbon (TOC) and total nitrogen (TN), active C (KMnO₄ oxidizable carbon), water extractable organic C and water extractable N (WEOC and WEN, respectively), and soil pH. Activities of soil microbial enzymes β -glucosidase and β -glucosaminidase were evaluated as biological indicators. Water stable aggregate content (WSA) was quantified to examine changes in physical soil properties. Few differences in soil quality parameters were observed in the ~1.5 years after harvest. However, two indicators, β-glucosaminidase activity and WEN, showed significant change after harvest. In CC treatments β-glucosaminidase activity decreased significantly at the 0-10 cm depth in January 2013 post-harvest collections when compared to the January 2013 NH values and pre-harvest CC treatment values

collected January 2011. On specific collection dates, WEN also decreased significantly in CC treatments at 0-10 cm depths from low and medium SNS soils. In the CC treatment, values in low SNS soils collected in January 2013 post-harvest were significantly lower in WEN than NH treatment values from January 2013 and pre-harvest (January 2011) NH values. Soil quality changes after harvest, were most pronounced in CC harvested sites, though CC and STS sites were rarely significantly different from each other. Within the timeframe studied, β -glucosaminidase did not show signs of rebounding to pre-harvest conditions in low or medium SNS soils at either depth. Additionally, WEN values in low SNS soil down to a depth of 20 cm exhibited a steady declining trend, though not completely below pre-harvest values. Thus, it is imperative that long-term monitoring of these trends continue due to the importance of nitrogen availability in forest soils. The research presented here indicates that β -glucosaminidase activity and WEN may be useful early indicators of soil quality changes in Missouri Ozark forest soils. Other indicators investigated may prove to be more valuable indicators of soil quality over time.

Chapter 1: Introduction, Objectives, and Literature Review

1.1 Introduction

Recent concern over sustainability has spurred interest in evaluating the influence of land management practices on soil health and soil quality. In research publications and instructional literature the terms "soil health" and "soil quality" are defined and used in slightly different manners. For the purposes of the research presented here, these terms are defined as follows. Soil health is considered to be "the capacity of soil to function as a vital living system within ecosystem land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health" (Doran and Zeiss, 2000). Soil health is a term often used interchangeably with soil quality but the definition of soil health emphasizes soil biological components which influence countless soil processes responsible for supporting life on Earth. Soil quality, as defined by Doran and Parkin (1994), is "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health". The definition of soil quality does not differ greatly from soil health as both terms emphasize the role soil plays in supporting ecosystems and the living organisms within them. The definition of soil health emphasizes the importance of considering chemical, physical, and biological components of soil and how they influence each other when evaluating or monitoring soil degradation. For the purposes of this paper, the term "soil quality" will be used when discussing parameters measured to evaluate if and how soils have changed over the

course of the study. The term "soil health" will be used when discussing the overall condition of the soil.

The properties examined to evaluate soils are referred to as indicators and used to indicate changes in soil quality and health. A plethora of different indicators are applicable for assessing soil health. Chemical soil quality indicators consist of: cation exchange capacity; soil pH; soil carbon, nitrogen, phosphorus, and calcium content; and electrical conductivity. Biological indicators may include: enzyme activity (β -glucosidase, urease, protease, and others); microbial carbon and nitrogen content; microbial diversity; and soil respiration. Physical indicators can consist of: soil bulk density; aggregate stability; water holding capacity; and soil strength. Complicating evaluation of soil health is the fact that numerous methodologies exist for measuring the same soil quality indictor. Additionally, identifying the most meaningful and analytically practical indicators to evaluate can be very challenging for any soil scientist or land owner. There is no guarantee that the chosen indicators will provide a timely response to soil disturbance or environmental change.

The pool of research dedicated to forest soil health and forest soils of Missouri in particular is significantly smaller than the amount of research dedicated to agricultural systems. This is unfortunate given that much of our country and a one-third of the State of Missouri is forested (Raeker et al., 2010). While forest soils do not sustain much in the way of human food sources, they do support air and water quality as well as the forestry industry itself. Missouri's timber industry contributes a total of \$4.32 billion per year to Missouri's total gross state product (Missouri Economic Research and Information Center, 2007). Considering population growth and the rising interest in biofuels, the

demands on Missouri forests to produce timber will only increase. Timber harvest and related industries employ many citizens in south-central Missouri. Unfortunately, the soils in this region are highly weathered and, in some areas, low in soil nutrients. Thus, it is necessary to evaluate current forest management practices to ensure that they provide a sustainable source of timber for future generations.

Why research into forest soils has fallen by the wayside is uncertain. Perhaps it is considered less important given that forest soils do not directly provide a food source for humans. It may be that forest soils, given their wide spatial and temporal variability, are so challenging to study that many soil researchers have avoided making an attempt. The poor accessibility of these soils and the quantity of samples required to accurately quantify changes in soil indicators may also be a deterrent. Although the reasons for the lack of previous research on forest soil health are unclear, it is certain that more research is needed if forests are to continue to be utilized sustainably.

In recognition of the vital role forests play in environmental health and sustaining forest harvest practices, the Missouri Ozark Forest Ecosystem Project (MOFEP) was implemented in 1989. This long-term study site in south-central Missouri is the study area for multiple soil researchers including the research presented here.

1.2 Objectives and hypothesis

The goal of this project was to examine possible changes in soil C and N pools (labile and total), soil microbial enzyme activity, and other indicators of soil quality in soils of differing nutrient status following timber harvest. This information will inform forest managers of useful indicators of soil quality, how quickly changes in soil quality

occur within Missouri Ozark forests, and how such change is related to common sawlog harvest practices (i.e. clearcut and single tree selection).

Objective #1: Quantify the influence of clearcutting and single-tree selection sawlog harvests on total organic carbon (TOC) and total nitrogen (TN) pools, and labile organic carbon (OC) and nitrogen (N) pools in Ozark Highland soils with differing initial nutrient status.

Hypothesis #1a: Due to changes in soil physical conditions (e.g., increased soil temperature and soil moisture) that encourage increased microbial activity, microbial utilization of labile C and N will increase. Although there may be an initial increase in labile C and N as organic compounds are leached from harvest slash, labile C and N will eventually decrease in the clearcut and single-tree selection treatments, relative to non-harvested sites.

Hypothesis #1b: Total organic carbon and TN will decrease with time as recalcitrant TOC and TN substrates are decomposed, but such changes will not be observed in the timeframe of this study (1.5 years post-harvest). The labile forms of C and N will be utilized first by soil flora and fauna, only after these pools are depleted will recalcitrant pools of C and N begin to be utilized.

<u>Objective #2:</u> Quantify changes in soil microbial enzyme activities following clearcutting and single-tree selection sawlog harvest.

<u>Hypothesis #2:</u> Changes in soil physical and chemical conditions that encourage microbial activity will result in soil microbial enzyme activity increases initially after

forest harvest, relative to non-harvested sites, but the activities will eventually decrease as labile C, N, and other soil nutrients become insufficient.

Objective #3: Quantify changes in soil pH and water stable aggregate (WSA) content following clearcutting and single-tree selection sawlog harvest.

Hypothesis #3: Due to the impact of heavy harvesting equipment, removal of the forest canopy, and anticipated mineralization of soil organic matter, it is expected that WSA concentration will diminish over the period of this project. Soil pH may decrease after harvest due to increased soil moisture resulting in the leaching of base cations through the soil profile and production of organic acids during soil organic matter (SOM) decomposition. The magnitude of the change in pH however may not be significant depending on site specific forest soil conditions.

1.3 Introduction and Literature Review

1.3.1 <u>Soil management and soil health.</u> The importance of responsible soil management and conservation has long been on the minds of American farmers and legislators. The 1935 Soil Conservation Act publicly emphasized the importance of effectively maintaining soils. While this legislation was primarily concerned with maintaining soils for the purpose of agricultural utilization, a more modern understanding elucidates the importance of comprehensive soil management. Soils influence and are influenced by all with which they contact, making for some very complex relationships as illustrated in Figure 1.1.

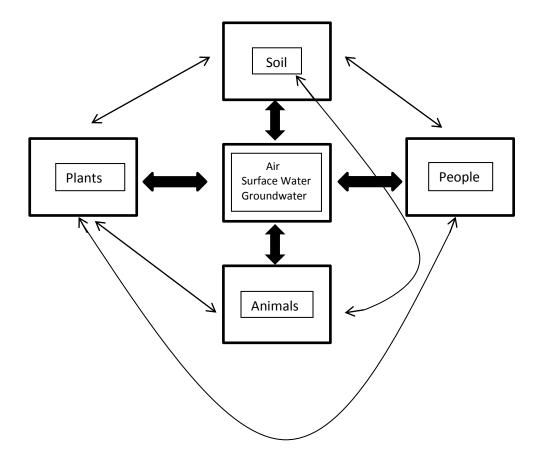


Figure 1.1 Connections between soil health, the environmental and living organisms. Direct (Thick arrows) and indirect (thin arrows) connections between soil, air, water, animals, and people (adapted from Harris et al. 1996).

The multiplicity of interactions and reactions that transpire within soils has forced the use of increasingly more specialized parameters in the evaluation of soil health. In agricultural settings, some farmers have taken the initiative to monitor soil health. A number of agricultural soil score cards and a variety of guidelines to aid farmers in the maintenance of their soils have been developed but even they are state specific (Ditzler and Tugel, 2002). Such score cards are also meant for use by farmers who lack access to laboratory equipment, resulting in highly subjective data records (Romig et al. 1996). Soil scientists studying soils have made significant progress identifying meaningful soil quality indicators.

While Karr (1981) and Larson and Pierce (1991) helped pave the way for a more thorough evaluation of soil health by implementing the unification of chemical, biological, and physical soil property components in soil health evaluation, Harris et al. (1996) and Karlen et al. (1997) exposed the importance of considering soil function when deciding precisely which soil parameters to measure. Christensen et al. (1996) emphasized the importance of also considering management goals when deciding upon soil health indicators. Management goals may be quite different from one system to the next and certainly quite different between agronomists and foresters.

1.3.2 <u>Forest Management Effects on Soil Quality Indicators.</u> The various chemical, biological, and physical parameters of soil health can all be altered by the act of removing trees from a forest. Understanding the effects of these alterations is key in deciding which soil quality indicators to utilize in monitoring soil health. Upon harvest, the forest floor is exposed to an increase in radiant energy and deprived of vegetation which draws water from the soil. Overall, these changes increase soil temperature and moisture, thus

encouraging soil microbial growth and activity (Vitousek et al., 1979). Li et al. (2007) and Lal (2005) suggested that these changes to the soil environment can increase decomposition of the more labile carbon fractions. The actively cycling carbon fraction is the regularly added leaf litter and debris that break down, adding nutrients and contributing to soil structural development. The precise chemical composition of this carbon fraction can vary from one forest type to the next (Kögel-Knabner, 2002) but generally consists of easily oxidizable carbon structures (Weil et al., 2003). The actively cycling carbon fraction in a given forest can significantly impact forest productivity (Ellert and Gregorich, 1995). Carbon, as related to organic matter, affects soil structure which influences root penetration and the suitability of microbial environments. Soil carbon is also vital to microbial nutrition. Microbial activity affects numerous other soil chemical reactions, which in turn influence site productivity (Nannipieri et al., 2012).

Water extractable organic carbon and dissolved organic matter represent the most bioavailable fractions of actively cycling carbon. Although water extractable organic carbon (WEOC) is not a perfect surrogate for dissolved organic matter (DOM), as WEOC would encompass DOM and weakly sorbed organics released into solution during the water extraction process, we would expect similar trends in the cycling of DOM or WEOC (Chantigny, 2003). In the course of this research, active carbon and WEOC were expected to fluctuate over the course of the study but possibly at different rates depending upon soil conditions.

It is difficult to confidently predict how DOM or WEOC will react post-harvest.

Only 10-44% of DOM in soil solution is available to microorganisms (Kalbitz et al.,

2000; Qualls et al., 2000), thus only subtle changes in active and WEOC concentrations

may be observed. Hughes et al. (1990) and Delprat et al. (1997) observed the mobilization of DOM/WEOM in forested sites two years after clear-cutting. However, Moore and Jackson (1989) and McDowell and Likens (1988) reported no notable change in DOC after clear-cutting. Without a clear understanding of how much carbon and organic matter is added to and lost from the soil following sawlog harvest, it is difficult to accurately ascertain timber harvest effects on SOM (Bresee et al., 2004; Chen et al., 2004).

Nitrogen is a key element in healthy productive soils. Nitrogen supports plant and microbial growth and can be a limiting factor in forest productivity (O'Connell et al, 2004; Fisher and Binkley, 2000). After harvest, soil N may be lost from the system through leaching, runoff, erosion, or it may be present but in a form inaccessible to plants (O'Connell et al, 2004). Precisely what happens to forest nitrogen after harvest is unclear. Prior studies have shown increased levels of nitrogen mineralization following forest harvest (Borman and Likens, 1979; Frazer et al., 1990; Prescott, 1997). However, a study by Idol et al. (2003) yielded no such results. At a study site in Indiana, Idol et al. (2003) found no increase in nitrogen mineralization due to harvesting in stands varying in age from one to 100 years in age and at depths up to 30 cm. Additionally, O'Connell et al. (2004) indicated that excess nitrogen in the soil after harvest was not utilized by the remaining trees but leached through the soil profile and not significantly affected by residue management. In fact, according to Idol et al. (2003), plant utilization of soil nitrogen does not reach significant amounts until 5-10 years after forest harvest. Moore and Johnson (1995) noted that increased microbial activity after harvest may preferentially utilize NH₄⁺ and deplete it from the system. Nitrate, however, is not as

easily utilized by trees such as white spruce, Norway spruce, and beech (Brenner et al., 2005). Studies by Larcher (1995) and Atlas and Bartha (1993), indicated that the activity of nitrate reductase in plants and microbes may be inhibited in the presence of excess ammonium, a situation which may or may not occur following forest harvest. Changes in the soil environment and the subsequent changes in plant biochemistry influence nitrogen forms which make the prediction of soil nitrogen content and forest productivity rather challenging.

Forest harvest affects biological components of soil as well as chemical components. Forest harvest can alter the soil environment by increasing water content, altering soil structure, and the exchange of gasses all of which may induce changes in microbial composition or activity rate (Grigal, 2000). Soil microbial populations alter soil carbon and nitrogen content as they fulfill metabolic processes. Respiration of CO₂ from soil microbes and plant roots are one of the largest terrestrial contributors of atmospheric CO₂ (Raich and Schlesinger, 1992; Schimel et al., 1994; Taneva et al., 2006). Monitoring soil biological changes post-harvest is expected to substantiate explanations of why and how soil properties change after harvest (Hassett and Zac, 2005).

Soil microbial enzyme activities are closely associated with soil carbon and nitrogen cycling (Hassett and Zac, 2005; Crecchio et al., 2001), thus correlation between enzyme activity and soil nutrient content is expected. However, precisely how forest harvest practices affect the abundance of certain soil microorganisms is still unclear (Levy-Booth and Winder, 2010). Jizheng et al. (2006) found no significant changes in bacterial diversity or composition after harvests consisting of varying residue retention regimes, and Carter et al. (2002) found no significant changes in microbial populations

under mechanical whole tree removal or hand-cut bole only forest regeneration methods. Another study, however, did report a decrease in enzyme activity after harvest (merchantable bole harvest, total tree harvest, and total tree harvest with forest floor removal) when compared to no harvest control sites 8-10 years post-harvest (Hassett and Zak, 2005). Additionally, Grayston and Rennenberg (2006) noted changes in microbial biomass and enzyme activity after intermediate and heavy forest thinning but results varied according to aspect and soil microbial population. These varied results imply that changes in soil microbial activity and diversity are dependent on numerous factors and reinforces the importance of gathering site specific data if forest harvest effects are to be gaged reliably.

In the forest harvest process, heavy machinery can compact soils and alter soil water content, damage soil aggregates, increase bulk density, and decrease soil porosity (Grigal, 2000). Decreases in soil organic carbon content can also affect soil structure weakening soil aggregation (Nambiar, 1996; Schoenholtz et al., 2000). Soil organic carbon helps bind small soil particles together and form aggregates (Tisdall and Oades, 2006). These aggregate formations encourage water and root penetration as well as gas exchange. Maintaining favorable physical soil conditions is important to maintaining soil health.

Soil pH is a chemical component of soils which can impact soil cation exchange capacity, and thus nutrient availability (McFee et al., 1977), as well as the biological processes that occur in soils (Rosso et al., 1995). Soil microbes typically thrive at pH 7 (Rosso et al., 1995) and soil nutrients are most bioavailable in quantity and variety within a pH range between 5.5 and 7 (Lucas and Davis, 1961). Should soil pH shift significantly

outside of a favorable range, the composition of the microbial population or microbial activity may be altered and potentially inhibit plant productivity (Bardgett et al., 1996; Waldrop et al., 2000; Dick et al., 2000). Changes in forest soil pH, especially small, temporary changes, may not necessarily inhibit forest productivity (Fisher and Binkley, 2000). Forest plant life is not typically as sensitive to soil pH as some agricultural crops but dramatic and enduring soil pH changes are definitely a concern. Acidification of nutrient poor soils can make soil nutrients scarcer as exchange sites are occupied by H⁺ ions and soil nutrients are leached from the system. Acidification of soils may also lead to aluminum toxicity in forests as H⁺ ions react with soil minerals and release aluminum from the mineral structure into soil solution (Hue et al., 1986).

Forest harvest influences soil pH by increasing soil moisture and temperature, consequently altering soil pH as soil biological activity increases in the more favorable soil environment. The decomposition of vast quantities of harvest slash and the resulting flush of bioavailable carbon can lead to increased microbial nitrification, a process which results in the addition of hydrogen ions to soil solution (Van Miegroet and Cole, 1984). In some environments, forest harvest may increase the concentration of acidic cations depending on harvest intensity (Olsson et al., 1996).

In acidic soils, fungal species typically thrive and may occupy a greater portion of the microbial community than they would at higher pH levels (Bardgett et al., 1996), potentially altering nutrient cycling rates. An increased fungal population creates the added complication that fungal activity is often inhibited by high concentrations of NH₄⁺ (DeForest et al., 2004). A flush of NH₄⁺ after forest harvest is not uncommon as newly

added materials decompose. These opposing conditions create uncertainty when considering the potential effect of harvest on soil health.

Soils in undisturbed forests persistently undergo countless chemical and biochemical reactions that influence nutrient cycling and soil properties. When forests are disturbed on a large scale, such as what happens during a forest harvest, a variety of changes to the soil environment occur triggering new or altered chemical and biochemical reactions to occur. Given that soil chemical, biological, and physical properties influence each other, site specific data illustrating these changes are the only way to enhance forest management and ensure sustainable forestry practices. An important first step in this endeavor is identifying soil quality indicators appropriate for a given system and location.

1.3.3 Soil quality indicators in forest soil. As soil scientists continue to evaluate appropriate soil assessment tools for forest soils, the highly variable nature of forest soils have led to a variety of approaches (Harris et al. 1996; Burger and Kelting, 1998; Powers et al. 1998; Andrews and Carroll, 2011). Some researchers have attempted to define and compare high quality and low quality sites (Karlen and Stotts, 1994). Others have suggested a specific number of soil attributes to evaluate (Warkenton, 1995; Doran and Parkin, 1994; Powers et al., 1998; Smith and Conkling, 2005) or focusing on productivity (Romig et al., 1996). Additionally, there is debate as to what defines a useful soil quality indicator (Moffat, 2003; Andrews and Carroll, 2011). Currently, many scientists advocate for the establishment of site specific information and management goals prior to determining the soil quality parameters necessary to monitor soil health (Harris et al., 1996; Karlen et al. 1997; Andrews and Carroll, 2011). For these reasons this thesis

explores the chemical, biological, and physical properties of Missouri Ozark forest soils within different forest management practices.

