ANTHROPOGENIC EFFECTS ON
SOIL PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES
IN THE MISSOURI OZARKS

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by

ALEXANDER W. TAYLOR

Dr. Keith Goyne, Dissertation Supervisor

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The undersigned, appointed by the dean of the Graduate School, have examined the
dissertation entitled

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SOIL PHYSICAL, CHEMICAL, AND BIOLOGICAL PROPERTIES
IN THE MISSOURI OZARKS

presented by Alexander W. Taylor,

a candidate for the degree of doctor of philosophy, and hereby certify that, in their
opinion, it is worthy of acceptance.

Professor Keith W. Goyne

Professor Stephen H. Anderson

Professor John M. Kabrick

Professor Robert J. Kremer

DEDICATION

As with most undertakings of a similar magnitude, this dissertation was not a singular event – many individuals contributed - providing sound advice and direction, moral support and technical assistance. First, I would like to thank my principal advisor, Keith Goyne, for taking a risk on a non-traditional (older) student and providing this opportunity to receive a second PhD. His guidance, patience, and intuitive sense of when to push and when to leave me to my own devices contributed greatly to the culmination of this project and the continued development of myself as a researcher. Furthermore, I would like to thank the other members of my committee - Stephen Anderson, Robert Kremer, and John Kabrick for their patience and advice. I have little doubt there were additional challenges to having an older student in their classes. Nevertheless, they took it in stride and managed to make this degree enlightening - and dare I say – an enjoyable experience. The folks at the USDA-ARS Cropping Systems and Water Quality Research Lab in Columbia, MO also deserve special mention. They opened their lab to me and provided much-needed training on soil enzyme activity and PLFA analysis.

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ABSTRACT

The maintenance of healthy forest ecosystems is critical to ensuring the sustainability of forest harvest activities. Forest soil health is a critical component of the knowledge required to manage a forest ecosystem; however, forest systems are understudied relative to agronomic systems with respect to soil health. The primary objective of this study was to enhance the understanding of forest soil health in the Missouri Ozarks via three research objectives: (1) quantification of the effects that clearcut and single-tree selection harvests have upon soil chemistry (2) enhance understanding of the effect forest harvest has upon the microbial populations responsible for driving nutrient cycling and (3) examine potential forest soil health indicators for their sensitivity to harvest at two post-harvest time intervals (5 and 22 years), eventually leading to the development of a forest soil health index specific to the Missouri Ozarks.

To better understand changes in soil nutrient concentrations, a series of zero tension samplers (ZTS) were installed (15 and 40 cm depths) in conjunction with throughfall samplers prior to a scheduled harvest in 2012 at the Missouri Ozark Forest Ecosystem Project (MOFEP) Site in the southern Missouri Ozarks. Soil solution samples were collected after rainfall events from pre-harvest until 2015. The samples were analyzed for Al_T ; Br^- ; Ca^{2+} ; Cl^- ; DOC (dissolved organic carbon); EC (electrical conductivity); F^- ; K^+ ; Mg^{2+} ; Na^+ ; NH_4^+ ; NO_2^- ; NO_3^- ; pH; PO_4^{3-} SO_4^{2-} ; and TN (total nitrogen). Soil samples were collected at 10 cm depth intervals to a total depth of 40 cm on a yearly basis from 2011 to 2016 and analyzed for Al_T ; exchangeable Ca^{2+} , K^+ , Mg^{2+} and Na^+ ; pH (via KCl and water extraction); TN; TOC (total organic carbon); sum of bases; base saturation; Bray-1 phosphorus and effective cation exchange capacity. The

data indicated the presence of a nutrient flush affecting concentrations of carbon, nitrogen, calcium, magnesium, potassium and phosphorous within soil solution beginning within 2 to 14 months after the clearcut and ceasing 17 to 31 months after the flush began. After the flush all nutrient concentrations returned to pre-harvest levels, with the exception of NO_3^- which remained elevated through the end of the study. Analysis of pre-harvest and yearly soil samples concentrations were either elevated or remained unchanged, with the sole exception of available phosphorus, which experienced a significant decline in concentration due to clearcut stem-only harvest.

Determination of the effect of forest harvest on microbial populations was accomplished via phospholipid fatty acid (PLFA) analysis and the measurement of four soil enzyme activities: β -glucosidase (β -glu); N-acetyl- β -D-glucosaminidase (NAG); arylsulfatase (Aryl); and acid phosphomonoesterase (AcdP). The following important soil geochemical properties were also analyzed: total organic carbon (TOC); active carbon (AC); total nitrogen (TN) and potentially mineralizable nitrogen (PMN); total sulfur (TS); Bray-1 extractable phosphorus (Bray1P) and soil pH. Carbon to nitrogen and total organic carbon to active carbon ratios were also computed as an indicator of substrate quality. Samples for this analysis were taken from the top 5 centimeters of the mineral horizon to maximize the capture of biological activity. Nominal to significant increases were seen in soil carbon, nitrogen and sulfur concentrations within the clearcut stem-only treatments. Paired with shift toward more neutral pH and improved substrate quality, as measured by C:N and TOC:AC ratios appear to indicate a healthy recovering environment within the clearcut environment. The sole exception is an inferred reduction in phosphorous cycling indicated by reduced Bray-1 P concentrations and acid

phosphomonoesteratase activity. Reduction in PLFA-derived fungal biomass and increases in arbuscular mycorrhizal fungi indicate changes in microbial communities linked to increased pH and better substrate quality. As a result, the fungal:bacterial and actinobacterial:fungal ratios indicate shifts in the dominant microbial communities away from fungal populations.