1.3.4 Chemical soil quality indicators: Soil organic carbon and soil organic carbon pools. Total organic carbon (TOC) is a vital source of energy for soil biota and essential in the chemical transformation of nitrogen (Johnson and Edwards, 1979; Davidson and Swank, 1987; Starr and Gillham, 1993). Organic matter, consisting predominantly of carbon, contributes to favorable soil structure allowing for movement of water and dissolved nutrients throughout the soil profile and the exchange of nutrients and dissolved ions from exchange sites (Pierzynski et al., 2005; Essington, 2004). Total carbon, active organic carbon, and water extractable organic carbon make up progressively smaller pools of soil organic carbon (Trumbore et al, 1995) (Fig. 1.2). Total organic carbon (TOC) is the quantifiable carbon released from a soil sample during high temperature combustion after removal of inorganic carbonates or when no appreciable concentration of inorganic carbonates are present, and TOC consists of recalcitrant, moderately stabile, and labile forms of soil carbon.

Active carbon pool consists of carbon containing material that is easily utilized by soil microbes and includes water extractable organic carbon as well. Active carbon is quantified using a method that employs an oxidation-reduction reaction involving soil carbon and a solution of potassium permanganate (KMnO₄). Water extractable organic carbon (WEOC) consists of organic molecules that readily dissolve in water and are considered to be most easily utilized/decomposed by soil microorganisms (Burford and Bremner, 1975; Qualls and Haines, 1992; DeLuca and Keeney, 1993). Readily available carbon within active and water extractable

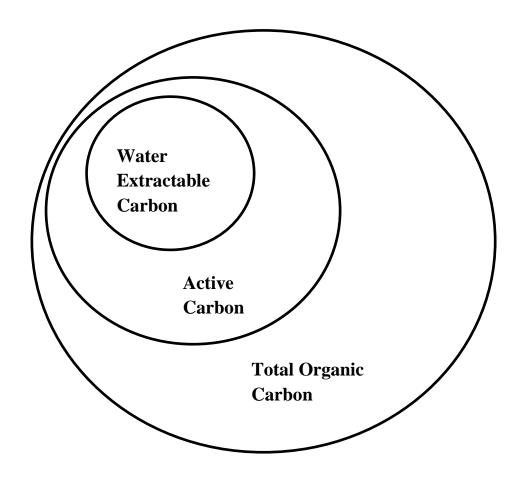


Figure 1.2 Schematic of progressively smaller carbon fractions within soils.

carbon pools can be limiting to soil microbes and the important processes microbes perform within ecosystems (Burford and Bremner, 1975). Better understanding of changes in carbon pools in response to sawlog harvest will provide insight into overall soil health changes after harvest.

1.3.5 <u>Chemical soil quality indicators: Soil nitrogen and soil nitrogen pools.</u> Nitrogen is a vital nutrient to forest systems and is frequently a limiting nutrient in forest productivity (Näsholm and Persson, 2001). Nitrogen is only available to plants in specific forms, i.e., inorganic ammonium (NH₄⁺) and nitrate (NO₃⁻). Changes in environmental conditions can influence microbial processes which in turn influences the form of soil nitrogen and soil productivity (Qualls et al., 2000). Nitrogen is often tightly cycled in forest systems as shown in Figure 1.3 (Lundgren, 1982). If soil conditions change and restrict microbial nitrogen fixation and/or ammonification, the nitrogen lost during forest harvest may not be replaced (Lundgren, 1982; Spratt, 2002). Should this occur over multiple harvests, forest productivity may be inhibited.

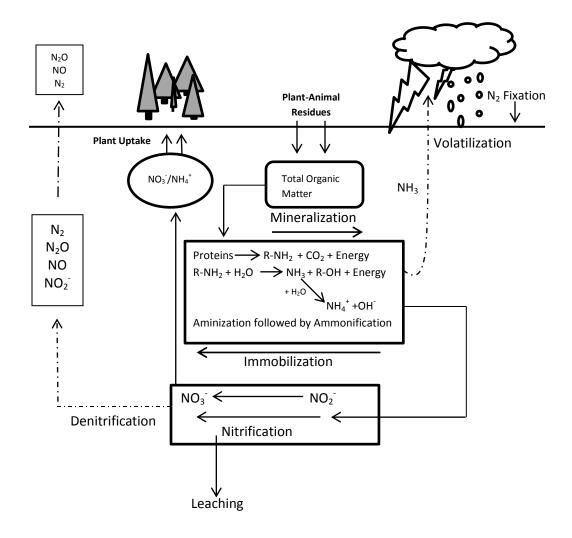


Figure 1.3 Diagram of nitrogen cycling in a terrestrial ecosystem. (Image adapted from the sesl_{tm}website,http://www.sesl.com.au/fertileminds/200805/Nitrogen.php.)

New additions to soil nitrogen, with the omission of plant residues and fertilizers, occur through microbial nitrogen fixation, lightning storms, and acid rain. Nitrogen-fixing soil microbes utilize N_2 in the atmosphere and transform it into ammonia. This process, however, occurs predominantly in the top 10 cm of the soil profile and is dependent on maintaining a favorable microbial environment. Changes in soil pH, soil moisture, and substrate availability all influence microbial activity. Nitrogen can be converted to nitrogen oxides in the presence of the intense heat of lightning and then dissolve into rain drops that eventually percolate through the soil for plant or microbial use. Acid rain contains dissolved atmospheric nitrogen (HNO₃) and adds to soil nitrogen pools, primarily in the northeastern part of the United States (Galloway and Likens, 1981). All atmospheric contributions to soil nitrogen pools however, contribute minimally to plant available soil nitrogen. Because additions of soil nitrogen are rare, maintaining soil nitrogen is vital to forestry sustainability.

1.3.6 <u>Chemical soil quality indicators: C/N ratios.</u> Monitoring changes in carbon/nitrogen ratios (C/N ratios), helps bridge the gap between chemical and biological components of soil health. Soil microorganisms require a C:N ratio of 24:1 to maintain the structural requirements of the cell body and the energy required for respiration (Brady and Weil, 2002). While C containing compounds are typically the source of energy for soil microbes, N is vital to protein synthesis. Availability of C and N, in the proper proportions is vital to microbial function (Haney et al., 2012). It is not surprising then,

that C:N ratios, WEOC/WEN ratios in particular, often correlate soundly with microbial activity (Haney et al., 2012).

Soil C/N ratios are additionally important to soil health given the impact of microbial activities on available soil nutrients when C and N are not available in suitable proportions. In an environment where the ratio of C to N exceeds 24:1, soil nitrogen may be immobilized quickly by a flush of microbial activity straining to utilize the abundance of available C (Hendrickson et al., 1985). Should an increase in microbial activity significantly deplete soil nitrogen stores, competing plants may become nitrogen deficient and inhibit forest productivity.

1.3.7 <u>Chemical soil quality indicators: pH.</u> Soil pH is a fundamental aspect of soil chemical and biogeochemical processes. Different forms of vegetation can be sensitive to particular soil pH values. In this way, soil pH can determine which kinds of plant life will thrive in an area. The balance of ion exchange between mineral exchange sites and soil solution, which determines soil pH, influences several critical components of soil health and productivity including the rate of mineral weathering (Uroz et al., 2009), enzyme activity (Parham and Deng, 2000), and aluminum toxicity (Hue et al., 1986).

While soil pH is important to how soils function on the whole and can be influenced by, and itself an influence on, a variety of factors, temporary fluctuations typically do not have a significant effects on forest productivity (Fisher and Binkley, 2000). For the research presented here, changes in pH were expected to help explain other observed phenomenon, or be used in the general accumulation of knowledge about forest soil pH in harvested forests.

1.3.8 <u>Biological soil quality indicators.</u> Microbial activity is vital to plant health because it facilitates the decomposition of complex molecules into utilizable forms of nitrogen, sulfur, phosphorus, and other important nutrients required for plant life (Dick, 1997). It is the enzymes specifically produced by bacteria, fungi, and plant roots that catalyze the chemical reactions required for decomposition and nutrient availability in the soil (Tabatabai, 1994). It has also been noted that there may be a strong correlation between enzyme activity and plant biomass productivity (Skujins, 1978).

Enzymes produced by soil microbes break down plant residues which are comprised of polysaccharides (starch, cellulose, hemicellulose, and pectin), which constitute about 50-60% of the plant residue; lignin (15-20%); and proteins, polyphenols, chlorophyll, cutin, suberin, lipids and waxes (10-20%) (Lutzow et al., 2006). The processes involved in the decomposition of the many components of organic matter require a variety of microbial enzymes. Analysis of any one enzyme activity requires a unique procedure, thus analyzing numerous enzyme activates typically requires significant time and expense. For the research presented in this thesis, activities of the enzymes β -glucosidase and β -glucosaminidase were measured.

The enzyme β -glucosidase degrades cellulose, a relatively large, organic carbon polymer found in plants. β -glucosaminidase degrades chitin, the second most abundant polymer on Earth found in fungal cell walls and insect exoskeletons, which serves as a source of carbon and nitrogen (Udawatta, et al. 2008). We expect the quantified enzyme activities to lend insight into nutrient cycling changes in the Ozark forest ecosystem in response to forestry practices. While there are no standard biological measurements of forest soil health, β -glucosidase and β -glucosaminidase enzyme activities were chosen

because they relate to the soil chemical components studied here and they are prevalent in Ozark forest soils (Eivazi and Bayan, 1996). Additionally, β-glucosidse activity was shown to contribute valuable information pertaining to carbon cycling when added to the Soil Management Assesment Framework (SMAF) (Stott et al., 2010).

- 1.3.9 Physical soil quality indicator. Water stable aggregates (WSA) are useful indicators of soil structure, which is reflective of soil quality. The innumerable microbial processes that occur within soil systems occur within and between soil aggregates. The larger and more stable the aggregates the greater the concentration of SOM and thus organic carbon (Chaney and Swift, 1984). Water stable aggregates encourage water infiltration, soil nutrient mobility, soil aeration, and root penetration (Bronick and Lal, 2005). Water stable aggregates form in the presence of SOM which binds soil particles together with organic polymers. Aggregation can also occur as soil particles are collected within fine root systems or fungal hyphae. The beneficial soil structure that accompanies soil aggregation encourages forest productivity and contributes to soil quality by facilitating the mechanical penetration of growing roots and providing a favorable environment for nutrient availability (Bronick and Lal, 2005).
- 1.3.10 <u>Soil quality indicators selected for this research</u>. The soil quality indicators chosen for this research are intended to provide valuable information about the changes occurring in Missouri Ozark soils after harvest, as well as the indicators themselves. This research examined carbon and nitrogen pools (TOC, Active carbon, WEOC, and TN and WEN) and enzyme activities that utilize C and N containing compounds (β-glucosidase and β-glucosaminidase respectively). Additionally, WSA content and pH were also monitored. By examining indicators that are easily affiliated with each other, data from

one or more soil quality indicator may help explain observations of another indicator. Water sable aggregate content and pH both influence microbial activity, while carbon content influences WSA content. Changes in enzyme activity may be explained by values of WEOC, WEN, or WEOC/WEN ratios. Changes in WSA concentration may be better understood by having data about MOFEP carbon pools, sampled at the same time as soil pH. Soil pH can influence enzyme activity rates, which in turn may influence the turnover or alteration of available soil C and N. The accumulation of data from these chemical, biological, and physical soil quality data helps create a broader understanding of harvested Missouri Ozark soils.

1.4 MOFEP Information and Background

The Missouri Ozark Forest Ecosystem Project (MOFEP) located in southeastern Missouri provides a unique and exceptional opportunity to study the effects of forest harvest on soil quality. Since 1989, the objective of MOFEP has been to evaluate the impact of different forest management regimes (even-age, uneven-age, and no harvest management) on the Ozark forest ecosystem (Brookshire et al., 1997). The 9,200 wooded acres (3,700 ha) of the MOFEP area are split into: three even-age management (EAM) sites where clearcutting is used as the regenerating method; three uneven-age management (UAM) sites where selection is the regenerating method; and three no harvest or control sites (NHM). The proposed research aims to gain insight into changes in forest soil health following sawlog harvest as indicated by changes in carbon, nitrogen, soil microbial enzyme activity, bacterial diversity, and water stable aggregates.

The Missouri forestry industry is centralized in the south-central part of the state in the Missouri Ozarks where soils are primarily comprised of Ultisols and Alfisols. These soils as examined by Meinert et al., (1997), Hammer, (1997), and Kabrik et al. (2000), vary in drainage class from moderately well-drained to excessively well-drained. The aforementioned studies have also noted significant variability in depth to bedrock from shallow to very deep and geologic strata underlying MOFEP are mostly sandstones and dolomites containing varying amounts of chert, (Fig.1.4, Meinert, 1997; and Fig. 1.5, Albers, 2010). The soil parent material is predominantly hillslope sediments, residuum, and hillslope sediments over residuum. Some loess can be found on stable summits, and alluvium has accumulated in lower areas of the landscape (Kabrick et al., 2000). Due to weathering, portions of the landscape have been worn away creating a hilly terrain with significant slope. Many soils of this region are low in exchangeable base cations, especially soils with great depth to bedrock (Kabrick et al., 2000). The drainage class and landscape slope contribute to the predominantly low nutrient levels within these soils as nutrients can be leached through the profile or eroded to lower landscape positions.

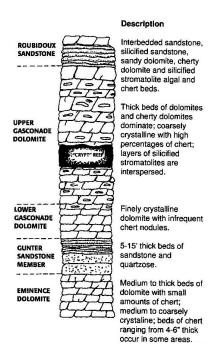


Figure 1.4. Stratigraphy of bedrock geology at MOFEP experimental sites (Meinert et al.,1997).

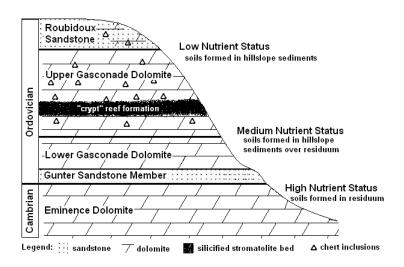


Figure 1. 5. Landform profile illustrating relationship between bedrock geology, soil nutrient status, and soil parent material (Albers, 2010).

1.5 Summary of MOFEP Literature Related to the Research Presented Here

Given that forest soils and the climate associated with a forest's location uniquely influence soil nutrient concentrations, it can be difficult to apply research findings from one site to another. Seasonal variability and annual climate fluctuations may even confound findings from the same sites. There have been several studies of soil nutrient cycling on the MOFEP sites which occasionally reveal slightly different results. Li et al. (2007) noted an increase in total carbon concentrations of mineral soils under UAM and no significant effect of EAM on total carbon (TC) from samples collected eight years after harvest. The authors also found that soil TN significantly correlated with TC. Research by Chen et al. (2004) reported significantly greater soil respiration rates in UAM sites compared to EAM and NHM, but no difference was observed between EAM and NHM sites. Increases in soil respiration in UAM sites indicate increases in microbial activity and possibly soil nutrients that drive such activity, however no such data are currently available to substantiate this postulation. Additionally, Xu et al. (2011) found no difference in soil respiration between EAM, UAM, and NHM sites where sampling took place in the months May through August for a period of five years. These findings may have been due to differences in annual soil moisture. Xu et al. (2011) did conclude that summer mean respiration was more sensitive to soil moisture in EAM and UAM sites than the NHM sites. Albers (2010) concluded that, in comparison to control samples, soil nutrients may be decreasing around stumps remaining after single-treeselection harvest in UAM sites and increasing in soils following clearcut harvests in EAM sites. The samples for Albers (2010) were gathered in July 2007, approximately ten years after harvest. The data presented by Albers (2010) however showed no statistically significant differences between the treated sites (CC sites or STS) relative to control sites

(NHM). All the research discussed above represents data from different times of the year and at different periods of time after harvest events and often at different slope positions. Even though all research occurred at the MOFEP sites, time and slope position can influence research results.

Research recently completed by Singh (2013) examined soil solution chemistry which revealed an increase in NO₃⁻ and TN concentrations in EAM sites, after harvest, when compared to values from UAM and NHM sites. Research by Singh (2013) also noted a significant increase in mean daily flux of NO3⁻ and Mg²⁺ for CC treatment sites after harvest when compared to all other pre and post-harvest treatments. The research for this thesis occurred over the same period of time and at the same locations as the work completed by Singh (2013). Research by Singh also indicated that extractable manganese (manganese oxides) was the most important factor in explaining variability in the three forms of phosphorus studied (total phosphorus, Mehlich-3 available phosphorus, and Bray-1 available phosphorus). Total organic carbon and CBD-Mn together explained 39.6% of the variation in total phosphorous values in the Ozark phosphorus pools. Given that Ozark soils likely have relatively small pools of nutrients like phosphorus and nitrogen, the importance of understanding nutrient cycling in this region seems even more imperative to sustainable forestry.

1.6 Summary

Forest soils are highly complex systems requiring multiple interactions within the soil environment and between environments if they are to "sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health" (Doran and Zeiss, 2000). Understanding these interactions becomes increasingly

important in highly weathered soils of the Missouri Ozarks that support the high demands of forest industry. The suite of soil quality indicators discussed for this thesis has not been examined together before in the MOFEP sites. It is the hope of this study that the indicators examined will provide useful information about the Missouri Ozark forest soils and aid in sustainable management.

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Chapter 2: Evaluation of forest harvest management effects on soil quality indicators in the Ozark Highlands.

2.1 Abstract.

Our country's needs for wood products, the rising interest in biofuels, and a global interest in soils as a carbon sink place high demands on forest soils. The highly weathered and nutrient-poor soils of the Missouri Ozarks are vulnerable to degradation, thus necessitating improved understanding of forest harvest impacts on soil quality. The objective of this study was to investigate changes in selected soil quality indicators following sawlog harvests. The research was conducted at the Missouri Ozark Forest Ecosystem Project (MOFEP), a long-term experimental study in mixed hardwood forests of southeast Missouri. Pre-harvest and post-harvest soil samples were collected at depths of 0-10 cm and 10-20 cm from sites harvested using clearcutting (CC) and single-tree selection (STS) and from no harvest (NH) management sites. Samples were collected from low (<20 % base saturation in diagnostic subsoil horizon) and medium (20-50 % base saturation in diagnostic subsoil horizon) soil nutrient status (SNS) soils. The chemical soil quality indicators examined included total organic carbon (TOC) and total nitrogen (TN), active C (KMnO₄ oxidizable carbon), water extractable organic C and water extractable N (WEOC and WEN, respectively), and soil pH. Activities of soil microbial enzymes β-glucosidase and β-glucosaminidase were evaluated as biological indicators. Water stable aggregate content (WSA) was quantified to examine changes in physical soil properties. Few differences in soil quality parameters were observed in the \sim 1.5 years after harvest. However two indicators, β -glucosaminidase activity and WEN, showed significant change after harvest. In CC treatments β-glucosaminidase activity

decreased significantly at the 0-10 cm depth in January 2013 post-harvest collections when compared to the January 2013 NH values and pre-harvest CC treatment values collected January 2011. On specific collection dates, water extractable nitrogen also decreased significantly in CC treatments at 0-10 cm depths from low and medium SNS soils. In the CC treatment, values in low SNS soils collected in January 2013 post-harvest were significantly lower in WEN than NH treatment values from January 2013 and preharvest (January 2011) NH values. Soil quality changes after harvest, were most pronounced in CC harvested sites, though CC and STS sites were rarely significantly different from each other. Within the timeframe studied, β-glucosaminidase did not show signs of rebounding to pre-harvest conditions in low or medium SNS soils at either depth. Additionally, WEN values in low SNS soil down to a depth of 20 cm exhibited a steady declining trend, though not completely below pre-harvest values. Thus, it is imperative that long-term monitoring of these trends continue due to the importance of nitrogen availability in forest soils. The research presented here indicates that β -glucosaminidase activity and WEN may be useful early indicators of soil quality changes in Missouri Ozark forest soils. Other indicators investigated may prove to be more valuable indicators of soil quality over time.

2.2 Introduction

Identifying sensitive, meaningful, quantifiable, and timely economic indicators of soil health has been the goal of agricultural and environmental soil scientists for a number of years. While there has been more research applied to agricultural soils than forested soils, neither focus has clearly determined which soil health indicators are most valuable for either general practice or specific sites. The response time and duration of response

for soil health indicators are also not well understood especially with respect to region and climate.

Recent research has provided some interesting revelations pertaining to soil health and soil health indicators. A growing number of studies suggest that the utilization of biological indicators, soil microbial enzyme activity in particular, can provide early indication of changes in soil health (Ndiaye et al., 2000; Pajares et al., 2011, Marinari et al., 2006). While some studies have demonstrated notable changes in soil enzyme activity after 7-10 years of treatment (Marinari et al., 2006). Pajares et al. (2011) noted changes in urease, protease, β-glucosidase and phosphatase activity in cultivated soils of Mexico just 4 years after new management implementation. Ndiave et al. (2000) noted changes in soil enzyme activities (arylsulfatase and β -glucosidase) as early as 1-2 years after new management implementation. A study by Yuqing et al. (2012) observed changes in protease and arylsulfatase activity in pitch pine forest soils within one year after disturbance, however there were no observed changes in β -glucosidase or β glucosaminidase activities. Whether these differences between enzyme activities are due to species specific biochemistry of the enzymes or ecological properties such as test site slope or aspect is unclear (Hojeong et al., 2009).

Biological indicators are useful not only as an indication of healthy, active microbial populations, but they also correlate well with soil nutrients which may not elicit measurable change as rapidly as biological indicators. Enzyme activity often correlates well with soil nutrient levels (Acosta-Martinez et al., 2004), most notably C/N ratios (Geisseler and Horwath, 2009; Michel and Matzner, 2003) and soil organic carbon (SOC) (Chaer et al., 2009). The study by Chaer et al. (2009) was conducted in the Pacific

Northwest and documented changes in phosphatase activity 50 years *after* harvest which accounted for 97% of SOC variation among sites. While more information is needed to clarify how rapidly after harvest phosphatase exhibits change, this study makes a strong case for phosphatase as a viable soil health indicator for the Pacific Northwest, this enzyme my not be equally useful for other regions. Soil microbial enzyme activity has been shown to correlate strongly with productivity in unmanaged or low input agricultural systems (Skujins, 1978; Stursova and Baldrian, 2011), and this soil quality indicator may react similarly in forested systems (as suggested by Eivazi and Bayan, 1996). In situations where the quantification of enzyme activity is inconvenient, soil microbial C may also provide an early response to disturbance (Ndiave et al. 2000; Sparling, 1997). Soil microbial carbon may be a good option if an informative enzyme soil quality indicator is unknown, requiring multiple enzyme assays. Soil microbial C has been shown to correlate positively with enzyme activity and provides quantification of a carbon source in soils as well as an indication of microbial proliferation.

There are still many questions to be answered with regard to the application of biological indicators. The activities of some soil microbial enzymes are not universal indicators that can be applied to every forest soil (Yuqing et al., 2012; Pajares et al., 2011). The duration of soil biological change is also unclear, as is the variability between agricultural and forested ecosystems. While enzyme activities can help provide early measurements of soil changes after perturbation, researchers must remember that several enzymes may be involved in the alteration of any given nutrient pool and not all alterations to soil nutrient pools are influenced by enzyme activity (Nannipieri et al.,

2012). However, where early indication of soil degradation or alteration is required, biological indicators and enzyme activities in particular seem to show promise.

The use of other forest soil health indicators (i.e., chemical or physical soil properties) may necessitate longer time periods of study. Studies of soil C and N have noted changes in forest soils occurring between 10 to 110 years after disturbance (Compton and Boone, 2000; Albers, 2010). Arshad and Martin (2002) have suggested that long-term studies be conducted for a minimum of 30 years with the anticipation that longer periods of study will explicate the duration of soil quality changes after disturbance, determine the possibility of rectification or mitigation of these changes, and clarify how changes immediately following disturbance correlate with long-term soil health effects.

From the point of view of the forester, the ultimate soil health indicator may be forest productivity. Unfortunately, measurements of productivity often are not measurable within the span of a study (Powers et al., 2005). While there have been a number of studies discussing total organic carbon and total nitrogen in respect to soil health (Albers, 2010; Powers, 2004; Grand and Lavkulich, 2012; Bedison and Johnson, 2009), this does not give a clear picture of microbial or plant available soil nutrients. The degradation of TOC and TN is highly relevant to maintaining soil structure and soils as a carbon sink, but these properties may not relate to site productivity as rapidly as other variables.

It is unfortunate that active and labile forms of soil nutrients such as carbon and nitrogen are often overlooked when evaluating soil quality (Amacher et al., 2007; Moffat,

2003; Andrews and Carroll, 2011). The much smaller, but significantly more bioavailable, pools of KMnO₄ oxidizable carbon and water extractable carbon and nitrogen have been shown to change relatively quickly in response to disturbance (Chantigny, 2003; Qualls et al., 2000; and Hannam and Prescott, 2003) and they are strongly correlated with soil microbial processes (Takuo, et al. 2004; Boyer and Groffman, 1996; and McDowell and Likens, 1988). After clearcutting, a study by Moore and Jackson (1989) noted a change in dissolved organic carbon (DOC) less than two years after harvest. Persistent changes in DOC after clearcut harvesting have also been documented 10 years after harvest (Meyer and Tate, 1983). Moore and Jackson (1989) found no changes in dissolved organic matter (DOM) after clearcut harvest, but there is often significant variability in ecosystem dynamics and time between harvest and sampling. The Moore and Jackson (1989) study in particular, collected samples 8-10 years post-harvest and analyzed soil solution samples rather than soil samples. These studies indicate the necessity of site-specific data but do not infringe upon the potential importance of labile nutrient fractions as soil health indicators.

Increased soil bulk density can be indicated by decreased content of larger water stable aggregates (WSA) which could inhibit plant root penetration (Taylor and Gardner, 1963; Grable and Siemer, 1967). Soil compaction may inhibit soil enzyme activity as was reported for protease and phosphatase (Xiau et al., 2008). In addition to compaction, soil aggregation is also influenced by soil chemistry, the state and presence of soil fungi, the activity of soil organisms, as well as climatic influences (Horn and Smucker, 2005; Oades, 1993). Changes in soil structure can occur locally, immediately following compression by biological, mechanical or environmental forces (Hamza and Anderson,

2005). Soil compaction, however, is not always significant enough to inhibit root growth (Beylich et al., 2010). In agricultural systems WSA content has been shown to increase significantly after approximately two years of management implementation (Perfect et al., 1990).

Soil pH is not as influential in forest soils as agricultural soils but can have a substantial impact following disturbance. Forest plants are typically not as sensitive to minor and temporary fluctuations in soil pH as food crops, though pH may affect soil microbial activity (Andersson and Nilsson, 2001). Enduring changes to soil pH may alter forest composition over time, selecting for species more tolerant to the new soil pH and limiting forest diversity (Falkengren-Grerup and Tyler, 1993; Falkengren-Grerup, 1995). Monitoring of soil pH as a parameter of forest soil health primarily serves two purposes. First, the measurement of soil pH on the study sites will contribute to the overall knowledge of these specific sites. Second, monitoring soil pH is important should it change dramatically and influence other soil parameters and plant productivity. Highly acidic soils can inhibit forest productivity by mobilizing soil aluminum and inducing aluminum toxicity in forest plants (Hue et al, 1986; Hue et al, 1986).

Much of the available literature indicates that harvest methods involving less biomass removal (i.e. STS) typically impart less impact to forest soils than more intensive harvesting (Jerabkova et al., 2011; Li et al., 2007; Olsson et al., 1996; Zheng, et al., 2000). Indeed, it appears that clearcut stem-only harvests are even less damaging to soils than whole tree harvests where no part of the tree is left to recycle forest nutrients (Mahendrappa et al., 2006). The intensive nature of biomass harvest has been shown to reduce tree growth for at least 20 years post-harvest due to poor P and N nutrition

(Thiffault et al., 2011). However, the changes to soil health indicators are highly variable by region and the impact of varying management regimes between specific sites can be difficult to predict or compare across regions.

Laporte et al. (2003) indicated that STS harvests were better at mitigating the efflux of CO₂ from soils than clearcutting in forest soils of the Turkey Lakes Watershed of northern Ontario. In a separate study, nine to ten years post- harvest, however, there were no notable differences in nutrient availability in the mineral soil layers or in tree nutrition between NH, partial cuts, and CC sites in British Columbia's Date Creek Research Forest (Kranabetter, and Coats, 2004). There were only small decreases in C/N ratios and soil moisture of the forest floor of the CC sites (Kranabetter, and Coats, 2004). Grand and Lavkulich (2012) noted greater SOC in the mineral horizon of CC sites than those of regenerating plots and control plots. These data however was gathered on Canadian Spodosols under Douglas firs 2-5 years post-harvest and may not be comparable to southern deciduous forests. Barg and Edmons (1999) also failed to show any significant difference in the rate of change for soil nitrogen or microbial biomass between CC, STS, and NH sites; however, sampling for this study took place 60-70 years post-harvest and only rates of change in nitrogen were reported, no comparison of preharvest and post-harvest soil nitrogen content was available. It is interesting that the rates of change for nitrogen and microbial biomass were not different between treatments, indicating the possibility that initial losses from harvest may not be regained over time. A study of Amazonian forest soils by McNabb et al. (1996) revealed a decrease in soil C and N sixteen years after harvest which was exacerbated by increasing harvest intensity. Similar results were found by Olander (2005) only a few months after forest harvest in

the Amazon, implying a sustained impact on Amazonian soil health. More studies documenting and comparing values of soil health indicators before harvest to those immediately following *and* distantly after harvest would help clarify our understanding of harvest influences on forest soil health.

2.3 Objectives and Hypotheses

The goal of this project was to examine possible changes in soil microbial enzyme activity, soil C and N pools (labile and total), and other indicators of soil quality in soils of differing nutrient status immediately following timber harvest. This information will inform forest managers of early indicators of soil quality, how quickly changes in soil quality occur within Missouri Ozark forests, how such change is related to forest management practices (i.e. clearcut and single tree selection), and provide a starting point for long-term monitoring of soil quality at the Missouri Forest Ecosystem Project (MOFEP). Ultimately, the incorporation of this research with past and future soil research at MOFEP will aid in future management decisions for Ozark forests and provide information about the suitability of particular soil health indicators for this region.

2.4 Sampling and Analysis

2.4.1 <u>Experimental site and sampling locations.</u> The 3,723 ha (9,200 acres) comprising MOFEP in Southeast Missouri provides an optimal opportunity to study ecological changes that may be associated with forest management. At MOFEP, there are nine permanent sites varying in size from 321 to 514 hectares with each site assigned one of three treatments: even age management (EAM) with clearcutting forest regeneration; uneven age management (UAM) with single tree selection forest regeneration; and no

harvest management (NH) (control) (Appendix J). Within *each* of the nine sites, we have established sampling locations on low and medium soil nutrient status (SNS) soils. Soil nutrient status was defined according to percent base saturation of the soil cation exchange capacity in the diagnostic subsoil horizon. Soils with less than 20% base saturation are defined as "low" SNS, those with 20-50% base saturation are "medium" SNS, and soils with greater than 50% base saturation are defined as "high" nutrient soils (Appendix I). Sites with high nutrient status soils were excluded from this study as none were proposed for harvest in 2011. This is likely due to the fact that soils of this type were positioned in areas with widely variable depth to bedrock, thereby, severely limiting site productivity.

The low and medium SNS soils were delineated by soil map units 80F (63F alternate) and 82F (75F alternate), respectively. The low nutrient soils (80F/63F units) comprise loamy-skeletal and loamy-skeletal/clayey textured soils with low base saturation. The medium nutrient soils (82F/75F units) are loamy-skeletal and loamy-skeletal/clayey textured soils with low to high base saturation (Meinert et al., 1997). All samples were gathered in close proximity to soil pits used by Gaddie (2012) and Singh (2013) for the study of soil solution chemistry changes following harvest. The locations of these soil solution sampling pits were chosen to ensure forest harvest would occur in 2011. Care was also taken to ensure that pits were dug in a low nutrient soil and a medium nutrient soil within each of the nine sites. To control as many influential factors as possible, each pit was dug on the backslope landscape position. In order to maintain consistency in soil composition and, when possible, convenience to an access road, precisely uniform percent slope could be not be rigidly maintained among pit locations.

2.4.2 <u>Soil sampling and processing.</u> At each of the 18 soil pits (nine low and nine medium nutrient status soils), triplicate subsamples were collected at two depths (0-10 cm and 10-20 cm). Samples were collected at the same elevation or slightly below the position of the soil solution sampling pits (within 25 m of the soil solution monitoring site). Approximately one quart-sized bag (~1200 to 1700 g) of soil was collected at each depth, labeled, and stored in a cooler until the end of the day, at which point the samples were removed from the coolers and stored in a refrigerator at approximately 4°C until the end of the sampling trip. During the 3-4 hour trip back to Columbia, MO, the set of 108 samples was stored in coolers. In the laboratory, samples were stored at 4°C until processed and analyzed.

Two sets of pre-harvest samples (January 2011 and March 2011) were gathered from all sites during two-to three-day-long sampling expeditions. The first set of post-harvest samples was collected 30-60 days after each individual plot was harvested. Due to differences in harvest times amongst the sampling sites, all sites were not sampled at the same time. Subsequently, the 30-60 days post-harvest sampling resulted in a staggered collection throughout the summer, fall and winter of 2011 and 2012. To account for such variations in the data set, soils of equivalent nutrient status within NH locations were sampled simultaneously. Once harvest was completed, post-harvest sampling of all 18 sites occurred in April 2012, July 2012, and January of 2013.

Processing of the samples was completed within seven days of sample collection.

Approximately one-half of each sample was air-dried for 48 hours and then sieved through a 2 mm sieve. Air-dried samples were analyzed for TOC, TN, WEOC, WEON, WSA, and active carbon. Remaining soil that was not dried was stored in the refrigerator

at 4°C for enzyme activity analyses. Enzyme analyses were performed prior to analyses involving air-dried samples to reduce changes to soil chemical and biological content as much as possible.

2.4.3 Analyses

While there is a suite of available soil indicators to choose from when evaluating soil health, those chosen for this research touch upon the spectrum of biological, chemical, and physical indicators, as well as indicators believed to illustrate immediate and long-term changes to soil health.

<u>C and N.</u> There are many methods available to measure SOC and TN, but the methods used here are precise and reasonably uncomplicated (Sikora and Stott, 1996). Total organic carbon and TN analyses measure recalcitrant and labile forms of carbon and nitrogen. Total organic carbon and TN were measured using a LECO TOC/TN analyzer (St. Joseph, MI, USA). Air-dried samples were ground using a mortar and pestle, they were oven-dried at approximately 105°C for approximately 1 hour. Some variability in oven temperature occurred, possibly due to the age of the oven. The samples of approximately 0.2 g were analyzed by combustion and the CO₂ released was measured using an infrared detector to quantify TOC (Burt, 2004). Total nitrogen was also quantified by combustion and N₂ released was measured using a thermal conductivity cell to quantify TN (LECO Corporation, 2008).

The WEOC/WEN analysis quantifies a small portion of active carbon fraction as it consists of carbon that readily dissolves in water and is most available to soil microfauna (Chantigny, 2003). For the WEOC and WEN analysis, we referred to the

general extraction method by Buford and Bremner (1975). Twenty grams of air-dried sample was combined with 40 mL of Barnstead ultra-pure water in a 50 mL polypropylene centrifuge tube. The tubes were shaken on the end-to-end shaker for 1 hour and centrifuged at 3,600 rpm for 20 min. Following centrifugation, the supernatant of each sample was filtered through filter paper of 0.45 µm nominal pore diameter into 25 mL glass vials. Prior to analysis, WEOC/WEN samples were pre-acidified to pH 2 with 10% H₃PO₄. Acidified filtrates were analyzed using a Shimadzu TOC-V_{CPH} (Kyoto, Japan) equipped with an ASI autosampler and TNM-1 total nitrogen module to quantify non-purgeable organic carbon and total N as measures of WEOC and WEN, respectively. In many samples the pre-acidification process caused a precipitate to form, presumably humic acid. To ensure a homogenous sample, the vials that contained precipitate were vortexed and agitated on an end-to-end shaker for approximately one hour before analysis. Occasionally values appeared unusually low, possibly because precipitation interfered with analysis. In such cases, the samples were re-extracted and analyzed. This procedure was sufficient for several data sets, but a revised procedure was developed that called for extraction and analysis to be performed in the same day. Samples were not acidified until immediately before analysis and samples were extracted and analyzed in small batches of twelve samples to minimize precipitation.

The potassium permanganate (KMnO₄) technique was used to estimate active carbon, a carbon pool readily available to microbes. Potassium permanganate analysis utilizes a redox reaction to colorimetrically identify active carbon content. Changes to this carbon fraction in other studies were useful indicators of soil quality (Islam and Weil, 2000). Concentrations of KMnO₄ similar to those used in this procedure have been shown

to oxidize the carbon fraction most closely correlated with properties associated with soil health (aggregate stability, infiltration rates, and effective cation exchange capacity) (Bell et al., 1998).

The KMnO₄ procedure performed here is a slightly modified version of the procedure described by Weil et al. (2003). The procedure involved adding 5 g of sieved, air-dried soil to 6 mL of 0.2 M KMnO₄ and 14 mL ultrapure water in a 50 mL polypropylene centrifuge tube. The suspension was then reacted on an end-to-end shaker for 15 minutes and centrifuged at 3,600 RPM for 5 min. Samples were diluted by combining 0.25 mL of the supernatant with 24.75 mL Barnsted ultrapure water. Samples were analyzed by reading absorbance at 550 nm using a Spectronic Genesys 8 UV/Visible spectrophotometer (Thermo-Fisher Sci., Madison, WI). The procedure was modified by incorporating greater volumes of 0.2 M KMnO₄ to account for the elevated quantities of carbon that occur in forest soil samples.