The determination of appropriate forest soil health indicators was performed via analysis of the previously collected data, limited to the top 10 cm of the mineral soil horizons for geochemical analyses and the top 5 cm of the mineral horizons for the biological analyses. Soil physical properties were also analyzed. Samples were collected at the MOFEP site and the adjacent Long Term Soil Productivity (LTSP) site, corresponding to a time interval of 5 and 22 years post-harvest. The LTSP site consists not only of a stem-only harvest, similar to MOFEP, but also has a whole-tree harvest treatment. Specifically the following soil properties were analyzed: aggregate stability; soil bulk density; fine fraction bulk density; percentage of coarse fragments; active carbon; base saturation; Bray-1 P; exchangeable Ca^{2+} , K^{+} , Mg^{2+} and Na^{+} ; carbon to nitrogen ratio; effective cation exchange capacity; pH; potentially mineralizable nitrogen, total nitrogen; total organic carbon; total sulfur; fungal to bacterial ratio; and β -glu; NAG; Aryl; and AcdP soil enzyme activities. At five years post-harvest, the following soil properties were found to have changed due to stem-only harvest: bulk density; percent coarse fragments; base saturation; Bray-1 P; C:N ratio; effective cation exchange capacity; exchangeable Ca^{2+} and K^{+} ; pH; total nitrogen; total organic carbon:active carbon ratio; total sulfur; Aryl and AcdP soil enzyme activities and the fungal:bacterial ratio. At 22 years post-harvest, only the C:N ratio and acid phosphomonoesterase

(reduction) varied by treatment. We suggest the following list of indicators for monitoring forest soil health as affected by harvest in the Missouri Ozarks: BS; CF%; exchangeable Ca^{2+} and K^{+} ; C:N; ECEC; pH; TN; TOC:AC; T; and Bray-1 P. Although fine fraction bulk density was not found to be sensitive to harvest in this study, we would recommend it be retained as part of a soil health assessment due to the strong influence it has on limiting root growth.

Short-term impacts were seen in soil chemistry and microbiological indicators as a result of stem-only harvest; however, all (with the exception of phosphorus) were consistent with the healthy cycling of a forest ecosystem. Concentrations either returned to pre-harvest levels, or were slight elevated above pre-harvest levels. These changes, paired with observed microbiological shifts represents a natural response to shifts in soil chemistry and microclimate as a result of clearcutting. However, reductions in available phosphorous and acid phosphomonoesteratase activity present a potential scenario of concern for the long-term prognosis for phosphorus cycling in the area with repeated forest harvest activities.

CHAPTER 1: INTRODUCTION, OBJECTIVES AND LITERATURE REVIEW

1.0 Introduction

The fact that forest harvest removes valuable nutrients from the ecosystem is largely undisputed. Grigal (2000) in his landmark paper on the effects of forest management on soil productivity considered this statement as axiomatic. The unanswered question is whether the rate of removal exceeds an ecosystem's ability to replace nutrients. Long-term, repeated harvesting activities can be considered unsustainable when the ratio of input to losses is less than 0.9, and if the stock of nutrients contained within live trees, the forest floor and the soil is insufficient for the next ten harvest rotations (Vangansbeke et al., 2015). Studies have shown that the long term effects of harvest on forest soils may be site-dependent necessitating careful consideration of harvest methods on a case by case basis (Hartmann et al., 2012; Ponder et al., 2012; Vangansbeke et al., 2015). Studies have also shown that the various forest management (e.g., even and uneven-aged) and slash management (e.g., removal, piling, dispersal) have differing impacts and site-dependent effects on soil nutrient concentrations (Achat et al., 2015; Johnson et al., 2016).

The research presented in this document represents the culmination of the efforts of several researchers over the last eight years studying nutrient cycling and the effects of forest management on cycling processes occurring at the Missouri Ozark Forest Ecosystem Project (MOFEP) site in Reynolds, Shannon and Carter counties of southcentral Missouri. This effort, sponsored by the Missouri Department of Conservation, is intended to inform the development of best management practices to protect the sustainability of Missouri forests and woodlands. It is estimated that over fifteen million acres of Missouri is covered in forest, making up nearly a third of the

landcover (Goff, 2018). The harvesting and processing of timber makes up a significant proportion of the economy of Missouri, generating \$3.2 billion dollars in 2016 (“Missouri Forest Facts,”) making the long-term sustainability of forests an important component of Missouri’s economic outlook.

1.1 Physiography of the Region

The Missouri Ozarks (physiographic description), or more geographically correct, the Salem Plateau region of the Interior Highlands is generally identified as that area bounding the Ozark dome and dominated by underlying Cambrian and Ordovician-aged rocks (Fenneman, 1938). The Salem plateau is considered to be within the Central Hardwood Forest category indicating that the floral succession is predominantly deciduous hardwoods (Fralish, 2003). The Salem Plateau region can be further subdivided into ecological classes, and the study sites are located within the Current River Hills subsection (Nigh and Schroeder, 2002). The field sites consist of the Missouri Ozark Forest Ecosystem Project (MOFEP) in the Current River and Peck Ranch Conservation Areas (CA), and the Long-Term Soil Productivity Research (LTSP) site in the Carrs Creek State Forest (roughly adjacent to the Current River CA).

1.2 Forests, Geology and Soils of the Region

The Missouri Ozark forest, prior to European influence, was likely dominated by shortleaf pine (*Pinus echinata* Mill.) with interspersed savannah barrens of multiple oak species (*Quercus* sp.) (Batek et al., 1999; Hanberry et al. 2014). Between 1880 and 1920, the majority of Missouri’s pine forests were logged to support economic development in the region (Guldin, 2008). In the place of the pine, a predominantly hardwood forest

grew, with oak (white oak, black oak, scarlet oak and post oak) dominating at approximately 71 % of the basal area, followed by shortleaf pine at 8% and various hickories at (*Carya sp.*) 12% (Kabrick et al., 2011).