Soil microbial enzyme assays Quantification of β-glucosidase required modification of the procedure established by Tabatabai (1994). In our procedure, 0.5 g of field moist soil, passed through a 2 mm sieve, was added to a 50 mL Erlenmeyer flask. Test samples (analyzed in duplicate) received 13.75 mL of modified universal buffer (MUB) at pH 6, 0.5 mL of 0.05 M p-nitrophenyl-β-D-glucoside (PNG), and 0.25 mL of toluene. Samples were swirled to thoroughly mix the contents, flasks were plugged with a rubber stopper, and samples were incubated at 37°C for 1 hour. A control sample was analyzed with the two test samples; the control sample contained 0.5 g soil, 14.25 mL of MUB at pH 6, and 0.25 mL of toluene (no substrate added). After incubation, each test sample and control received 1.0 mL of 1.0 M CaCl₂ and 4.0 mL of THAM pH 12

(tris(hydroxymethyl)aminomethane). All samples were swirled again, filtered through a 0.2 µm nominal pore diameter nylon syringe filter and absorbance was measured using a spectrophotometer at a wavelength of 410 nm. The total liquid volume used for this procedure is 19.5 mL with 0.5 g of soil. The original method by Tabatabai (1994) called for a total liquid volume of 10.25 mL with 1 g of soil; however, this ratio of liquid to soil produced very high absorbance readings. Dissolved organic carbon and fine clay particulates also appeared to alter the absorbance readings. The larger reagent volume, more concentrated flocculating agent, and a refined filtration method acted to provide a more consistent absorbance reading and thus more accurate data.

The β -glucosaminidase activity procedure used for this research was modified from Parham and Deng (2000). Our procedure reacted 0.5 g of sieved (< 2 mm), field moist soil with 7.0 mL of 0.1 M acetate buffer of pH 5.5 and 0.5 mL of 10 mM P-nitrophenyl-N-acetyl- β -D-glucosaminide (P-NNAG) solution in a 50 mL Erlenmeyer flask. Control samples were run concurrently with test samples. Two replicate test samples and one control were analyzed for each individual soil sample and incubated for 1 hour at 37°C. Once incubation was complete, test and control samples received 1.0 mL of 0.5 M CaCl₂ and 7.0 mL of 0.5 M NaOH solutions resulting in a final volume of 15.5 mL. The control sample also received 0.5 mL P-NNAG after the addition of CaCl₂ and NaOH rather than at the beginning of the reaction. After filtration with a nylon syringe filter (0.2 μ m pore size), samples were analyzed using a spectrophotometer at a wavelength of 405 nm (Parham and Deng, 2000).

The decision to increase the volume of the reacting solution was made because at a smaller volume the absorbance readings were too great to be reliable. To maintain

consistency between the analysis of the two enzymes and save time, these samples are also filtered using a syringe filter rather than a vacuum filtration system which is commonly used in other protocols. Additionally, samples analyzed for β -glucosaminidase activity were filtered and absorption readings were read in groups of three. When all samples were filtered at once and before absorbance readings were taken, a white precipitate formed in the samples. This resulted in an artificially high absorbance reading that skewed the data.

Soil pH analysis. Soil pH was analyzed using the 1:2 (w/v) CaCl₂ procedure described by Burt (2009). To quantify soil pH, each soil sample was first air-dried and sieved to < 2 mm. Twenty grams of each sample was weighed into an 8 oz. plastic cup and combined with 20 mL ultrapure DI water. The samples were initially stirred and then allowed to sit for 1 hour with periodic stirring. After the hour passed, 20 mL of 0.02 M CaCl₂ was added to each sample and stirred for 30 seconds. After waiting one minute upon cease of stirring, soil pH was measured with a calibrated AR60 Accumet Research Dual Channel pH meter and Thermo Scientific Ross sure-flow epoxy-body electrode with BNC connector. The most reliable results were obtained if the cup was not moved after the slurry sat for that one minute. Otherwise, the meter took notably longer to stabilize. Each sample collected from the field was analyzed only once for pH. Because subsampling is built into the method of collection, additional repetition was deemed superfluous.

Water stable aggregate analyses. Water stable aggregates were quantified using a method based on the Kemper and Rosenau (1986) procedure, modified by Angers and Mehuys (1993). Ten grams of field moist soil were sieved to ≤ 2 mm and air-dried

overnight. The samples were spread over a 250 μm sieve and placed on the wet sieving apparatus. The sieve was lowered into the water and the samples were allowed to wet by capillarity for 10 min. Samples were wet sieved for 10 min at 29 strokes per min. Once complete, aggregates remaining on the sieve were washed into a 250mL Erlenmeyer flask and dried at 105°C and weighed. Subsequently, approximately 50 mL of 0.5% sodium hexametaphosphate was added to each flask and shaken on a side-to-side shaker for 45 min. The dispersed aggregates were washed onto a 250 μm sieve and rinsed briefly. The material was transferred back into the flask and dried again at 105°C overnight or until dry. Water stable aggregate values are reported by weight of oven-dried soil.

2.4.4 Statistical Analysis. SAS Enterprise Guide 5.1 Software Version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. APROC GLIMMIX (generalized linear mixed model) code was first used to compare all data collected throughout the study (two pre-harvest and four post-harvest collections). Second, PROC GLIMMIX was used to compare one pre-harvest data set (January 2011) with one post-harvest set of data (January 2013) (Appendix A.I). The PROC GLIMMIX model was a spatially-repeated split-plot model used to create a split by treatment, soil nutrient status, depth, and harvest. Data were analyzed separately for each dependent variable. The main effects of this analysis included: (1) treatment (trt) which evaluated differences between the CC, STS and NH treatments; (2) soil nutrient status (sns) evaluating differences in sample data from 0-10 cm depths and 10-20 cm depths; (4) harvest, evaluating differences between pre and post-harvest samples; (5) block, evaluating differences in the spatially repeated treatment samples; (6) completion, evaluating differences between

complete sample collections and the sporadic collections that took place as harvest occurred; and (7) event, evaluating differences in the time periods in which the collections occurred.

The PROC UNIVARIATE code (Appendix A.II) was used to determine normality of data distribution as well as the most applicable transformation to use if the data were not normally distributed. The applied transformations are noted in Tables 2.1 and 2.2. The Tukey-Kramer least squared differences LSMEANS test was also applied to the data to compare differences in mean values with respect to harvest, depth, and the harvest*treatment interaction.

The 95% confidence intervals around mean values of CC, STS, and NH site data were compared over time with respect to SNS and depth. The trends in mean values of the data compilation were also analyzed using a linear regression analysis; mean values for each treatment (NH, CC, and STS) and sampling period (January 2011 pre-harvest, March 2011 pre-harvest, 30-60 days post-harvest, April 2012 post-harvest, July 2012 post-harvest, and January 2013 post-harvest) were evaluated. The 95% confidence interval was calculated for the slope of each treatment with respect to SNS and sampling depth and compared against each other. This analysis however, indicated no significant trends or differences. The results of this analysis can be found in appendix B.

2.5 Results and Discussion

Statistical results displayed in Table 2.1 summarize the PROC GLIMMIX analysis investigating the influence of the main effects on soil quality indicators studied across all sampling periods. Table 2.2 displays PROC GLIMMIX results limited to

analysis of pre- and post-harvest samplings from January 2011 and January 2013, respectively. Analyses for Table 2.2 were completed to eliminate suspicion of temporal influence that may have masked treatment effects.

There were no significant effect of treatment (trt), soil nutrient status (sns), and the interaction of treatment and soil nutrient status (Tables 2.1 and 2.2). From analysis of the complete data set (Table 2.1), it is also observed that there were no significant interaction of depth with treatment. In Table 2.2, no significant differences were observed for the interactions of depth by soil nutrient status, soil nutrient status by harvest, and depth by harvest. The lack of an effect associated with treatment may be due to a general lack of differences between the three treatments (CC, STS, NH); however, it is also important to note that the statistical analysis was performed on a compilation of all pre-treatment and post-treatment data. Thus, only large changes associated with treatment could be observed under such a scenario. No observation of significant differences for the soil quality parameters between the soils of differing nutrient status (low and medium nutrient status) is likely attributable to the shallow sampling depth employed in this study. Soil profile and soil characterization data (Gaddie, 2012) indicate that the upper soil profile is similar between the soils studied due to the presence of hillslope sediments at the soil surface.

Of the main effects and interactions between main effects, depth and harvest show the greatest number of significant differences for the soil quality parameters evaluated. While the influence of harvest (pre- versus post-harvest data) appears to be a very influential effect, it is important to consider that temporal variations and weather could be strongly influencing the results. Additionally, the data analyzed also includes NH data in

the post-harvest data set (harvest simply designates whether the data were collected before or after harvest was completed at MOFEP, not whether or not a site was actually harvested). Evaluation of the Tukey-Kramer LSMEANS results indicate that values for the soil quality indicators were generally less after harvest. We attribute this to drought conditions occurring January, July, and October of 2011 and again from March 2012 through September 2012 as well as November through December of 2012 (NOAA, 2014) when many post-harvest samples were collected.

Of the remaining interactions, the most relevant for evaluating the influence of CC and STS on the soil quality indicators is the interaction of treatment by harvest. This interaction permits separation of the harvest treatment (SS, STS, and NH) effects to data collected pre- and post-treatment. Soil quality indicators exhibiting statistical significance associated with this interaction in Tables 2.1 and 2.2 will be discussed in the following sections, however, no significant differences were observed between CC and STS treatments in any soil quality indicators studied.

Lastly, the effect of depth is also consistently significant. Comparison of least squares means shows a decrease in the values from all analyses as depth is increased. This is not surprising as soil nutrients and biological components can decrease with increased depth (Jobbágy and Jackson, 2001).

Table 2.1 Type 3 Tests of Fixed Effects, evaluating treatment (trt), soil nutrient status (sns), depth, and harvest effects on soil properties at MOFEP. Dependent variables include water extractable organic carbon (WEOC), KMnO₄oxidizable carbon (Active C), total organic carbon (TOC), total nitrogen (TN), total organic carbon/ total nitrogen ratio (TOC/TN), water extractable organic carbon/water extractable nitrogen (WEOC/WEN) ratio, β -glucosidase enzyme activity (β -glu), β -glucosaminidase enzyme activity (β -glumin), water stable aggregate concentration (WSA), and soil pH (pH). Significant values (p-values <0.05) are placed in bold. The "†" indicates a gamma distribution of the data set and "‡" indicates a lognormal distribution.

Analyte											
p-values											
Source	WEOC †	Active C	TOC ‡	WEN	TN ‡	TOC/TN	WEOC/WEN	β-glu	β-glumin	WSA †	pН
Trt	0.6249	0.8446	0.0590	0.5846	0.3780	0.1735	0.4913	0.9996	0.4761	0.2437	0.5382
Sns	0.6221	0.3712	0.2279	0.2712	0.6197	0.4538	0.5449	0.5135	0.5862	0.9827	0.5747
trt*sns	0.8085	0.4441	0.9298	0.6770	0.09308	0.8987	0.9611	0.4941	0.3166	0.7012	0.9296
Depth	< 0.0001	< 0.0001	0.0401	< 0.0001	< 0.001	0.0130	0.1352	< 0.0001	< 0.0001	0.0743	0.2621
depth*trt	0.0848	0.1315	0.6126	0.2943	0.2940	0.7950	0.8588	0.4270	0.2343	0.9625	0.1673
depth*sns	0.5098	0.2320	0.4038	0.5350	0.8575	0.6851	0.6422	0.3653	0.0315	0.8422	0.1763
Harvest	< 0.0001	0.0126	< 0.0001	< 0.0001	< 0.0001	0.0005	0.8293	< 0.0001	< 0.0001	0.4303	< 0.0001
trt*harves	t 0.0102	0.7083	0.1009	0.5118	0.9446	0.0009	0.8745	0.1475	0.9979	0.3557	0.9757
sns*harve	st 0.1712	0.7067	0.2827	0.3293	0.8543	0.0219	0.4565	0.8472	0.5611	0.6589	0.8890
depth*har	vest 0.4000	0.9865	0.7161	0.0697	0.8453	0.6205	0.8703	0.0982	<0.0001	0.9862	0.0188

Analysis of all pre and post-harvest soil sampling data.

Table 2.2 Type 3 Tests of Fixed Effects, evaluating treatment (trt), soil nutrient status (sns), depth, and harvest effects on soil properties at MOFEP. Dependent variables include water extractable organic carbon (WEOC), KMnO4 oxidizable carbon (Active C), total organic carbon (TOC), total nitrogen (TN), total organic carbon/ total nitrogen ratio (TOC/TN), water extractable organic carbon/water extractable nitrogen (WEOC/WEN), β-glucosidase enzyme activity (β-glu), β-glucosaminidase enzyme activity (β-glumin), water stable aggregate concentration (WSA), and soil pH (pH). Significant values (p-values <0.05) are placed in bold. The "†" indicates a gamma distribution of the data set and "‡" indicates a lognormal distribution.

Analysis of January 2011 pre-harvest and January 2013 post-harvest soil sampling data.

p-values											
Source	WEOC †	Active C	TOC ‡	WEN	TN ‡	TOC/TN †	WEOC/WEN	β-glu †	β-glumin †	WSA	pН
Trt	0.5642	0.6941	0.3260	0.7024	0.3936	0.0596	0.7918	0.8798	0.2654	0.0949	0.5382
Sns	0.7976	0.6898	0.3180	0.3202	0.4963	0.2440	0.1920	0.4453	0.6301	0.7794	0.5747
trt*sns	0.8837	0.7356	0.6070	0.6960	0.9721	0.9580	0.7943	0.9915	0.3720	0.9520	0.9296
depth	<.0001	<.0001	0.0229	0.0052	<.0001	0.0004	<.0001	<.0001	<.0001	0.0355	0.2621
depth*trt	0.0094	0.8495	0.2090	0.3665	0.7508	0.0261	0.6333	0.2984	0.5632	0.9486	0.1673
depth*sns	0.295	0.6458	0.9630	0.7734	0.4839	0.4451	0.7903	0.6700	0.0344	0.5609	0.1763
Harvest	<.0001	0.0399	<.0001	0.0015	<.0001	0.0005	0.1239	<.0001	<.0001	<.0001	< 0.0001
trt*harvest	0.6444	0.8259	0.1532	0.1896	0.3779	0.0008	0.2905	0.2460	0.0015	0.1131	0.9757
sns*harvest	0.1868	0.9821	0.0941	0.2893	0.3293	0.9582	0.8785	0.8025	0.3222	0.8771	0.8890
depth*harvest	0.2992	0.3710	0.5962	0.5003	0.6866	0.0631	0.0519	0.0384	0.1152	0.8429	0.0188

2.5.1 β-glucosaminidase. Soil microbial enzyme activity, particularly β-glucosaminidase, was one of the first soil quality indicators to respond to forest harvest. Figures 2.1, 2.2, 2.4, and 2.5 show mean values of β-glucosaminidase activity at depths of 0-10 cm and 10-20 cm from soils collected at low and medium SNS sites. In general, all treatments at both depths exhibited a declining trend in β-glucosaminidase activity as a function of time (Appendix D). However, activity in the NH treatment typically began to increase at later sampling times; whereas, activity in the harvest treatments appears to be reaching some degree of stability at values less than sample values collected prior to harvest. Differences between treated values (CC and STS) and the NH values are further illustrated by Tukey-Kramer least squares analysis of treatment*harvest interaction performed with pre and post-harvest data from January 2011 and January 2013 (Fig. 2.3).

Comparison of significant differences, based on the 95% confidence intervals (CI), also reveal some interesting findings. Prior to harvest, there were no statistically significant differences in β-glucosaminidase amongst the three treatments, although the data are more variable in the March 2011 sample set. During January 2013 (post-harvest), β-glucosaminidase activity in the low SNS soils at both depths is significantly diminished in the CC treatment relative to the NH treatment (Figs. 2.1 and 2.2). Additionally, activity of this enzyme in the CC treatment during January 2013 is significantly less than the CC and NH treatment in January 2011 (pre-harvest) for both 0-10 and 10-20 cm depths in low SNS soils. Similar observations are also reflected with the Tukey-Kramer least squares analysis of treatment*harvest interaction performed with pre and post-harvest data from January 2011 and January 2013 (Fig. 2.3). The least squares means illustrate a decrease in β-glucosaminidase activity after harvest but no significant differences

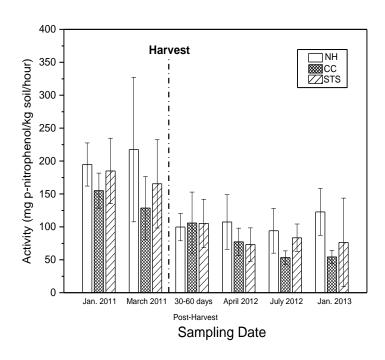


Figure 2.1 Activity of β -glucosaminidase in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

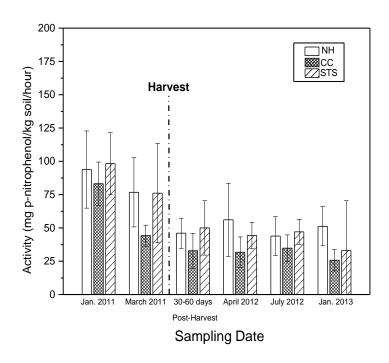


Figure 2.2 Activity of β -glucosaminidase in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

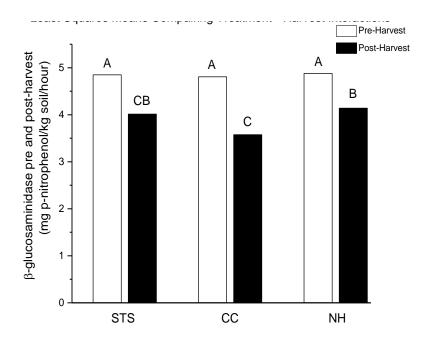


Figure 2.3 Comparison of least squares means from Tukey-Kramer analysis of β -glucosaminidase activity treatment*harvest interaction. Analyzed data includes values from January 2011 (pre-harvest) and January 2013 (post-harvest) only. Treatments include: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Bars marked with the same letter(s) are not considered significantly different.

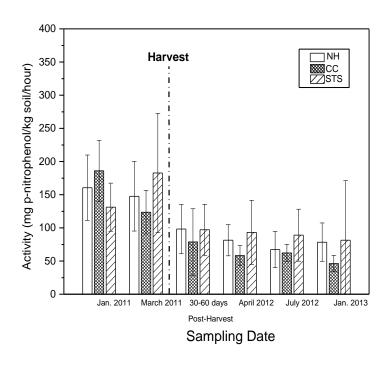


Figure 2.4 Activity of β-glucosaminidase in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

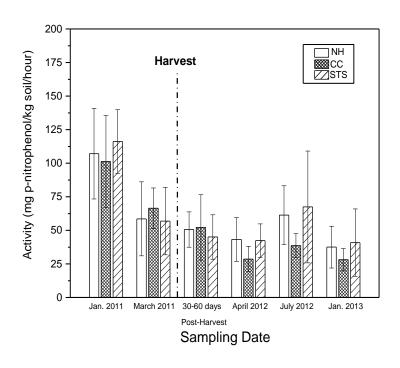


Figure 2.5 Activity of β -glucosaminidase in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

between treatments. Comparison of enzyme activity for samples collected at the 10-20 cm depth in low SNS soils, indicates that values for the STS treatment in April 2012 and July 2012 were significantly less than values for the STS treatment measured in the January 2011 (pre-harvest) samples. However, the January 2013 post-harvest STS treatment mean was not significantly different from January 2011 values, indicating that differences in April 2012 and July 2012 may be due to temporal changes.

In medium SNS soils at the 0-10, and 10-20 cm depths, β -glucosaminidase activity decreased in all treatments after the completion of harvest, including the NH treated sites (Figs. 2.3 and 2.4). The trend in data does not indicate a clear rebound in enzyme activity for either harvest treatment. No significant differences between CC and NH treatments were observed during the same sampling period. However, samples collected from CC treatment sites in January 2013 exhibited significantly lower β -glucosaminidase activity than samples collected in January 2011 (pre-harvest) at both depths (Figs. 2.4 and 2.5). A similar observation is made for the STS treatments between the January 2011 and 2013 dates, but only at the 10-20 cm depth.

The values of enzyme activity reported for the research discussed here are somewhat greater than observed in other studies, but many of the studies investigating β -glucosaminidase have been conducted in agricultural settings (Parham and Deng, 2000; Udawatta et al., 2008) and enzyme activities are typically greater in forested soils than agricultural soils (Trasar-Cepeda et al., 2008). The average values of β -glucosaminidase activity for this research range between 25 and 300 mg PNP kg⁻¹ soil hour⁻¹. Parham and Deng (2000) reported values between 29 and 40 mg PNP kg⁻¹ soil hour⁻¹ in agricultural soils. Udawatta et al. (2008) examined agroforestry soils and reported β -glucosaminidase

values between 124.9 and 131.5 mg PNP kg⁻¹ soil hour⁻¹ but the samples were air-dried prior to analysis.

While studies have found soil enzyme activity to be useful as soil quality indicators, not every enzyme in every study site has been found to be responsive within the first few years following forest disturbance or harvest (Yuqing et al., 2012; Pajares et al., 2011). β -glucosaminidase activity appears to be a good early indicator of soil health for Missouri Ozark soils. The decrease in β -glucosaminidase activity confirms that CC treated sites are being impacted by harvest and these changes can be observed within two years of harvest especially in low SNS soils.

2.5.2 β -glucosidase β -glucosidase activity did not exhibit obvious and easily understood changes after harvest. Figures 2.6 to 2.9 show values of β -glucosidase activity at depths of 0-10 cm and 10-20 cm from low and medium SNS sites. Generally, all treatments at both depths exhibited a decrease in enzyme activity after harvest. However, activity in the 0-10 cm samples appears to increase at later sampling times in all treatments including NH (Figs. 2.6 and 2.8). Comparison of significant differences based on the 95% CI also revealed interesting findings. Prior to harvest only low SNS soils at 0-10 cm depth illustrated significant differences in β -glucosidase activity amongst the three treatments. Comparison of enzyme activity for samples collected at the 10-20 cm depths in low SNS soils indicates that values for the CC treatments in April 2012 and July 2012 were significantly less than values for the CC treatments measured in the January 2011 and March 2011 (pre-harvest collection) (Fig. 2.7).

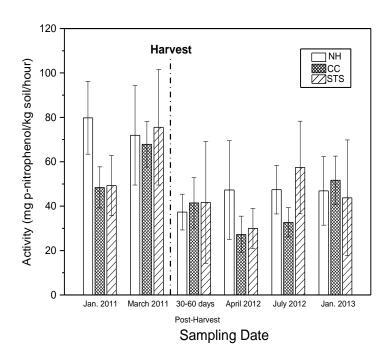


Figure 2.6 Activity of β -glucosidase in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

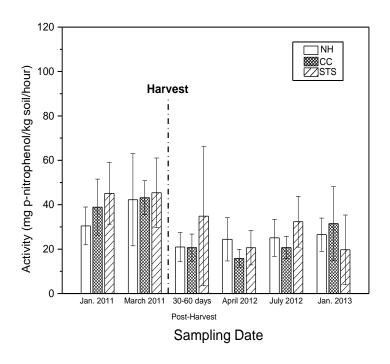


Fig. 2.7 Activity of β -glucosidase in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

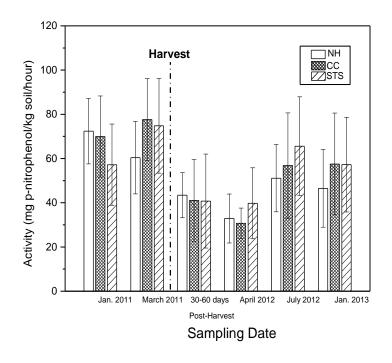


Fig. 2.8 Activity of β -glucosidase in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

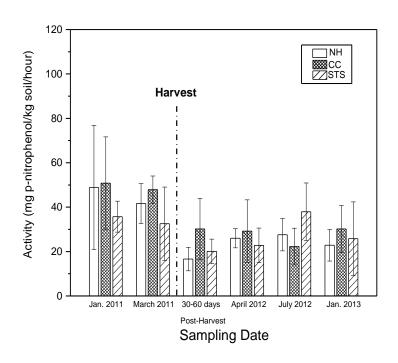


Fig. 2.9 Activity of β -glucosidase in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

In medium SNS soils at 0-10 cm depths, β -glucosidase activity decreased in all treatments at both depths after the completion of harvest, including the NH treated sites (Fig. 2.8). However, a rebound in enzyme activity also appears to occur over the course of the four post-harvest data sets for all treatments. No significant differences between treatments were observed however NH treatments collected 30-60 days post-harvest and in April 2012 were significantly lower in β -glucosidase activity than NH treatments samples January 2011 prior to harvest. Clearcut treatments sampled in April 2012 were significantly lower in β -glucosidase activity than CC treatments sampled before harvest.

In medium SNS soils at 10-20 cm depths, β -glucosidase activity was lower in STS treated sites collected 30-60 days post-harvest than STS treatments prior to harvest in January 2011 (Fig. 2.9). However, β -glucosidase activity was also lower in NH treatments sampled 30-60 days post-harvest when compared to March 2011 NH samples indicating that the observed decrease in enzyme activity may be part of normal fluctuations that occur in this system.

Average values of β-glucosidase for this research ranged between 15 and 110 mg PNP substrate kg⁻¹ dry soil hour⁻¹. Eivazi and Bayan (1996) reported β-glucosidase activity between 33 and 48 mg PNP substrate kg dry soil⁻¹ hour⁻¹ in soils from southeastern Missouri under oak-hickory forest. While our measured values are greater, the Eivazi and Bayan (1996) samples were also collected during the months of November, April, May, and September. The samples collected for this thesis included the months of January, March, and July (Appendix C) and some collections took place during a drought year. Seasonal effects on enzyme activity may explain the discrepancy

between these two data sets which were both assembled from research on Missouri soils under oak-hickory forest.

While some significant changes in β -glucosidase activity were observed, the effect was not lasting. Given that enzyme activity rebounded relatively quickly after harvest, β -glucosidase as a soil quality indicator does not illustrate the long term effects of harvest that have been elucidated by other studies (Eivazi and Bayan (1996). β -glucosidase activity does not appear to be sufficiently sensitive or enduring in Missouri Ozark forest soils to serve as an early soil quality indicator for forest harvest. This is somewhat surprising as β -glucosidase activity has been consistently useful in monitoring agricultural soil health and has been incorporated into the Soil Management Assessment Framework (SMAF) for the evaluation of agricultural soil systems (Stott et al., 2009). Further research will be needed to determine if changes become apparent after a greater period of time has passed after harvest.

2.5.3 <u>WEN.</u> Water extractable nitrogen (WEN) exhibited nominal differences between treatments which were interesting but not significantly different. At the 0-10 cm depth in low SNS soils, WEN in CC treatments collected in January 2013 (post-harvest) was significantly lower than NH and STS treatments (Fig. 2.10). At 10-20 cm depth in NH treatments before harvest, mean WEN values were significantly different when comparing January 2011 and March 2011 collections, but CC and STS treatments were not different. Single tree selection treatments were significantly lower than CC treatments 30-60 days post-harvest at the 10–20 cm depth, but no other significant changes were observed in low SNS soils (Fig. 2.11).

After harvest CC and STS treatments also showed evidence of a rebound in WEN content for the medium SNS soil at the 0–10 cm depth (Fig. 2.13). Water extractable nitrogen in CC and STS treatments at the shallow sampling depth within medium SNS soils was significantly lower during 30-60 days post-harvest collections than the NH treatment (Fig. 2.12), and CC treatment samples collected 30-60 days post-harvest and April 2012 collections were lower in WEN than CC treatments from pre-harvest collections (Fig. 2.12). The CC treatments from the 30-60 days post-harvest collection in medium SNS soils at 0-10 cm in depth exhibited the lowest values of WEN observed in this study.

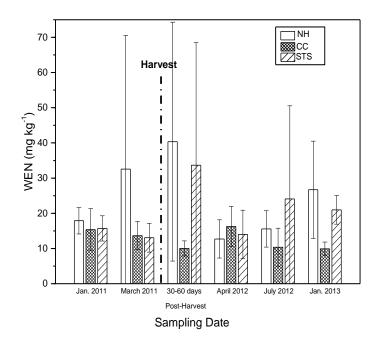


Figure 2.10 Mean values of water extractable nitrogen (WEN) in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

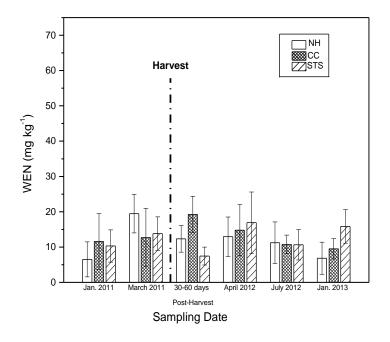


Figure 2.11 Mean values of water extractable nitrogen (WEN) in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

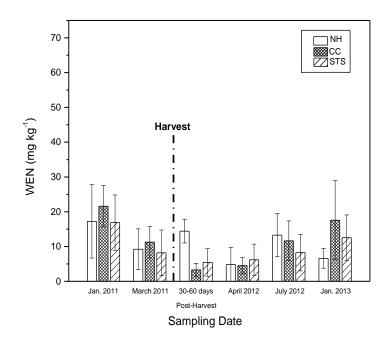


Figure 2.12 Mean values of water extractable nitrogen (WEN) in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

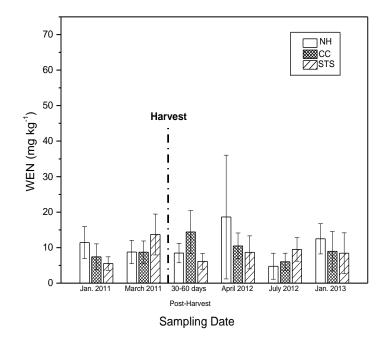


Figure 2.13 Mean values of water extractable nitrogen (WEN) in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

Values of WEN observed in this research fell within the range of values reported by Huang and Schoenau (1998). Average values of WEN content in this study ranged from 3 to 40 mg kg⁻¹; whereas, Huang and Schoenau (1998) reported WEN values that ranging from 0.83 and 306 mg kg⁻¹. Research by Huang and Schoenau (1998) was conducted in an aspen stand over several months but included organic and mineral layers of the soil profile, which likely explains the wide range of their data.

While changes in WEN were not distinct between treatments they at least provide some indication of soil quality soon after harvest. In CC treatments from low SNS soils at 0-10 cm depths WEN collected post-harvest in January 2013 is significantly lower in CC sites when compared to NH values. In medium SNS soils at 0-10 cm CC treatments 30-60 days post-harvest were lower in WEN when compared to NH treatments from the same collection and CC treatments from January 2011 pre-harvest. This gives evidence that WEN makes a good early soil quality indicator for Missouri Ozark forest soils especially at the 0-10 cm depth.

The January 2013 collection illustrated lower amounts of β-glucosaminidase activity in low SNS soils as well as WEN. The decrease in enzyme activity may be because of the decreased WEN content. Normally we would expect a spike in labile C or N pools or enzyme activity early after harvest. This was not clearly observed in this study. It is possible that the flush occurred before the first post-harvest samples were taken. The 10-20 cm depths of both SNS soils did show an increase in WEN, WEOC, and active carbon (discussed in greater depth in future sections) in CC treatments 30-60 days post-harvest when the 0-10 cm depth did not. It may be that the carbon spike occurred in the upper depth earlier than the 30-60 days post-harvest collection but leached down to

the 10-20 cm depth shortly after harvest. The increase in enzyme activity may have occurred at the same time as the C spike in the 0-10 cm depth samples and was not observed in our analyses. No spike in enzyme activity was observed at the 10-20 cm depth because most soil enzyme activity typically decreases with depth (Šnajdr et al., 2008).

2.5.4 <u>WEOC.</u> Water extractable organic carbon concentrations in low SNS soil samples collected from CC and STS treatments prior to harvest were not significantly different from each other (Figs. 2.15 and 2.16). Figure 2.14 fails to illustrate significant differences between treatments when comparing least squares means. Comparison of significant differences of mean values based on the 95% CI reveals more interesting findings.

In low SNS soils at 0-10 cm depth, STS treatments 30-60 days post-harvest were significantly greater in WEOC content than CC or NH treatments (Fig. 2.15). Water extractable organic carbon in STS treatments from the July 2012 collection was significantly lower than the March pre-harvest STS collection and NH treatments from July 2012. Single-tree-selection treatments from July 2012 were also significantly lower than the January 2013 (post-harvest) sampling. This likely indicates spatial and temporal variability of WEOC in this system, as the other treatments do not necessarily follow the same pattern. Mean WEOC increased in CC treatments 30-60 days post-harvest for the low SNS soil and was significantly greater than STS treatments from the same sampling (Fig. 2.16). In the STS treatment 30-60 days post-harvest, WEOC concentrations were also significantly less than NH values determined for the same sample set and from March 2011 (pre-harvest). The January 2011 and March 2011 NH pre- harvest averages

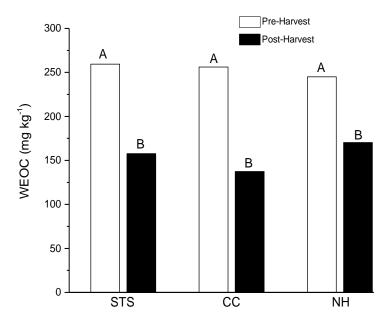


Figure 2.14 Comparison of least squares means from Tukey-Kramer analysis of WEOC values from treatment*harvest interaction. Analyzed data includes all values from all collections pre and post-harvest. Treatments include: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Bars marked with the same letter(s) are not considered significantly different.

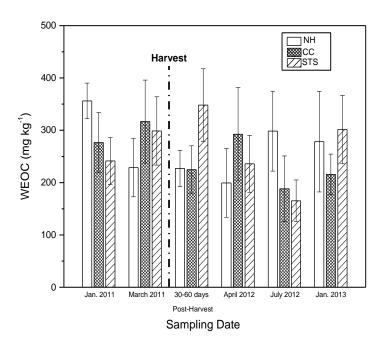


Figure 2.15 Mean values of water extractable organic carbon (WEOC) in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

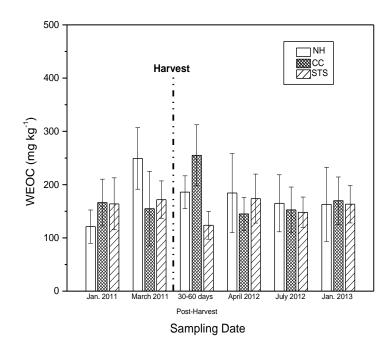


Figure 2.16 Mean values of water extractable organic carbon (WEOC) in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

of WEOC were significantly different from each other, but NH treatment values from March 2011 (pre-harvest) and 30-60 days post-harvest were not significantly different.

When examining the SAS ANOVA analysis there were no significant differences between treatments. When examining the 95% CI, pre-harvest collections from March 2011 of medium SNS soils did exhibit significant differences in WEOC values between treatments. After harvest, CC and STS treatments from the 30-60 days post-harvest collection contained lower quantities of WEOC than the NH treatment, and CC and STS treatments from January 2011 pre-harvest collections (Fig. 2.17). In the January 2013 collection, WEOC in STS treatments was significantly greater than in STS treatments sampled in March pre-harvest.

At 10-20 cm in medium SNS soils showed no significant differences between treatments or when comparing pre- and post-harvest means (Fig. 2.18). No harvest treatments collected January 2011 were significantly higher in WEOC than March 2011 pre-harvest NH samples, indicating the seasonal variability of WEOC in these sites. Single-tree selection treatment values collected 30-60 days post-harvest were also significantly less than STS values collected post-harvest in January 2013 (Fig. 2.18).

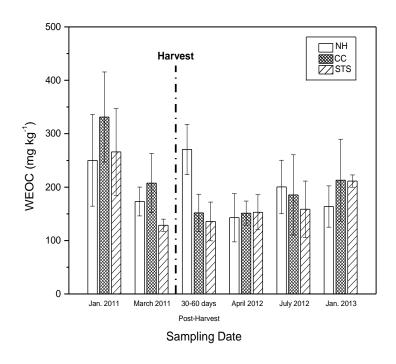


Figure 2.17 Mean values of water extractable organic carbon (WEOC) in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

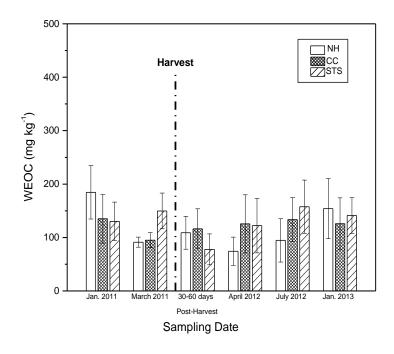


Figure 2.18 Mean values of water extractable organic carbon (WEOC) in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

At the 0-10 cm depths there appears to be more variability in WEOC content as well as more obvious changes in WEOC after harvest. Table 2.1 further illustrates the effect of depth on WEOC content. This is not surprising since the majority of microbial activity occurs close to the soil surface (0-30 cm) (Taylor et al., 2002). At greater depths WEOC may not be as vulnerable to degradation and prone to change. The contribution of organic matter added to the soil surface from leaf litter and the decrease in microbial activity easily explain the decrease in WEOC content with depth, as shown by comparison of lest squares means (Appendix D). The greater variability in WEOC content observed in the 0-10 cm depth may have been caused by the combination of the early mobilization and leaching of SOM after harvest as well as drought conditions. If we assume that at least some WEOC was leached from the top 10 cm and into the lower 10-20 cm depth this may replace any loses from the 10-20 cm depth that moved even deeper, while removing WEOC from the top 10 cm of the soil profile. Additionally, drought conditions following forest harvest may have periodically inhibited additional decomposition of harvest slash at the soil surface where microbial communities are most exposed to changes in temperature and moisture. Further additions of new WEOC to the 0-10 cm depth may have been sporadic in the period following harvest.

Water extractable organic carbon values reported above are consistent with other reports of WEOC content in forest soils. Hishi et al. (2004) examined WEOC content in a mountain forest of Japan and reported values between 39.9 and 328.4 mg WEOC kg⁻¹dry soil in samples collected from the top five centimeters of the soil profile. Water extractable organic carbon from this thesis research ranged between 74.3 and 356.2 mg WEOC kg⁻¹dry soil and was collected from the 0-10 cm and 10-20 cm depths. Some

differences are to be expected between sites due to differences in climate, vegetation, topography, and other influences that differ between any two sites, but reported WEOC values by Hishi et al. (2004) and this study are quite similar.