The primary stratigraphic units outcropping in the MOFEP/LTSP area are Lower Ordovician rocks (Ibexian Series) composed primarily of dolomite, chert and quartz sand (Overstreet et al., 2003). Underlying the Ordovician rocks is the Cambrian-aged Eminence Dolomite Formation. The Eminence Dolomite is a light to medium gray cherty, sandy dolomite that was originally deposited as a limestone, but was altered during the Paleozoic to a dolomite (Unklesbay and Vineyard, 1992; Weary and Weems, 2005). The Gasconade formation of early Ordovician age unconformably overlies the Eminence formation and is composed of 5 distinct members (Gunter sandstone, the lower, middle and upper portions of the Middle Gasconade and the Upper Gasconade) containing dolomite, chert sandstone and orthoquartzite. The Gunter Sandstone member at the base of the Gasconade is a light grey to white quartz sandstone (occasionally an orthoquartzite) with interbedded thin bedded dolomites. The middle unit of the Gasconade is subdivided into three parts, (i.e., the lower, middle and upper parts) is a light grey to a very light gray dolomite with a persistent oolite bed near the base grading upwards to a cryptozoon chert bed. The Upper Gasconade member is a light gray thick bedded vuggy dolomite. Overlying the Gasconade formation is the Roubidoux Formation, also of early Ordovician age. The Roubidoux is a series of multicolored dolomites, ranging from gray to pale orange and pinkish gray interbedded quartz sandstones. Stromatilitic, oolitic and fossiliferous chert commonly replaces the dolomite. The contact between the Roubidoux and the Gasconade is commonly quartz sandstone or

orthoquartzite (Weary and Weems, 2005). A generalized stratigraphic column of Ordovician-aged and older rocks can be seen in Figure 1.1, below.

Figure 1.1 Generalized Stratigraphic Column of the Lower Ordovician and Cambrian in the Missouri Ozarks (Weary and Weems, 2005).

Soils in the area are primarily of the orders Alfisols and Ultisols, deeply weathered, and interspersed with soils that have a shallow depth to bedrock, containing fragipans (Meinert et al., 1997). A list of soil series and their associated taxonomic family mapped to the region can be seen in Table 1.1(Kabrick et al., 2011).

Table 1.1 Soil Series and Taxonomic Family Descriptions for the MOFEP Project Area (Kabrick et al., 2011)

Series Name	Taxonomic Family Description
Midco	Loamy-skeletal, siliceous, superactive, nonacid, mesic Typic Udifluvents
Tonti	Fine-loamy, mixed, active, mesic Typic Fragiudults
Scholten	Loamy-skeletal, siliceous, active, mesic Typic Fragiudults
Bendavis	Loamy-skeletal, siliceous, active, mesic Typic Hapludults
Bender	Loamy-skeletal, siliceous, active, mesic Typic Hapludults
Clarksville	Loamy-skeletal, siliceous, semiactive, mesic Typic Paleudults
Poynor	Loamy-skeletal over clayey, siliceous, semiactive, mesic Typic Paleudults
Hercules	Clayey-skeletal, mixed, superactive, mesic Cumulic Hapludolls
Alred	Loamy-skeletal over clayey, siliceous, semiactive, mesic Typic Paleudalfs
Rueter	Loamy-skeletal, siliceous, active, mesic Typic Paleudalfs
Mano	Loamy-skeletal over clayey, mixed, semiactive, mesic Oxyaquic Hapludalfs
Ocie	Loamy-skeletal over clayey, mixed, semiactive, mesic Oxyaquic Hapludalfs
Arkana	Very-fine, mixed, active, mesic Mollic Hapludalfs
Bardley	Very-fine, mixed, active, mesic Typic Hapludalfs

Two soil series that could be said to be representative of the soils in the study area are the Clarksville and the Alred soil series. The Clarksville soil is a loamy-skeletal, siliceous, semiactive, mesic Typic Paleudult. The Clarksville soil is described as very deep and somewhat excessively well-drained, and formed in hillslope sediments and the underlying clayey residuum derived from cherty limestones and dolomites. The Alred soil series is a loamy-skeletal over clayey, siliceous semiactive, mesic Typic Paleudalf. It is described as very deep, well-drained and formed in cherty hillslope sediments and underlying clayey residuum (Simmons et al., 2006). Figure 1.2 illustrates typical associations of the Clarksville and Alred soil map units in Reynolds County, Missouri.

Figure 1.2. Clarksville and Alred soil associations in Reynolds County, Missouri (Simmons et al., 2006).

1.3 MOFEP and LTSP Experimental Design

It is fortunate that two separate study sites focused on forestry are co-located in the Current River and Peck Ranch CAs within the southern Missouri Ozarks. The MOFEP study was initiated in 1991 with the most recent harvest experiment in 2011. The second site is the LTSP site, which was initiated in 1993. This resulted in an opportunity to examine the effects of harvest on the soil environment at the 5 and the 22 year post-harvest intervals, simultaneously. The location of the MOFEP and LTSP sites within Shannon, Reynolds and Carter counties in Missouri can be seen in Figures 1.3 and 1.4. The layout of the treatments for MOFEP and LTSP can be seen in Figures 1.5 and 1.6, respectively.

Figure 1.3. County map of the state of Missouri upon which the counties containing LTSP and MOFEP are highlighted.

Figure 1.4. Location of field areas in southcentral Missouri.

Figure 1.5. Missouri Ozark Forest Ecosystem Project (MOFEP) study sites and treatments (Sork et al., 2005).

Figure 1.6. Missouri Long Term Soil Productivity program plot layout (Ponder, 2008).