While there were some instances in the research for this thesis where changes in WEOC were observed, those changes did not persist for the duration of the study. This was not surprising as changes in water extractable organic matter (WEOM) are often not enduring (Chantigny, 2003). The lack of significant difference between treatments however was not entirely expected. While there have been studies comparing NH, CC, and STS treatments that also found no differences between treatments (Barg and Edmonds, 1999), the studies examined rates of change in soil nitrogen and microbial biomass, not WEOC content. The Barg and Edmonds study (1999) also took place 60-70 years after harvest. Other studies in the Missouri Ozarks did indicate differences between treatments when comparing the values of the various soil properties; those studies found that UAM stands (under STS harvest) significantly increased total carbon 8 years after harvest Li et al. (2007). Chen et al. (2004) reported greater rates of soil respiration in UAM stands (STS harvest) when compared to EAM (CC harvest) and NH management stands. However, data reported by Albers (2010) implied that there may be some differences in SOC between treatments but no statistically significant claims could be made. For the data presented here, the most likely causes for the lack of treatment effect may be the timing of sampling after harvest or climate conditions. Post-harvest data was largely collected in 2011 and 2012 which were drought years. This climatic anomaly may have affected decomposition of harvest slash and the release of WEOC for all treatments.

The magnitude of climatic effects compared to measurable effect of treatment is difficult to gage.

WEOC occur, STS treatments seem to illustrate significant differences more often than NH or CC treatments. These differences in WEOC content may be temporal; however if that is the case we would expect NH treatments would demonstrate similar changes over time. Therefore, small but cumulative differences in topography or soil composition may be introducing greater variability into the data collected. Ultimately it seems WEOC is not a good early indicator of Ozark forest soil health. Future research may indicate different conclusions with increased time after harvest.

2.5.5 <u>WEOC/WEN.</u> Comparison of WEOC/WEN mean values (± 95% CI) over time revealed few significant differences (Figs. 2.19 – 2.22). There were no significant differences between pre- and post-harvest values for either depth in low SNS soils (Figs. 2.19 and 2.20). In medium SNS soils at a depth of 0-10 cm, mean values exhibited no significant differences between treatments or between pre and post-harvest value of WEOC/WEN ratios (Fig. 2.21). However, there was a very dramatic increase in WEOC/WEN in the 30-60 days post-harvest set for CC sites, although the difference is not statistically significant due to substantial variability in the measurements. This spike does not show up in the TC/TN ratio (Fig. 2.37) or WEOC (Fig. 2.17). Water extractable nitrogen, however, appears to be at its lowest recorded value in CC sites of medium SNS soils at 0-10 cm in depth in the 30-60 days post-harvest set.

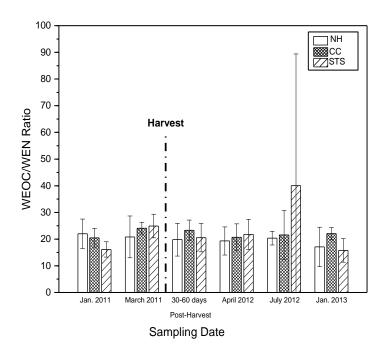


Figure 2.19 Mean values of water extractable organic carbon /water extractable nitrogen ratios (WEOC/WEN) in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

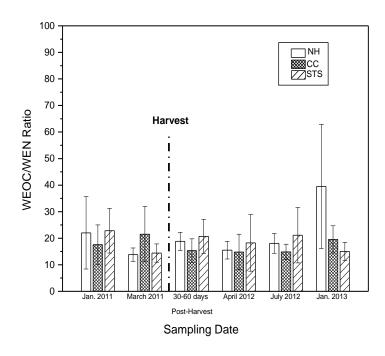


Figure 2.20 Mean values of water extractable organic carbon /water extractable nitrogen ratios (WEOC/WEN) in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

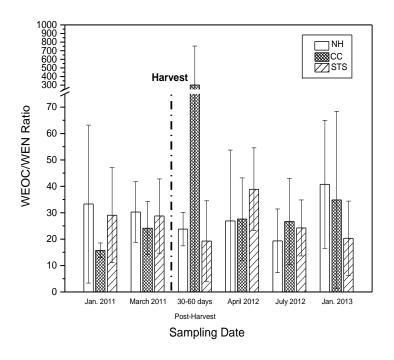


Figure 2.21 Mean values of water extractable organic carbon /water extractable nitrogen ratios (WEOC/WEN) in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

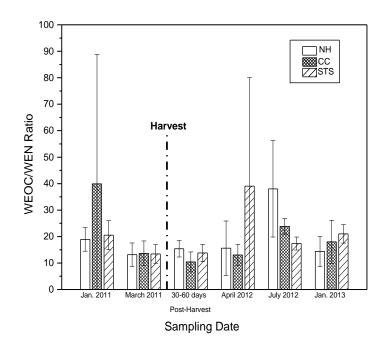


Figure 2.22 Mean values of water extractable organic carbon /water extractable nitrogen ratios (WEOC/WEN) in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

At the 10-20 cm depth in the medium SNS soils, mean values of WEOC/WEN ratios for CC treatments collected July 2012 had significantly greater ratios of WEOC/WEN than CC, STS and NH treatments collected in March 2011 pre-harvest and 30–60 days post-harvest (Fig. 2.23). However, NH treatments from the July 2012 collection also had significantly greater values of

WEOC/WEN than all treatments in March 2011 (pre-harvest) and 30–60 days post-harvest, indicating a possible temporal influence on the WEOC/WEN ratio. There were no significant differences in the WEOC/WEN ratio in the STS treatment over time with exception for comparison of January 2013 post-harvest and the March 2011pre-harvest samples.

Few significant differences in harvest or treatment were illustrated when examining WEOC/WEN ratios. Differences that were observed were not long lasting, which is consistent with other reported trends in WEOM (Chantigny, 2003). We expected WEOC/WEN ratios to correlate with enzyme activity (Haney et al., 2012), but there does not appear to be clear correlation with either β -glucosidase or β -glucosaminidase activity. The lack of relationships between the ratio of WEOC/WEN and soil microbial enzyme activity may be associated with insufficient changes in the WEOC/WEN ratio to elicit change in the microbial response. Nitrogen availability is frequently low in forest soils, thus soil N and N dependent enzyme activity may be better soil quality indicators than carbon and C dependent enzyme activity for Missouri Ozark forest soils.

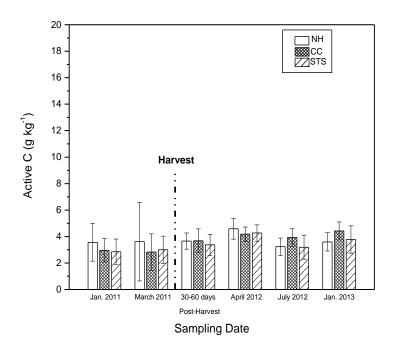


Figure 2.23 Mean values of active carbon in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

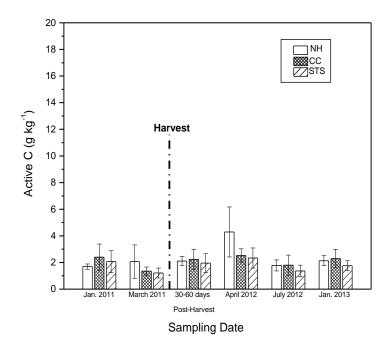


Figure 2.24 Mean values of active carbon in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

2.5.6 <u>Active Carbon.</u> Active carbon in low SNS soil at the 0-10cm depth (Fig.2.23), showed no significant differences between treatments or between pre- and post-harvest collections. At the 10-20 cm depth in the low SNS soil, active carbon contents in samples collected from the STS and CC treatments during April 2012 were significantly greater than CC and STS treatments in March 2011 (pre-harvest) (Fig. 2.24). Active carbon content within the NH samples collected in April 2012 was not significantly different from March 2011 pre-harvest samples, but the active carbon content within the NH samples collected in April 2012 were greater than NH January 2011 pre-harvest values (Fig. 2.24). There were no differences between treatments when comparing the two pre-harvest collections, including the NH treatments.

In medium SNS soils at the 0-10 cm depth there were no significant differences in mean values of active carbon between treatments or between pre and post-harvest samplings (Fig. 2.25). The active carbon values from CC treatments from the 30-60 days post-harvest samples showed a sharp increase, but the variability within the replicate samples for this treatment and sampling period were quite large. Thus, this average is only nominally greater than active carbon content observed in other treatments and sampling periods.

In medium SNS soil at 10-20 cm depths there were no enduring statistically significant differences between treatments. Active carbon in CC and NH treatments were significantly greater in 30-60 days post-harvest samples than CC and NH treatments, respectively, collected during March 2011 pre-harvest (Fig. 2.26). Samples collected from CC treatments during January 2013 after harvest were significantly greater in active

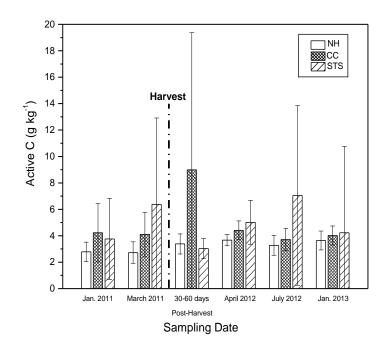


Figure 2.25 Mean values of active carbon in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

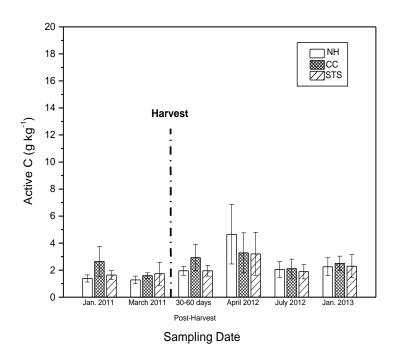


Figure 2.26 Mean values of active carbon in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

carbon when compared to CC samples collected in March 2011(pre-harvest) but not CC samples obtained in January 2011 (pre-harvest). There were no significant differences observed in active carbon amongst the pre-harvest samples.

Average values of active carbon reported by Weil et al. (2003) ranged between 0.6 and 1.58 g kg⁻¹ dry soil in agricultural soils, and Jiang and Xu (2006) observed approximately 2.5 to 5.8 g kg⁻¹ dry soil in forest soils of China. Khanna et al. (2001) reported KMnO₄ oxidizable carbon values greater than 13.5 g kg⁻¹ in forest soils from various sites and depths using the Blair et al. (1995) method which utilizes 0.333 M KMnO₄. Average active carbon values reported here range between 1 and 9 g kg⁻¹ dry soil, which seem reasonable compared to other reported concentrations of active carbon. However, there was a great deal of variability in our results, and widely variable temperature and precipitation throughout the duration of this study could be the cause. Samples collected after harvest had been exposed to periods of rain, cold, and drought, all influential to soil carbon cycling.

Few significant differences in active carbon were observed and those changes that were observed did not endure throughout the entire study. This may indicate that changes in active carbon occurred before the first post-harvest sampling collection 30-60 days post-harvest. Alternatively, CC and STS treatments may not significantly impact active carbon pools shortly after harvest (< 2 yrs).

Studies investigating active or KMnO₄ oxidizable carbon have primarily focused on agricultural systems. Frequently, soil disturbance results in a decrease in active carbon content after a flush of active carbon from decaying organic matter (Chantigny, 2003).

Active carbon has not been widely studied in forest soils and those studies which included active carbon had mixed results. Luan et al. (2010) found significant increases in active carbon in an 18 year old fir plantation when compared to a natural forest, but a slight, though not significant, decline in active carbon in an 18 year old regenerated forest. Active carbon consistently correlates well with a variety of microbial indices (microbial biomass, soluble carbohydrates, basal respiration, substrate-induced respiration) (Weill et al., 2003), but further research is needed to determine if active carbon is itself an informative soil quality indicator for forest soils. For Missouri Ozark soils it appears that active carbon is not a good early indicator of forest soil quality.

2.5.7 <u>TN, TOC, and TOC/T</u> Total organic carbon, TN, and TOC/TN ratios do not appear to be practical early soil quality indicators for Missouri Ozark forest soils. While there were some notable changes in TOC, TN, and TOC/TN ratios there were very few significant changes observed. There were no statistically significant differences between harvest treatments or pre and post-harvest values for TN analysis (Figs. 2.27-2.30).

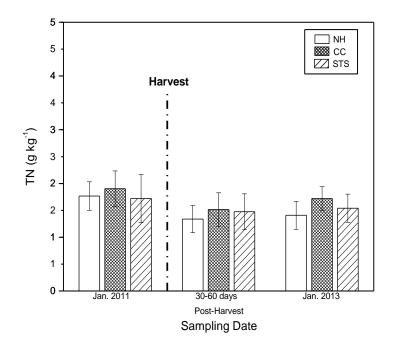


Figure 2.27 Mean values of total nitrogen in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

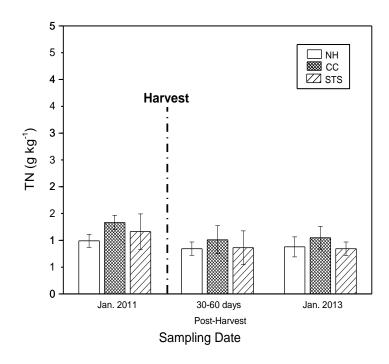


Figure 2.28 Mean values of total nitrogen in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

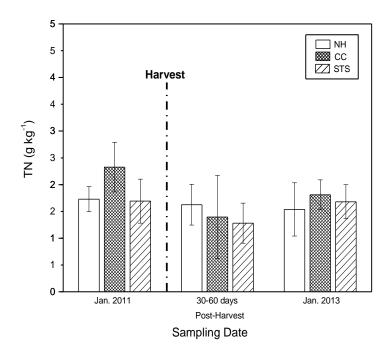


Figure 2.29 Mean values of total nitrogen in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

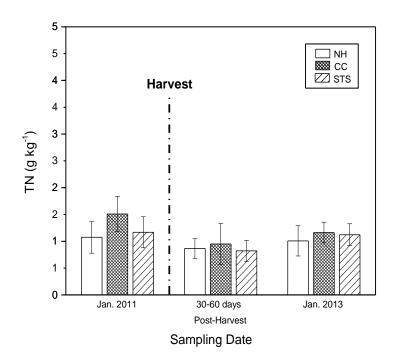


Figure 2.30 Mean values of total nitrogen in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

The values reported in this study are consistent with other forest soil values of TN. O'Connell et al. (2004) reported average TN values between 1.9 and 12.1 g kg⁻¹. Total nitrogen values reported here range between 1 and 3 g kg⁻¹. It was not surprising that no significant changes in TN were observed over the course of this study, but prior studies in forest soils do show varied results. Total nitrogen includes all forms of soil nitrogen including forms that are not readily available to microbes or plants. Idol et al. (2003) observed no significant increase in TN 1 to 100 years after forest harvest and, rates of N cycling had not recovered 30 years after harvest. Neill et al. (1997) did observe increases in TN when land was converted to pasture in five of the 18 pastures studied and one of the 18 pastures decreased in TN content. Given that TN is predominantly a more recalcitrant pool of N and other studies have not observed significant changes in TN shortly after disturbance, it is not unreasonable that no significant changes were observed in this study.

Comparison of low SNS soils at the 0-10 cm depth indicate one significant difference in TOC content. Samples collected 30-60 days post-harvest from NH and CC treatments were significantly lower in TOC than NH treatments before harvest (Fig. 2. 31). Samples collected at the 10-20 cm depth in the low SNS soil indicate that STS treatments collected in January 2013 post-harvest were significantly lower in TOC than STS treatments sampled before harvest in January 2011 (Fig. 2.32). Similar observations were noted by Albers (2010), implying depletion of TOC in STS treatments may begin within 1.5 years after harvest and endure at least 10 years after harvest.

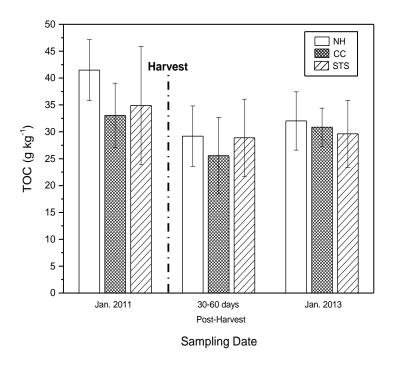


Figure 2.31 Mean values of total carbon in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

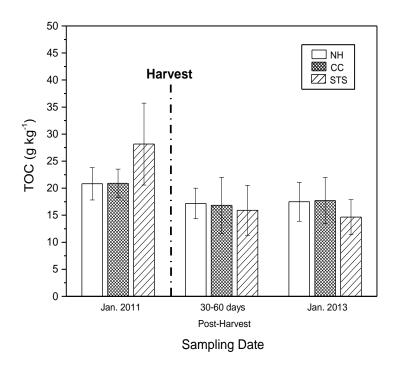


Figure 2.32 Mean values of total carbon in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

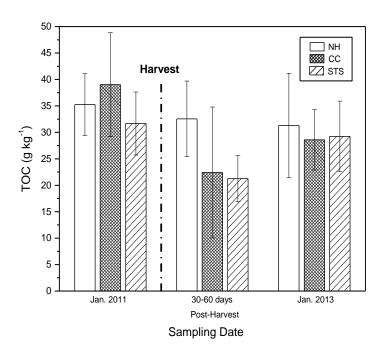


Figure 2.33 Mean values of total carbon in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

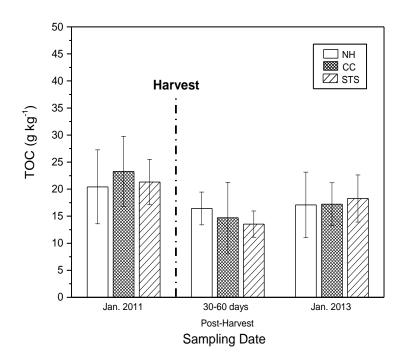


Figure 2.34 Mean values of total carbon in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

The medium SNS soils at 0-10 cm and 10-20 cm depths (Fig 2. 33 and 2.34) showed a significant decrease in TOC for STS treatments in the 30-60 days post-harvest collection when compared to the pre-harvest STS values. There were no significant differences between treatments within a given collection period.

Soil carbon values reported here are similar to other publications. Lal (2005) reported soil carbon values ranging between 27 and 162 g kg⁻¹ in forest soils. Total organic carbon values from this study range between 15 and 42 g kg⁻¹. The significant changes in TOC observed in this study indicated a decrease in TOC under STS harvest which was consistent with findings by Albers 2010. Research by Li et al. (2007) found that STS treatments contained less coarse woody debris 8 years after harvest when compared to CC treatments but more coarse woody debris than NH treatments. Li et al. (2007) also observed an increase in mineral soil carbon content in STS treatments when compared to NH treatments 8 years after harvest. While studies by Albers (2010) and Li et al. (2007) and the research for this thesis were all conducted on the MOFEP sites of southern Missouri, the variability of forest soils can still create discrepancies between studies. Environmental and temporal conditions impact rates of decomposition and affect soil TOC content. It may be that environmental conditions in the different years during which these studies were conducted lead to differing conclusions. While some interesting changes were observed, TOC would not make a reliable early soil quality indicator for Missouri Ozark forest soils. While STS treatments changed after harvest, NH treatments also changed in post-harvest collections and were not significantly different from STS or CC treated values from the same collections.

The TOC/TN ratios in low and medium SNS soils at the 0-10 cm depth from all collections were significantly lower in CC treatments than NH treatments (Fig. 2.35 and 2.37). Comparison of least squares values also illustrate decreased values in CC treatments when compared to NH treatments pre and post-harvest (Fig. 2.39). The difference between treatments, evident in the pre-harvest collection, was maintained after harvest. At the 10-20 cm depth in both SNS soils, pre-harvest treatment values were all significantly different from each other but after harvest there were no longer significant differences between the treatments (Fig. 2.36 and 2.38).

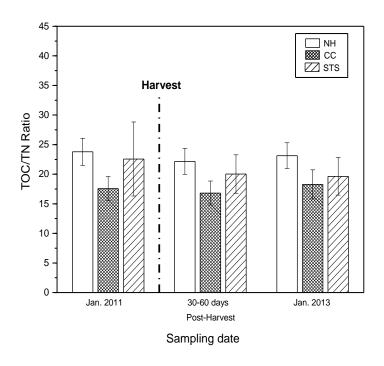


Figure 2.35 Mean values of total organic carbon/total nitrogen ratio in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

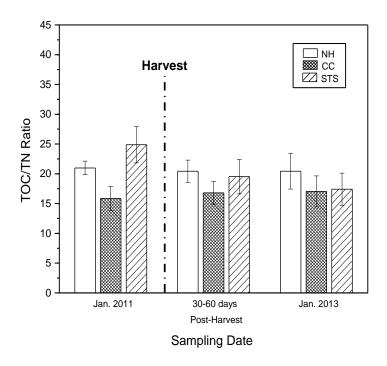


Figure 2.36 Mean values of total organic carbon/total nitrogen ratio in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

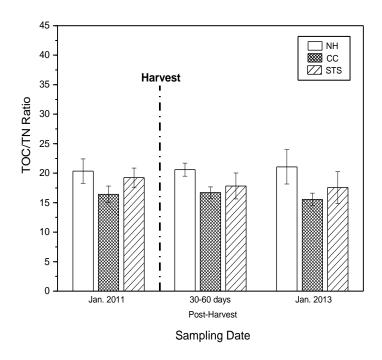


Figure 2.37 Mean values of total organic carbon/total nitrogen ratio in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

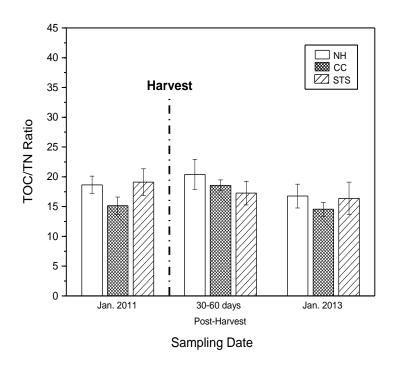


Figure 2.38 Mean values of total organic carbon/total nitrogen ratio in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

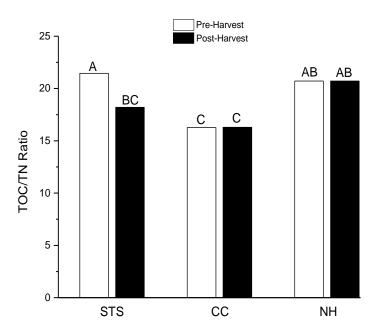


Figure 2.39 Comparison of least squares means from the Tukey-Kramer analysis of TOC/TN ratios from treatment*harvest interaction. Analyzed data includes all values from all collections pre and post-harvest. Treatments include: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Bars marked with the same letter(s) are not considered significantly different at $\alpha = 0.05$.

Carbon to nitrogen ratios typically correlate with enzyme activity (Geisseler and Horwath, 2009), however that was not the case with this research. TOC/TN values from post-harvest CC and STS treatments were not significantly different from CC and STS treatments after harvest. Because TOC and TN contain labile and recalcitrant forms of C and N, it is not unreasonable to expect that a greater length of time after harvest may need to pass before significant effects of harvest will be observed. Other studies have also not observed changes in TOC or TN years after harvest (Kranabetter and Coats, 2004).

2.5.8 <u>Soil pH.</u> Post-harvest values for pH were significantly greater in STS treatments than NH treatments at 0-10 cm depths in low SNS soils (Fig. 2.40). There were no significant differences in pH between the STS pre-harvest and post-harvest samples or the STS and CC treatments. Additionally, soil pH in CC treatments was not significantly different from NH treatment. In medium SNS soils at the 0-10 cm depth, CC treatments significantly increased in pH after harvest (Fig.2.41). However there were no significant differences between treatments for a given collection date. There were no significant differences observed at the 10-20 cm depth for either the low or medium SNS within a particular sampling date.

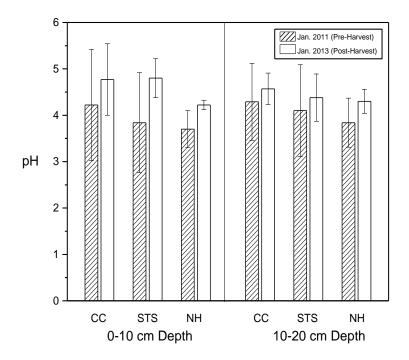


Figure 2.40 Mean values of soil pH in low soil nutrient status (SNS) soils sampled at 0-10 cm and 10-20 cm depths pre-harvest and post-harvest: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

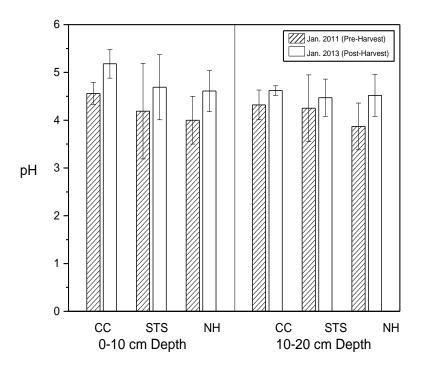


Figure 2.41 Mean values of soil pH in medium soil nutrient status (SNS) soils sampled at 0-10 cm and 10-20 cm depths pre-harvest and post-harvest: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

While there were some changes to soil pH, pre and post-harvest collections did not differ as much as might be expected. It seems likely that the lack of change in soil pH is due to the already low pH levels. The soil quality index assembled by Amacher et al. (2007) considers soils with pH 3.01 to 4.0 to be strongly acidic and many pre-harvest soil samples were already within that range. No sample averages were greater than the pH range of 4.01 to 5.5 (moderately acidic). Singh (2013) observed a decrease in throughfall pH in CC treatments of both low and medium SNS soils. He also recorded significant increases in hydrogen ion activity of soil solution collected 15 cm below the soil surface of both CC and STS treatments. Soil pH may be slower to change after treatment than soil solution pH values. It is possible that with more time soil pH will change but further research will be needed to elucidate this change.

2.5.9 <u>WSA.</u> In low SNS soil at the 0-10 cm depth, all treatments increased nominally in WSA content after forest harvest but then decreased significantly (Fig. 2.42). However, WSA content in this subset of samples was not significantly different between treatments under pre-harvest conditions. At the 10-20 cm depth of the low SNS soil, there is again a nominal increase in WSA content for all treatments followed by a decrease to significantly lower values one year after harvest (Fig. 2.43). Although WSA content in the NH pre-harvest samples does not differ significantly from NH one year after harvest, the same is not true for the CC and STS treatments. Within the harvested treatments, WSA content is significantly reduced one year after harvest relative to pre-harvest conditions.

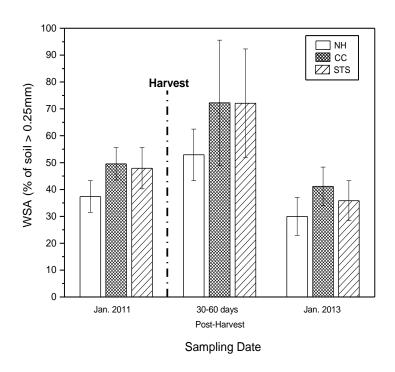


Figure 2.42 Mean values of water stable aggregates (WSA) in low soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

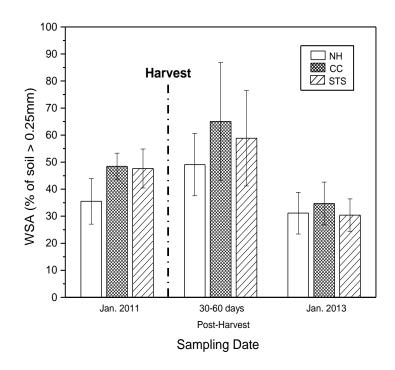


Figure 2.43 Mean values of water stable aggregates (WSA) in low soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

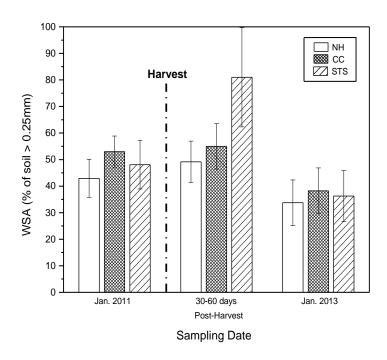


Figure 2.44 Mean values of water stable aggregates (WSA) in medium soil nutrient status (SNS) soils sampled at the 0-10 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

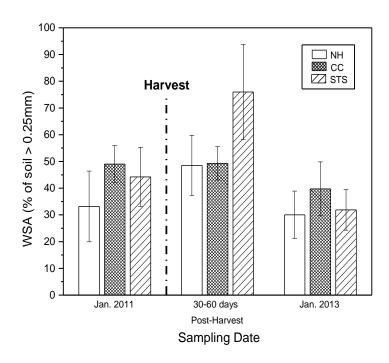


Figure 2.45 Mean values of water stable aggregates (WSA) in medium soil nutrient status (SNS) soils sampled at the 10-20 cm depth as a function of sampling date for the different harvest treatments: clearcuts (CC); single-tree selection (STS); and no harvest (NH) controls. Error bars represent 95% confidence intervals. The vertical line indicates separation of pre- and post-harvest samples. Actual dates of the 30 to 60 day post-harvest sampling vary amongst the sites due to different timings of harvest, thus explaining the lack of dates for this sampling period.

In medium SNS soils at the 0-10 cm depth, WSA content in the STS treatment increased significantly after harvest but then returned to pre-harvest values in January 2013. Water stable aggregate content in the CC treatment did not change significantly until the January 2013 collection where WSA content decreased below pre-harvest values (Fig. 2.44); however, WSA content in the NH treatment followed a similar pattern. At the 10-20 cm depth in medium SNS soils, STS treatments collected 30-60 days post-harvest were significantly greater in WSA content than pre-harvest values but values then returned to pre-harvest level in January 2013 (Fig. 2.45).

Average values of WSA content observed for this research ranged between 30 and 80 percent with most of the values below 60 percent. This data is similar to other publications reporting ranges between 22 and 48 percent (Kushwaha et al., 2001). Comparison of WSA content between studies can be difficult due to differences in the methodologies employed to determine WSA content (Arshad et al., 1996). The few observed changes in WSA concentration in this research do not imply a lasting effect, though further research is required to know conclusively. Because there were no lasting significant differences between harvested and NH treatments, WSA content may not be a valuable early indicator of soil quality in the Missouri Ozarks. Increased soil compaction and damage to WSA is often observed after harvest. This was not observed in this research possibly due to the great coarse fragment content in the soils studied. It may also be that changes in other parameters associated with WSA content (e.g., TOC) have not yet changed substantially.

2.5.10 <u>Summary</u>. Data from this research indicates that β -glucosaminidase activity and WEN content are applicable early soil quality indicators for Missouri Ozark forest soils.

Observed reductions in β -glucosaminidase activity and WEN content raise concern for sustainable forestry in the Missouri Ozarks due to the nature of nitrogen cycling in forest ecosystems. Utilization of PROC GLIMMIX analysis indicated no significant differences between harvest treatments but assessment of mean values and surrounding 95% confidence intervals illustrated instances of significant difference between CC pre and post-harvest values and CC and NH values. Analysis of other soil quality parameters explored in this research (TOC, TN, active carbon, TOC/TN ratios, WEOC, WEOC/WEN ratios, β -glucosidase activity, WSA content, and soil pH) did not demonstrate sustained significant changes after forest harvest.

While p-values from the PROC GLIMMIX analysis (Tables 2.1 and 2.2) indicate that harvest impacted all soil quality indicators examined in this study (except WEOC/WEN ratio), harvest alone fails to account for the value changes in NH (control) treatments after the harvest period. Additional environmental influences other than harvest must be influencing these changes. Therefore, the most compelling evidence for identifying a suitable soil quality indicator would be significant differences between pre and post-harvest values collected from the same sites coupled with significant differences between the treated site and NH sites collected at the same collection time. With this in mind, the most useful soil quality indicators in this study were β -glucosaminidase activity and WEN content.

The research illustrates instances where β -glucosaminidase and WEN content significantly declined after forest harvest. The strongest evidence for the use of β -glucosaminidase activity as a measure of soil quality in the Missouri Ozarks is observed in low SNS soil samples collected approximately two years post-harvest at 0-10 and 10-

20 cm depths. In these collections, values from CC treatments collected January 2013 were significantly lower than those from NH treatments sampled at the same sample period and from CC and NH treatment values collected January 2011 prior to harvest. The decrease in β-glucosaminidase activity was observed in medium SNS soils at both depths as well but the instances where decreases in activity were observed, were not statistically significant between CC and NH samples of the same sample period. Significance occurred at both depths with the comparison of CC treatment values from both pre-harvest collections to April 2012, July 2012, and January 2013 post-harvest collections. Unfortunately, April 2012 and July 2012 samples experienced drought conditions which are expected to influence soil enzyme activity. With this information, B-gluosaminidase activity would not be considered an informative soil quality indicator for medium SNS soils.

Comparison of WEN values from low SNS soils at 0-10 cm depths in the post-harvest collection from January 2013 reveal a significant decrease in CC treatment values when compared to NH treatment values. There were however, no significant difference observed between pre and post-harvest CC treatment values of WEN from low SNS soils at 0-10 cm. Clearcut treatments in medium SNS soils at the 0-10 cm depth exhibited a significant depletion of WEN in the 30-60 days post-harvest collection when comparing CC treatments to NH, and pre-harvest collections of CC treatments. Interestingly, WEN appears to rebound before the last sampling. Low SNS soils do not exhibit a rebound in WEN and may continue to decrease as in the future. The same potential for continued decrease can be said for β -glucosimindiase activity in low SNS soil especially at the 0-10 cm depth. Because WEN exhibited at least one instance where depletion was significant

between CC and NH treatments as well as pre and post-harvest values, WEN may be a useful soil quality indicator for medium SNS.

It is well understood that nitrogen is often a limiting nutrient in forest systems. It seems reasonable that the most informative soil quality indicators for this forested system would include a pool of soil nitrogen and nitrogen utilizing enzymes. Because this study examined soil changes soon after harvest it also seems reasonable that WEN, a labile pool of nitrogen would respond measurably to forest harvest even when TN did not. The other soil quality indicators examined in this study often exhibited a rebound or stabilization sometime after harvest. It seems concerning that the decrease in labile, plant available, nitrogen in low SNS soils is not only exhibited relatively soon after harvest but it also appears to persist. The decrease in readily available nitrogen may inhibit forest growth and productivity, thus inhibiting future harvests. Future research would help elucidate how long this depletion lasts. Though other soil quality parameters examined in this study did not exhibit significant or enduring responses to forest harvest in the time allotted for this research, furture work may provide additional information.

3.0 Conclusions

Overall, β -glucosaminidase activity and WEN content provided significant evidence that CC harvest methods can diminish soil quality at the 0-10 cm soil depth shortly after forest harvest. There was insufficient evidence to illustrate significant depletion of β -glucosamindase and WEN content in STS treatments after harvest compared to NH or pre-harvest STS treatments. These depletions were not observed in every collection but in low and medium SNS soils β -glucosaminidase activity failed to

return to pre-harvest levels. Water extractable nitrogen values in low SNS soil at 0-10 cm also appear to be declining. While WEN values did not fall below pre-harvest values during the span of this research further WEN depletion may be observed with future sampling. As previously mentioned, drought conditions in 2011 and 2012 encourage speculation. The timing of the drought conditions meant that much of the post-harvest data was collected under drought conditions while only one of the two sets of pre-harvest data sets (January 2011) was collected under drought conditions. It seems likely that the severe and prolonged drought occurring throughout post-harvest sampling may have had a greater effect on the soil quality indicators measured here than the shorter drought period the January 2011 pre-harvest samples experienced. Thus, statistical differences between NH and harvested treatments of the same collection may be considered more meaningful than differences between CC or STS treatments compared before and after harvest. The full extent of drought and drought duration on the quantified soil parameters examined in this research would be difficult to estimate. Further research on MOFEP sites after harvest may elucidate the effect of harvest more clearly, especially if climatic conditions are moderate.

Currently there is no data to indicate that the observed reductions in β-glucosaminidase activity and WEN will persist or if these depletions will become evident at greater depths. The long-term effect of these depletions on forest productivity in the Missouri Ozarks has not been thoroughly documented but the importance of nitrogen cycling in forested systems in general is widely accepted. Depletion of WEN from 0-10 cm depths may or may not affect growth rates of immediate future forest generations but persistent depletion of forest nitrogen is likely to hinder long-term sustainable forestry.

It is difficult to predict the effect of nutrient loss from surface soils on forest mast and the animal populations dependent on forest productivity. While this research did not observe changes in all soil quality parameters studied or between CC and STS treatments, such changes may become evident in future soil research and greater passage of time. Additional future studies focusing on the biological and ecological impact of the harvest methods practiced at MOFEP would also provide greater depth and meaning to the changes in soil quality observed in this and future soil research at MOFEP.