1.4 Forest Management

Forest silvicultural methods have had a long history of application (and misapplication) in the Central Hardwoods region of the United States. Previously in this region, little thought was given to silvicultural methods as the harvests were primarily

economic in focus. These harvests were largely carried out by clear-cutting, and high-grading (removal of only the best timber) of timber was the norm. Little thought was given to forest regeneration or sustainability and many areas, including the Missouri Ozarks suffered, as a result (Johnson et al., 2009). Nearly all of the original shortleaf pine in the Missouri Ozarks was cut during the 1880s-1920s logging boom, leaving little left for regenerative purposes, allowing the remaining oak and to a lesser extent, hickory, to predominate most of the Ozark region (Flader, 2004).

The even-aged and uneven-aged management practices generally used in the Missouri Ozarks have differing impacts on tree regeneration, soil productivity and aesthetic impact (Johnson et al., 2009). Clear-cutting is well-documented to be preferable to selection harvesting for the purposes of promoting a healthy and diverse stand. Clear-cutting involves the removal of the majority of the overstory to allow sunlight to filter in unimpeded. Regeneration can occur from seed banks on the forest floor, transported seed from nearby, or from stump-sprouting – a common feature in many oak species. Group selection harvest creates a preference for fast-growing shade-intolerant species, in the case of large openings, and shade-tolerant species in the case of smaller openings; whereas, single-tree selection harvest creates a preference for shade-tolerant species. The dominant oak species in the Ozarks have a greatly reduced ability to regenerate from oak stump sprouts when light levels are reduced from significant overstory, as is the case with single-tree selection and small group selection harvests (Dey et al., 2008).

1.5 Literature Review

This project focuses on determining the health of the soil by examining the functioning of the important biogeochemical cycles within the soil environment. These cycles, specifically carbon, nitrogen, sulfur and phosphorous, are critical for supporting productive forest ecosystems. Anthropogenic alterations to these cycles will be evaluated by looking at specific indicators of the cycles themselves, and on the supporting physical, chemical and microbiological environments which enable the cycles. A common theme discovered throughout the literature process was that forest harvest effects are highly dependent on management practices and are site specific.

1.5.1 Forest Management Effects on Soil Nutrient cycling

1.5.1.1 Carbon

The carbon cycle is arguably the most important biogeochemical cycle operating in terrestrial ecosystems. In terrestrial forested ecosystems, trees and other vegetation absorb carbon from the air and soil, using it to increase net primary productivity. Once the vegetation dies, various components of the plant (e.g., cellulose, hemicellulose and lignin) are decomposed by the action of numerous enzymes produced by soil bacteria and fungi. Through respiration and mortality, the bacteria and fungi create substrates, ultimately releasing CO₂ back into the atmosphere (Sylvia et al., 2005).

The primary effect of forest harvest on the terrestrial carbon cycle is the physical removal of carbon from the system (Laiho et al., 2003). The intensity of effect is directly related to the harvest and biomass removal intensity. Clear-cutting with whole tree removal has the greatest effect on the soil physical, chemical and biological environment;

whereas, clear-cutting with bole-only removal, and single or group tree selection harvest techniques have a lesser effect (Keenan and Kimmins, 1993). Although harvest removes biomass from the system, it also has the effect of leaving behind slash (except in the case of whole tree removal) which decomposes. These decomposing residues potentially create a short-term increase of carbon (and other nutrients) in the soil (Knoepp and Swank, 1997), often referred to as the “Assart effect” (Tamm, 1964). Singh (2013) studying the effects of clearcut harvest found short term increases in dissolved organic carbon concentration, which subsided quickly after harvest. Meta-analysis of soil carbon content studies showed that on average, clear-cutting with stem-only removal increased soil carbon content by 18%; whereas, whole tree harvest reduced soil carbon content by 6% (Johnson and Curtis, 2001). Dissolved organic carbon in soil solution has been found to be greater at the forest floor as well as in the A and B horizons of underlying soils of clear-cuts (Qualls et al., 2000).

Secondary effects of clear-cutting, specifically increased soil temperature (Londo et al., 1999) and soil moisture (Rogerson, 1976), can increase rates of tree litter decomposition and release nutrients more rapidly (Prescott, 2002).

1.5.1.2 Nitrogen

Nitrogen is a critical component of all life, being integral to both amino acids (e.g., protein compounds) and nucleic acids (e.g., DNA and RNA) (Sylvia et al., 2005). Soils deficient in nitrogen are unable to break down carbon sources in the soil, and resident vegetation cannot produce chlorophyll at requisite rates (Brady and Weil, 2013).

Clear-cutting with whole tree harvest is well documented to remove nitrogen from the forest system, at a greater rate than bole-only clear-cut harvest. The amount of nutrients lost from a site via physical removal of boles and slash is considered to be greater than leaching losses of nitrogen. Although losses via leaching and physical removal can be relatively large, several researchers have concluded that atmospheric replacement of nitrogen will be sufficient to maintain soil nitrogen reserves for several harvest rotations (Johnson et al., 1982). In some cases, the concentrations of nitrogen in soil leachate and in solid phase soil samples were found to be elevated for significant periods of time (e.g., 15 years) post-harvest ((Binkley, 1984; Knoepp and Swank, 1997).

1.5.1.3 Sulfur

Most research on the effect of forest management on sulfur cycling is related to the venerable (established in 1955) Hubbard Brook Experimental Forest in New Hampshire, U.S.A. Sulfur research at this site has been summarized in the works of Mitchell et al. (1989) and Likens et al. (2002). Mitchell et al. (1989) found significant increases in adsorbed sulfate in the B and E horizons under whole-tree harvest clear-cuts two years post-harvest; whereas, organic S pools remained relatively unchanged. The authors attributed these changes to increased soil acidity. Likens et al. (2002) carried the research further and found that vegetative disturbance generally had the effect of increased rates of organic matter deposition and nitrification, which increased the acidity of soil and streamwater pH, enhancing sulfate adsorption on mineral soil particles. When vegetative regrowth occurred, the adsorbed sulfate was released from the soil and, subsequently, it was found in adjacent surface water pools. The study also found that atmospheric deposition of sulfur has diminished since the project's inception in 1955.

Atmospheric sulfur deposition is expected to experience additional declines – potentially giving rise to a situation where sulfur deficiencies in soil are possible (Dick et al., 2008).

1.5.1.4 Phosphorus

As with many other soil nutrients, whole tree harvesting can cause depletion in phosphorous levels in soil, due to the physical removal of phosphorous from the system (Johnson et al., 1982). Other harvest methods, such as saw-log-only clear-cuts and selection harvest cause significantly less (or even negligible) depletion (Johnson et al., 1982; Singh, 2013). Yanai (1991), using zero tension sampler lysimeters at the Hubbard Brook Experimental Forest, tracked changes in total P in soil solution passing through the forest floor (Oa) and B horizon for 2 years post whole-tree harvest. Yanai (1991) found that total mean P concentrations in soil solution were greater under whole-tree harvest plots, relative to control sites; whereas, the total P concentration remained unchanged within deeper soil mineral horizons (Bh and Bs). Phosphorous flux from the forest floor to lower mineral horizons within whole tree harvest plots were found to exceed P flux via stream water, but the total amount being redistributed was very small relative to forest soil stores present at the site.

Generally, extractable phosphorous levels are a relatively small percentage of total phosphorous, and P depletion from plant uptake has little effect on total phosphorus in the soil. However, this may not hold true in areas where the soil is naturally very low in phosphorous (Johnson et al., 1982). The highly weathered soils in the MOFEP field area have relatively reduced total P concentrations, making monitoring of the soil phosphorous concentration important (Singh et al., 2015)

1.5.1.5 Base Cations

The scientific study of timber harvest effects on base cations began in the middle of the 20th century (McColl, 1978; Johnson et al., 1982; Adams et al., 2000). It is generally accepted that whole-tree harvest effectively removes more of base cations from the soil than stem-only harvest, due to the sequestration of these nutrients within the plant's cellular structure and the subsequent physical removal of the entirety of the plant from the site (Johnson et al., 1982). In general, initially after harvest, the concentration of available base cations increases (Knoepp and Swank, 1997) in soil solution, assuming sufficient litter is left behind. Although many species of trees have significant concentrations of nitrogen, phosphorus and potassium in the foliage, this trend does not necessarily hold true for oak and hickory (Johnson et al., 1982). Calcium appears to be a particular issue in hardwood oak and hickory forests, as a significant amount of calcium can be sequestered in the woody tissue of these trees (Ovington, 1959; Johnson et al., 1982; Zetterberg et al., 2016). However, McLaughlin (McLaughlin, 2014) in a meta-analysis concluded that in the short to medium term, forest management practices do not appear to trigger losses in soil Ca^{2+} pools. McLaughlin further stipulates that forest floor Ca^{2+} concentrations appear to increase post-harvest. McLaughlin suggests that rapid internal cycling of Ca^{2+} by fast-growing successional species retains Ca^{2+} released from decomposing woody material. Conversely, Zetterberg et al. (2016) examined nearly 40 years of Ca^{2+} flux at three coniferous sites in Sweden and found that over the long term, Ca^{2+} losses were significant for both bole-only and whole-tree harvest. Initially, differences between the two harvests were significant, but over time the differences in Ca^{2+} loss were diminished. The authors inferred that either harvest type triggers enhanced

Ca²⁺ depletion (Zetterberg et al., 2016). Although the Swedish sites were not calcium-limited, long-term trends in Europe of calcium depletion in forest soils due to atmospheric acidic deposition could impact forest sustainability.

Romanowicz et al. (1996) examined exchangeable K⁺ concentrations in soil solution via “tension free” lysimeters post whole-tree harvest at the Hubbard Brook Experimental Forest found there was an identifiable pulse of K⁺ concentration in soil solution after harvest, which coincided with increased NO₃⁻ concentration and a decrease in pH. Soil-exchangeable potassium pools were initially elevated above reference levels within three years, but had decreased to pre-harvest levels after 8 years at higher elevations. At lower elevations, a drop in K⁺ concentration was noted after 8 years; however, the overall level was still 20% above reference for the duration of the experiment (i.e., for 10 years). The authors also found that although removal of K⁺ from harvesting and from leaching into streams was great relative to overall soil pools, increased mineralization of soil organic matter and enhanced mineral weathering increased the overall pool of exchangeable K⁺ above pre-harvest levels.

The fate of the cations is highly dependent on the particular soil environmental parameters present at the site (Mroz et al., 1985; Johnson et al., 1988). Soils with lesser initial nutrient concentrations, lesser cation exchange capacity and highly weathered soils are generally considered to be more sensitive to nutrient depletion from harvest activities (Adams et al., 2000; Johnson et al., 2009), although other studies have shown greater harvest impacts occurring in higher nutrient status soils (Mroz et al., 1985). Limitations in the research design of Mroz et al. (1985) led to poor understanding of ultimate nutrient

fate. The researchers were unclear whether the lost nutrients (potassium and nitrogen) leached to groundwater or merely mobilized to deeper soil horizons.