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APPENDIX A

A. Statistical Models in SAS

A.I Code for split-plot generalized linear mixed model in SAS software. Each block is split by treatment (trt), soil nutrient status (sns), and is repeated by depth

```
Proc GLIMMIX data=(DATA SET) maxopt=2000 pconv=1e-4 plots=studentpanel;
class depth trt SNS block depth harvest completion event subsample;
model (DEPENDENT VARIABLE) = trt|SNS|depth|harvest @2/ dist=lognormal
link=identity;
*distribution codes must be changed in above statement for each
dependent variable*
Random int sns trt sns*trt/subject=block;
random depth/type=sp(pow)(depth) subject=trt*SNS*harvest(block);
lsmeans trt sns depth trt*harvest /pdiffadjust=tukeylinesilink cl;
Run;
```

A.II Code for Proc Univariate analysis

```
proc univariate data=(DATA SET) normalplot;
var (DEPENDENT VARIABLE);
histogramlargeoak/ normal;
histogramlargeoak/lognormal (theta=-.001);
histogramlargeoak/gamma (theta=-.001);
histogramlargeoak/ exponential (theta=-.001);
run;
```

APPENDIX B

B. Regression Tables

B.I Regression Tables for Biological Soil Quality Indicators

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosaminidase activity at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-20.7	-47.7	6.4
CC	-21.6	-27.8	-15.5
STS	-23.4	-38.8	-8.1

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosaminidase activity at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	-8.6	-17.2	-0.08
CC	-9.0	-18.3	0.2
STS	-12.0	-18.9	-5.0

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosaminidase activity at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

			<u>6 CI</u>
Treatment	Slope	Lower	Upper
NH	-19.1	-30.8	-7.3
CC	-25.8	-42.3	-9.3
STS	-15.3	-34.4	3.9

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosaminidase activity at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-9.9	-22.2	2.4
CC	-13.5	-22.5	-4.5
STS	-9.9	-26.1	6.2

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosidase activity at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>% CI</u>
Treatment	Slope	Lower	Upper
NH	-6.5	-15.0	2.0
CC	-3.0	-12.9	7.0
STS	-2.7	-13.6	8.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosidase activity at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-1.9	-6.8	2.9
CC	-3.1	-10.1	3.9
STS	-5.2	-9.5	-0.8

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosidase activity at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>% CI</u>	
Treatment	Slope	Lower	Upper
NH	-4.8	-12.6	3.0
CC	-3.9	-15.7	8.0
STS	-0.8	-11.0	9.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of β -glucosidase activity at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-4.7	-11.0	1.7
CC	-5.2	-9.7	-0.6
STS	-0.9	-6.1	4.4

B. II Regression Tables for Chemical Soil Quality Indicators

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable nitrogen (WEN) (mg kg⁻¹dry soil) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-1.0	-8.9	7.0
CC	-0.9	-2.6	0.9
STS	1.1	-4.5	6.7

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable nitrogen (WEN) (mg kg⁻¹dry soil) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	-0.6	-4.1	2.8
CC	-0.6	-3.1	1.9
STS	0.8	-1.7	3.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable nitrogen (WEN) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>% CI</u>
Treatment	Slope	Lower	Upper
NH	-1.4	-4.4	1.5
CC	-0.5	-5.8	4.8
STS	-0.6	-3.7	2.5

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable nitrogen (WEN) (mg kg⁻¹dry soil) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	0.1	-3.4	3.6
CC	-0.1	-2.3	2.0
STS	0.1	-2.0	2.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon (WEOC) (mg kg⁻¹dry soil) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>% CI</u>
Treatment	Slope	Lower	Upper
NH	-5.9	-48.0	36.1
CC	-17.8	-45.6	10.0
STS	-6.1	-53.1	41.0

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon (WEOC) (mg kg⁻¹dry soil) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	-1.3	-32.4	29.8
CC	-2.8	-32.8	27.2
STS	-0.7	-14.7	13.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon (WEOC) (mg kg⁻¹dry soil) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	-13.7	-46.1	18.8
CC	-18.8	-60.7	23.0
STS	-4.7	-43.5	34.0

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon (WEOC) (mg kg⁻¹dry soil) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-5.1	-35.7	25.6
CC	2.2	-8.2	12.7
STS	3.5	-17.1	24.1

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon to water extractable nitrogen ratio (WEOC/WEN ratio) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	-0.8	-1.4	-0.1
CC	-0.1	-1.1	1.0
STS	1.3	-5.1	7.7

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon to water extractable nitrogen ratio (WEOC/WEN ratio) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>% CI</u>
Treatment	Slope	Lower	Upper
NH	2.8	-3.0	8.6
CC	-0.3	-2.3	1.7
STS	-0.6	-3.0	1.8

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon to water extractable nitrogen ratio (WEOC/WEN ratio) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	0.2	-5.3	5.8
CC	-4.9	-88.0	78.3
STS	-1.1	-6.2	4.1

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water extractable organic carbon to water extractable nitrogen ratio (WEOC/WEN ratio) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	1.5	-5.2	8.1
CC	-2.2	-9.7	5.3
STS	1.1	-5.7	8.0

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of active carbon (g kg⁻¹ dry soil) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	-0.0	-0.3	0.3
CC	0.3	0.1	0.5
STS	0.2	-0.1	0.5

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of active carbon (g kg⁻¹ dry soil) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

	<u>95% CI</u>		
Treatment	Slope	Lower	Upper
NH	0.1	-0.6	0.8
CC	0.0	-0.3	0.4
STS	-0.0	-0.3	0.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of active carbon (g kg⁻¹ dry soil) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>% CI</u>
Treatment	Slope	Lower	Upper
NH	0.2	0.0	0.4
CC	-0.2	-1.7	1.3
STS	0.2	-0.9	1.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of active carbon (g kg⁻¹ dry soil) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	0.3	-0.6	1.1
CC	0.0	-0.4	0.5
STS	0.1	-0.2	0.5

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total nitrogen (TN) (g kg⁻¹ dry soil) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-0.2	-2.0	1.6
CC	-0.1	-2.3	2.1
STS	-0.1	-1.2	1.1

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total nitrogen (TN) (g kg⁻¹ dry soil) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>6 CI</u>
Treatment	Slope	Lower	Upper
NH	-0.1	-0.7	0.6
CC	-0.1	-1.5	1.2
STS	-0.2	-1.2	0.9

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total nitrogen (TN) (g kg⁻¹ dry soil) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-0.1	-0.1	-0.0
CC	-0.3	-5.2	4.7
STS	-0.0	-3.0	3.0

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total nitrogen (TN) (g kg⁻¹ dry soil) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-0.0	-1.3	1.2
CC	-0.2	-3.0	2.7
STS	-0.0	-2.4	2.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon (TOC) (g kg⁻¹ dry soil) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-4.7	-60.1	50.7
CC	-1.1	-47.9	45.7
STS	-2.6	-27.3	22.0

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon (TOC) (g kg⁻¹ dry soil) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-1.7	-16.1	12.8
CC	-1.6	-19.7	16.5
STS	-6.8	-47.0	33.5

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon (TOC) (g kg⁻¹ dry soil) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>6 CI</u>	
Treatment	Slope	Lower	Upper
NH	-2.0	-7.3	3.3
CC	-5.2	-88.8	78.3
STS	-1.2	-68.8	66.3

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon (TOC) (g kg⁻¹ dry soil) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-1.7	-18.8	15.4
CC	-3.0	-43.7	37.7
STS	-1.5	-47.5	44.4

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon to total nitrogen ratios (TOC/TN ratio) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>% CI</u>	
Treatment	Slope	Lower	Upper
NH	-0.3	-9.8	9.2
CC	0.4	-7.8	8.5
STS	-1.5	-9.4	6.5

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon to total nitrogen ratios (TOC/TN ratio) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-0.3	-2.3	1.8
CC	0.6	-2.0	3.2
STS	-3.7	-15.7	8.2

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon to total nitrogen ratios (TOC/TN ratio) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>	
Treatment	Slope	Lower	Upper
NH	-0.4	-0.4	1.1
CC	-0.4	-5.6	4.8
STS	-0.8	-5.1	3.4

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of total organic carbon to total nitrogen ratios (TOC/TN ratio) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>6 CI</u>
Treatment	Slope	Lower	Upper
NH	-0.9	-20.7	18.8
CC	-0.3	-27.5	26.9
STS	-1.4	-5.0	2.2

B. III Regression Tables for Water Stable Aggregates

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water stable aggregate concentration (WSA) (% of soil greater than 0.25mm in diameter) at the 0-10 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>			
Treatment	Slope	Lower	Upper		
NH	-3.7	-144.7	137.3		
CC	-4.2	-201.6	193.2		
STS	-6.0	-227.8	215.8		

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water stable aggregate concentration (WSA) (% of soil greater than 0.25mm in diameter) at the 10-20 cm depth of low soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>			
Treatment	Slope	Lower	Upper		
NH	-2.2	-117.8	113.4		
CC	-6.9	-178.9	165.1		
STS	-8.6	-154.0	136.7		

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water stable aggregate concentration (WSA) (% of soil greater than 0.25mm in diameter) at the 0-10 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95% CI</u>		
Treatment	Slope	Lower	Upper	
NH	-4.6	-84.1	74.9	
CC	-7.4	-76.0	61.2	
STS	-5.9	-290.7	278.8	

Slope and upper and lower 95% confidence intervals (CI) of regression line fitted to mean values of water stable aggregate concentration (WSA) (% of soil greater than 0.25mm in diameter) at the 10-20 cm depth of medium soil nutrient status (SNS) soils collected through time from one of three treatments: clearcuts (CC); single-tree selection (STS), and no harvest (NH) management.

		<u>95%</u>	<u>6 CI</u>
Treatment	Slope	Lower	Upper
NH	-1.6	-125.7	122.5
CC	-4.6	-40.7	31.4
STS	-6.2	-284.4	272.1

APPENDIX C

C. Harvest and Collection Dates

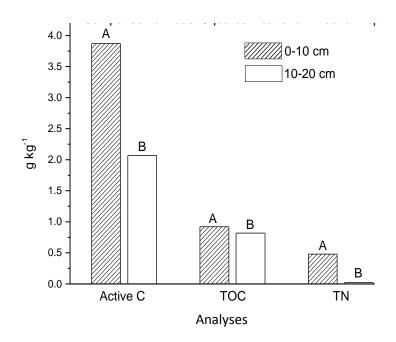
Site numbers 1, 6, and 8 are not noted in the 30-60 days post-harvest set because they are no harvest (NH) sites but they were sampled along with harvested sites. Only harvested sites, i.e. clearcut (CC) and single-tree selection (STS) sites were noted for each soil nutrient status (SNS). Sites of the same number are geographically close to each other.

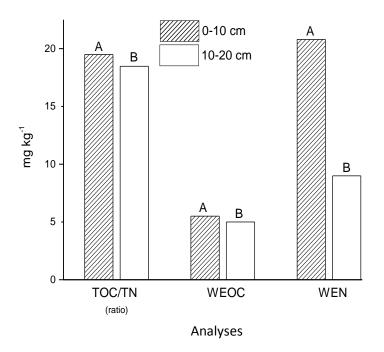
Collection Set	Collection Date	Harvest Date
Concetton Set	Dute	Trui vest Dute
Pre-Harvest (Full set)	1/28/2011	NA
Pre-Harvest (Full set)	3/30/2011	NA
30-60 days Post-Harvest:		
Site #2 Low SNS (STS)	8/16/2011	7/14/2011
Site #2 Medium SNS (STS)	8/16/2011	7/9/2011
Site #3 Low SNS (CC)	11/6/2011	10/7/2011
Site #3 Medium SNS (CC)	11/6/2011	9/29/2011
Site #4 Low SNS (STS)	5/17/2011	3/31/2011
Site #4 Medum SNS (STS)	8/16/2011	6/17/2011
Site #5 Low SNS (CC)	8/16/2011	6/16/2011
Site #5 Medium SNS (CC)	11/6/2011	10/7/2011
Site #7 Low SNS (STS)	2/4/2012	12/26/2011
Site #7 Medium SNS (STS)	11/6/2011	9/28/2011
Site #9 Low SNS (CC)	10/8/2011	8/15/2011
Site #9 Medium SNS (CC)	10/8/2011	8/15/2011
Post-Harvest (Full set)	4/21/2012	NA
Post-Harvest (Full set)	7/15/2012	NA
Post-Harvest (Full set)	1/16/2013	NA

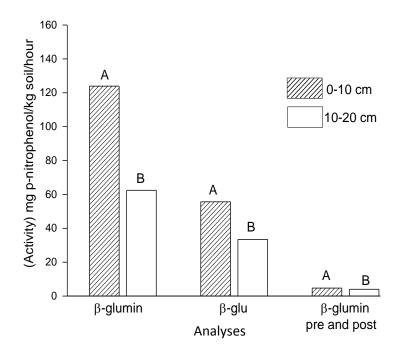
APPENDIX D

D. Comparison of least squares means for the effect of depth for various analyses

Note, comparisons cannot be made between analyses, i.e. values of active carbon at 0-10 cm in depth cannot be assumed to be statistically similar to TOC values at 0-10 cm in depth.







APPENDIX E

E. Modified Active Carbon Procedure

- -Weigh out 5.0 gm pre-sifted, air dried soil (sifted through 2.0 mm (#10) sieve) into 50.0 mL polypropylene centrifuge tube. Run each sample in triplicate.
- -Add 14.0 mL 0.2M KMnO₄ and 6.0 mL Barnstead ultrapure DI water to tube containing soil.
- -Set on side-to -side shaker for 15 min. at low speed.
- -Centrifuge at 3600 RPM for 5 min.
- -Combine 0.25 mL supernatant with 24.75mL Barnsted ultrapure DI water and mix well.
- -Read on spec. at 550nm.
- *Check calibration of pipettes before starting and make adjustments when needed.
- *Any samples reading <0.05 nm should be adjusted to a more concentrated dilution and the dilution should be noted in the log for calculations. Increased variability in instrument precision at such low concentrations necessitates higher absorbance readings to maintain reliability.
- *Run a standard curve each day of measurements. Make up standards before running samples.

APPENDIX F

F. Modified β-Glucosaminidase Activity Procedure

*Each field sample is run in duplicate with one control.

*Control samples receive 0.5 mL P-NNAG *after* incubation and the addition of CaCl₂ and NaOH.

To 50.0 mL Erlenmeyer Flask add:

-0.5 gm field moist soil filtered through #10 (2mm) sieve.

-7.0 mL Acetate Buffer pH 5.5

-0.5 mL 10 mM P-NNAG – Omit from control flasks until after incubation and addition of $CaCl_2$ and NaOH.

Swirl contents.

Stopper.

Incubate at 37 C° for 1 hour.

Un-stopper each flask and add:

-1.0 mL 0.5M CaCl₂

- 7.0 mL 0.5M NaOH

-*Add 0.5 mL 10 mM P-NNAG to control flasks only.

Swirl contents.

Filter contents through 0.2 um nominal pore syringe filters into disposable cuvetts.

Read at 405 nm, report activity by soil dry weight.

Subtract absorbance values of control samples from the two non-control samples before averaging duplicate values of enzyme activity.

APPENDIX G

G. Modified β-Glucosidase Activity Procedure

- -In a 50.0 mL Erlenmeyer flask weigh out 0.5 gm field moist, sifted (2.0 mm #10 siev) soil.
- -For each soil sample weigh out 1 control and 2 repetitions for a total of 3 flasks per soil sample.
- -To each test flask add:
 - -13.75 mL MUB pH 6
 - 0.25 mL Toluene
 - -0.05 M PNG (P-nitrophenyl-P-D-glucoside)
- -To each control flask add:
 - -14.25 mL MUB pH 6
 - -0.25 mL Toluene
- -Swirl each flask to ensure soil/solution contact.
- -Cap each flask with a rubber stopper and incubate for 1 hour at 37 C°.

After Incubation-

- -Add to all samples;
 - -1.0 mL 1M CaCl₂
 - -4.0 mL THAM pH12
 - -Swirl again
- -Filter through 0.2 um nominal pore syringes filter into cuvettes.
- -Read on Spec. at 410 nm, report activity by dry soil wt.
- -Standard curves can be made using solutions described in Dr. Kremer's procedure book for β -Glucosidase. The solutions can be wrapped in foil and kept in the fridge and used for several months. Standard curve solutions should be room temperature before measuring

APPENDIX H

H. Water Extractable Organic Carbon / Water Extractable Nitrogen Procedure

- -Weigh 20 gm air-dried soil into 50.0 mL polypropylene centrifuge tubes.
- -Add 40.0 mL DI water.
- -Put tubes on side-to-side shaker for 1 hour on low setting.
- -Centrifuge at 3600 rpm for 20 min.
- -Decant supernatant into onto Buchner funnel of filtration apparatus with 0.45 um filter paper and filter directly into 25.0 mL glass Shimadzu vials for WEOC/WEN analysis.
- -Pre-acidify samples with 85% H₃PO₄ to pH 2 (approximately 2 drops 85% H₃PO₄)
- -Analyze on Shimadzu NPOC with TN analyzer.

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APPENDIX I.

I. MOFEP Soil Nutrient Status Data by Treatment

				Textural	CEC		% Base S	aturation	рΗ	% Total	NH ₄	CI Extr	actable	Bases	s (cmolc/kg)	ECEC	% Base
NH Low	Depth (cm)	% Clay	% Sand	Class	cmolc/kg	% Al	NH₄OAc	% TOC	Salt	Nitrogen	Ca	Mg	Na	K	Sum of bases	NH₄CI	Saturation
	0-10	6.067	46.533	SL	9.8	49.67	15	2.8	4.2	0.162	1.533	0.333	0.033	0.2	2.1	5.733	32
	10-20	6.567	44.1	SL	4.167	62.33	11.333	0.867	4.1	0.033	0.233	0.133	0	0.133	0.567	2.433	25
	20-30	7.367	42.333	SL	3.567	71.33	7.667	0.433	4	0.015	0.167	0.1	0	0.1	0.367	3.4	11
	30-40	9.2	42.8	L	3.567	68	12	0.2	3.8	0.008	0.2	0.133	0	0.1	0.467	3.1	15
NH Med																	
	0-10	12.533	32.167	SL	8.633	26	24.667	2	4.1	0.108	1.267	0.6	0.033	0.167	2.1	5.133	43.333
	10-20	28	22.267	CL	9.467	49.67	25.333	0.667	4	0.038	1.733	1.833	0	0.167	3.733	7.23	33.667
	20-30	33.333	20.733	CL	12.333	42.33	32	0.367	4	0.018	3.167	2.933	0	0.167	6.267	10.233	36
	30-40	39.367	18.833	SCL	13.6	27.33	44	0.333	4.1	0.016	4	3.967	0	0.2	8.167	10.767	49.667
UEAM Low																	
	0-10	10.433	30.467	SIL	7.267	53.67	13.667	1.467	4.4	0.088	0.567	0.367	0.067	0.167	1.133	4.567	23
	10-20	10.167	28.433	SIL	5.167	58.33	13.333	0.733	4.4	0.041	0.333	0.3	0	0.133	0.8	3.7	19
	20-30	11.667	26.867	SIL	5.067	63.67	13	0.367	4.1	0.019	0.233	0.333	0	0.133	0.667	3.433	18.333
	30-40	15.067	26.367	SIL	5.8	51	20.667	0.267	4.1	0.014	0.467	0.567	0	0.133	1.133	3.933	28.333
UEAM Med																	
	0-10	8.633	32.5	SIL	7.433	25.67	28.333	1.4	4.7	0.099	1.533	0.667	0	0.2	2.4	5.833	36.667
	10-20	10.133	28.067	SIL	7.967	37.67	30	0.733	7.8	0.037	1.233	1.2	0	0.133	2.567	5.8	40.667
	20-30	15.667	21.7	SIL	12.467	39.33	33	0.433	4.5	0.024	2.267	2.367	0	0.167	4.9	10	43.667
	30-40	23.133	15.667	SIL	14.667	39	35.333	0.3	4.6	0.019	2.6	2.367	0	0.2	5.233	11.333	44.333
EAM Low																	
	0-10	9.8	21.167	SIL	9.933	13.33	38.667	2.133	5.5	0.15	2.8	0.7	0	0.3	3.833	6.6	53
	10-20	10.7	18.3	SIL	6.433	23.67	31.667	0.933	5.2	0.057	1.03	0.533	0	0.433	2.033	5.133	38.667
	20-30	14.2	15.867	SIL	6.333	44	18.333	0.4	4.5	0.028	0.4	0.633	0	0.267	1.3	4.967	27
	30-40	15.8	18.5	SIL	6.567	58	16.333	0.267	4.1	0.017	0.2	0.7	0	0.267	1.2	4.667	23.667
EAM Med																	
	0-10	12.467	18.333	SIL	9.067	18.33	27.667	1.633	4.8	0.125	1.6	0.667	0	0.2	2.533	5.2	46
	10-20	11.833	16.9	SIL	6.733	34	23.333	0.867	4.6	0.07	0.733	0.467	0	0.167	1.433	4.2	33.333
	20-30	14.867	15.5	SIL	6.533	29.33	26.333	0.5	4.5	0.04	0.567	0.667	0	0.167	1.4	5.133	28.333
ĺ	30-40	19.4	14.9	SIL	6.933	29	34.667	0.333	4.6	0.026	0.9	1	0	0.167	2.1	6.067	35.333

APPENDIX J

J. Location of MOFEP sites in three Missouri counties and management treatments (adapted from MOFEP.MDC.MO.GOV)