1.5.1.6 Larger scale geochemical studies

Qualls et al. (2000) used porous cup suction lysimeters to evaluate the dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorous (DOP) concentrations in soil solution at the Coweeta Hydrologic Laboratory, N.C., for two years after a clear-cut operation (whether whole tree harvest or bole-only was not specified). The authors found that DOC and DON concentrations in the slash leachate, O, A, and B horizons were greater in the harvested plots relative to control. Concentrations of DOP in harvested plots were greater in the slash leachate and O horizons, but unchanged in the deeper mineral horizons. They also found that, despite greater fluxes of DON from the harvested plots, more than 99% of the DON draining from the forest floor was removed from solution before entering the C horizon. The DON was assumed to be adsorbed in the mineral horizons rather than being removed completely from the soil via leaching.

Achat et al. (2015) conducted a meta-analysis of over 100 harvest management studies examining differing levels of biomass removal (i.e., stem-only, whole tree harvest, and complete slash removal). Overall, complete residue removal led to significant loss of soil organic matter content and decreases in nitrogen, phosphorous, and calcium concentrations. The loss of soil organic matter and nitrogen were found to be greater in temperate climates, as compared to colder climates. No effect was noted for potassium and magnesium. Within complete biomass removal treatments, increases in the carbon:nitrogen ratio and decreases in microbial activity (as inferred from microbial

biomass, soil respiration and other methods) were observed. A salient point was the finding that soil and vegetation responses to increasing levels of biomass removal varied greatly among the individual case studies, dependent upon pre-existing site conditions and the intensity of the residue removal.

1.5.2 Previous Soil Chemical and Biochemical Studies at MOFEP and LTSP

A number of studies examining soil chemical effects associated with forest harvest have been performed at the MOFEP and LTSP sites since their respective inceptions. An overview of the more germane research follows.

Spratt (1997) analyzed total and organic sulfur in the A horizon post clear-cutting and single-tree harvest at MOFEP and nearby Deer Run State Park. His research showed significant losses of both sulfur forms in the clear-cut plots. These losses occurred at the 2 to 3 and the 8 to 10 year post-harvest intervals. Additionally, carbon to sulfur ratios increased markedly during those same periods, with the largest changes occurring at the 2 to 3 year post-harvest interval.

Spratt (2002) examined changes in total carbon, nitrogen and sulfur, exchangeable potassium and magnesium, along with two microbial activity indicators – ^{14}C -lignocellulose mineralization to determine microbial catabolic activity and the integration of $^{35}\text{SO}_4$ into organic compounds. It was found that total carbon (35%), nitrogen (no relative percent change reported), sulfur (40%), and exchangeable potassium (75%) all experienced reductions in concentration two years post-harvest within the upper soil horizons of clear-cuts. Conversely, lower soil horizons experienced an increase in total sulfur concentration (75%). Lignocellulose mineralization was found to initially increase

post-harvest in clear-cuts but had declined 2 years post-harvest. Organic sulfur production as measured by $^{35}\text{SO}_4$ integration was found to be reduced by 80-90%. A weakness of the work conducted by Spratt (2002) is associated with sample collection. Soil samples were collected by combining horizons, specifically the top horizon was a combination of the Oa and A organic and mineral horizons, and the lower sample interval was formed by combining the E and B mineral horizons. The processes related to nutrient cycling are significantly different in the organic versus mineral soil horizons, and microbiological factors, such as soil enzyme activity and total microbial populations, can change rapidly with depth in the soil profile (Lladó et al., 2017). Therefore, some caution should be exercised when comparing the results of other studies, including work included here, with that obtained by Spratt.

Albers (2010) examined harvest effects in the soil solid phase at the MOFEP site at 10 years after the 1996 harvest. Samples collected at 10 centimeter intervals from top 30 centimeters of three nutrient status (low, medium and high) mineral soil horizons were analyzed for pH, base saturation, Ca, Mg, K, cation exchange capacity, exchangeable acidity, total organic carbon, total nitrogen, and stable and labile nitrogen pools. Albers found that soils under clear-cuts experienced increases in exchangeable Ca, TOC, TN, stable and labile nitrogen relative to controls, and decreases in these same properties in single-tree selection treatments relative to control. The effects were found to be greater in the upper mineral horizons and accentuated in high nutrient status soils. Albers (2010) attributed the changes to differences in slash distribution at the study sites. Slash at single tree selection sites was located well away from the base of the tree being harvested;

whereas, slash from clear-cut sites was more uniformly distributed throughout the harvested area.

Gaddie (2012) and Singh (2013) as a part of the predecessor studies to this research, completed research into nutrient cycling as impacted by forest harvest at the MOFEP site. Gaddie focused on the use of soil column data and oversaw the establishment of a soil solution sampler network at the MOFEP study area. Many of the pre-harvest readings were collected over the course of Gaddie's involvement with the project. Gaddie also evaluated nutrient leaching with the use of soil column experiments to characterize pre-harvest conditions at the site. Using three representative soils (low, medium and high base saturations) column experiments were set up with varying temperatures (21, 23 and 26 °C). Leachate was collected on a weekly basis after simulated rainfall events and was tested for pH, electrical conductivity, and concentrations of K^+ , Na^+ , NH_4^+ , Ca^{2+} , Mg^{2+} , Al_T , Cl^- , NO_3^- , SO_4^{2-} and PO_4^{3-} . Microbial activity was measured via determination of β -glucosidase activity. Gaddie (2012) found that NH_4^+ concentration (greatest at 23°C) and β -glucosidase activity (greatest at 21°C) were affected by temperature. Based upon pre-harvest soil solution studies collected by zero tension soil solution samplers, Gaddie found that there were seasonal fluctuations in pH, base cations, DOC and TN from both low and medium nutrient status soils. Overall, Gaddie concluded that there was little difference in cycling in undisturbed soils in low and medium nutrient status soils at the MOFEP site.

Singh (2013), in a follow-on study using the same soil solution sampler network as Gaddie (2012), collected samples from pre-harvest to approximately two years post-harvest in control, clear-cut and single tree selection plots at the MOFEP study site. Singh

(2013) analyzed soil solution data for pH electrical conductivity and concentrations of cations (K^+ , Na^+ , NH_4^+ , Ca^{2+} , Mg^{2+} , Al_T) and anions (F^- , Br^- , Cl^- , NO_2^- , NO_3^- , SO_4^{2-} and PO_4^{3-}). Samples were also analyzed for dissolved organic carbon (DOC) and total nitrogen. Singh (2013) found that in clear-cuts, relative to control, significant increases were observed in soil solution for NO_3^- , Mg^{2+} , TN and in the electrical conductivity values. Soil nutrient status did not significantly affect the examined variables. There were no observable differences between single tree-selection sites and control. Singh also found that concentrations of Ca^{2+} , Mg^{2+} , and K^+ in solution increased initially post-harvest. However, concentrations of Ca^{2+} decreased rapidly; whereas, K^+ and Mg^{2+} concentrations remained elevated to the end of the study period. Due to the shortened sampling period the length of time these ion concentrations remained elevated could not be determined; however, other researchers have noted greater cation concentrations in forest floor leachates two years after harvest, with declines beginning to occur in third year post-harvest (Hendrickson et al., 1989; Weis et al., 2006). Generally, increases in soil solution concentrations of Ca^{2+} , K^+ and Mg^{2+} are noted as decomposition of tree litter increases post-harvest; however, it is thought that most of these cations are not lost but retained within underlying mineral horizons (Piirainen et al., 2004).

Hatch (2014) in the only study focusing on soil quality indicators and forest harvest disturbance at the MOFEP site. This study examined changes in TOC, TN, active carbon, water extractable organic carbon and nitrogen, soil pH, and activities of the soil microbial enzymes β -glucosidase and β -glucosaminidase. Samples were collected pre- and post-harvest from control, single-tree selection harvest and clear-cut harvests at low and medium nutrient status sites at the 0-10 and 10-20 centimeter depth intervals. At 1.5

years post-harvest significant differences (decreases in both cases) in water extractable nitrogen concentration and in β -glucosaminidase activity were observed, as compared to control and pre-harvest values.

At the adjacent Long Term Soil Productivity site, a number of studies related to forest harvest activities effect on soil chemical and biological properties have been performed. Although the experimental design of LTSP was somewhat different than the MOFEP study site, several of the treatments are similar enough to consider using them as part of a chronological examination of the changes in soil properties at 20 to 25 years post-harvest. A summary of these various studies and findings follow.

Powers et al. (2005) reported the effects of stem-only, whole-tree and biomass harvests with varying compaction intensity levels upon soil properties at 10 years post-harvest across all LTSP sites in North America. Overall, the authors found that complete biomass removal (OM2 treatment) led to decreased soil carbon concentrations to a depth of 20 centimeters and reduced nitrogen and phosphorus availability in soils. However, more significant to the research presented in later chapters is that individual LTSP sites experienced varying levels of response to the measured indicators, indicating a site-specificity to forest harvest activities.

Ponder (2005) looked at CO₂ efflux rates at four years post-harvest at the Missouri LTSP site as a measure of microbial respiration. Ponder (2005) found that CO₂ efflux was positively correlated with temperature and that the treated plots had significantly lower respiration than the control plots. No significant differences in microbial respiration were noted among sites treated to forest floor removal, soil compaction or weed control treatments.

Ponder and Eivazi (2008) examined the activities of five soil enzyme activities eight years post-harvest at the LTSP site in southern Missouri. The surveyed enzymes were acid and alkaline phosphomonoesterase, β -glucosidase, β -glucosaminidase, and arylsulfatase. The authors also tested the bulk soil for carbon, pH, phosphorous and sulfur concentrations. Between control and harvested plots, significant differences were only noted in β -glucosidase activity (an increase in harvested plots) and in Mehlich-3 extractable phosphorous (a decrease in harvested plots). Organic matter removal did not appear to significantly affect soil enzyme activity. Although not germane to my study, weed control treatments did appear to negatively impact soil enzyme activity.

1.5.3 Effects of forest management on microbial communities and activity

Ponder and Tadros (2002) performed phospholipid fatty acid analysis (PLFA) to examine shifts in microbial populations at the Missouri LTSP site four years post-harvest. The authors collected 30 subsamples from the 0-10 centimeter depth interval per treatment plot, which were then composited to form a single composite sample per plot. Statistical analysis showed no change in overall bacterial biomass; however, individual groups did show significant differences by treatment. Specifically, gram positive and gram negative bacteria increased in biomass. Fungal populations and fungal:bacterial ratio did not change significantly. Ponder and Tadros (2002) did not test any additional soil properties, so it is difficult to determine specific factors influencing the microbial community structure. The authors concluded that soil disturbance triggered by forest harvest activities have the potential to favor certain microorganisms over others.

Most recent research into the analysis of soil microbial population changes due to forest harvest via PLFA methods are associated with forests in more northern climates.

Chatterjee et al. (2008) examined changes in soil carbon mineralization and microbial community composition via PLFA in Ponderosa and Lodgepole pine forests in Wyoming, USA. They found that harvest activities largely had negative impacts on mineralization rates and microbial populations.

Fichtner et al. (2014) examined ancient oak forests as compared to former forests that had been farmed and then reclaimed in Lower Saxony, Germany (latitude equivalent to southern Canada). They found that the disturbance of microbial population in German oak forests strongly affected saprophytic fungi, arbuscular mycorrhizal fungi, ectomycorrhizal fungi and actinobacteria groups, and that these effects could last up to 100 years post-disturbance event.

Lewandowski et al. (2015) examined the effects of group selection harvest on soil microorganisms in sugar maple-dominated northern hardwood forests in Northern Wisconsin. They found that initially arbuscular mycorrhizal fungi decreased; whereas, gram positive bacteria and anaerobic microorganism populations increased. No initial changes were noted for the saprophytic fungi and actinobacteria populations. However, in later years, saprophytic fungi populations increased and actinobacteria populations decreased. These findings suggest greater competitive interaction between actinobacteria and saprophytic fungi with time after group selection harvest. The authors concluded, based upon the recovery at four years post-harvest, that the effects of group selection harvest were not likely long-term in nature.

Lewandowski et al. (2016) studied the effects of three biomass harvest methods on trembling aspen dominated forests in northern Minnesota. Three levels of slash retention were studied – whole-tree harvest (no slash retention), 20% slash retention, and

bole-only harvest (100% slash retention). They found that harvest activity (regardless of slash retention level) increased arbuscular mycorrhizal fungi populations. However, they did find that the abundance of arbuscular mycorrhizal fungi, gram positive bacteria and actinobacteria were greater in the 100% slash retention plots relative to the other slash retention treatments. The authors concluded that increased slash retention may help ameliorate the effects of harvest activity of soil microbial populations.

1.5.3 Forest Soil Health (or Quality)

At the onset of this research, in 2013, soil quality was defined by the Soil Science Society of America (SSSA) as “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health (Karlen et al., 2008)”. Initially differentiation between soil health and soil quality was in dispute as some soil researchers and end-users considered the terms to be synonymous (Larson and Pierce, 1991; Bone et al., 2010). It has been observed that soil scientists use the term soil quality; whereas, other researchers (e.g., agronomists) prefer the term soil health (Bone et al., 2010). Many prefer the term soil health because it evokes a “living dynamic system” (Doran and Parkin, 1996). Doran and Zeiss (2000) suggested an expanded definition of soil health: “the capacity of a soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health”. The current definition of soil health as cited on the USDA-NRCS website is “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans”. The website states that soil health and soil quality are now considered to be synonymous (“Soil Health | NRCS Soils, 2018”).

As can be seen, the SSSA definition has proven to be contentious. Some scientists believe an overreliance on soil quality assessment, over a focus on appropriate soil management techniques misdirects valuable research time and money – insinuating that soil quality assessment is essentially impossible due to the complexity of the processes governing soils (Karlen et al., 1997, 2008). For the purposes of this project, the term soil health will be used as defined by the SSSA, above. When the term soil quality appears, it is a relict of a previous terminological usage in published manuscripts.

Doran and Parkin (1996) recognized five features that good quantitative soil quality (health) indicators should possess:

- They should correlate well with ecosystem processes.
- They should integrate physical, chemical and biological properties and processes, and/or serve as basic input needed for estimation of soil properties or processes which are more difficult to measure.
- They should be easy to use in the field and assessable by both soil specialists and by agricultural producers.
- They should be sensitive to variation in management and climate.
- They should be components of existing soil databases, whenever possible.

Soil health in agricultural systems has a long history and could be considered relatively robust (Karlen et al., 2008). There are a number of different models which can be used to determine the relative quality of agricultural soils, including the Soil Management Assessment Framework (Andrews et al., 2004) and the Cornell Soil Health Test. These models include soil physical, chemical and biological parameters to define

the overall soil quality on a dynamic basis (Karlen et al., 2008). Initially, the Cornell test was generally considered to be more accessible to the agricultural practitioner; whereas, the SMAF was more appropriate to the soil researcher, due to the SMAF model containing indicators that are more difficult to interpret (e.g., β -glucosidase activity). However, the most recent incarnation of the Cornell test, the Comprehensive Assessment of Soil Health, now contains more complex parameters (e.g., soil protein and microbial respiration rates), lowering the independent utility by some practitioners.

1.5.4 Forest Soil Health (Quality) Indices

The definition of soil health infers productivity, so it is expected that many indices can be seen as measures of productivity for a particular species of vegetation, rather than a more abstract soil health measurement. Early research into soil health was integrally tied to crop productivity and aimed at one or two end goals: 1) evaluating the responsiveness of a given crop to management and/or 2) predicting the yield of a crop on a certain soil type (Simonson and Englehorn, 1939). These developments occurred simultaneously with the “Dust Bowl” era. The combination of unusual climatic conditions and poor agricultural management created a historically unprecedented environmental disaster. A nascent Soil Conservation Service, formed in 1935, was charged with advancing scientific understanding of erosion processes and developing management practices to conserve soil resources (“More Than 80 Years Helping People Help the Land: A Brief History of NRCS | NRCS,”).

The development of soil quality indices for forested systems can be traced back to the 1930’s -concomitant with the creation of the Soil Conservation Service in the United States. Coile (Coile, 1935) built upon earlier research on forest soil properties to develop

what became known as the Soil-Site equation. This method related soil characteristics (soil depth, soil texture, and underlying geology) to forest site index to allow determination of the forest site index for soils upon which no trees reside. Forest site index is an estimate of the capacity of a forest to grow trees, based upon the height of dominant and co-dominant at 50 years of age (Carmean et al., 1989). Soil texture, in particular the fine fraction and the thickness of the soil horizons, were found to be correlated with forest site index. A number of subsequent studies by Coile's students and other researchers applied the same methodology to various regions around the United States (Young, 1954). By the mid 1970's, the Soil-Site equation was in common usage around the U.S., often without subsequent testing for accuracy (McQuilkin, 1976). McQuilkin performed an accuracy assessment of the Soil-Site Equation in the Missouri Ozarks and found poor correlation between the developed model and the unknown test areas (an R^2 value of 0.01), thus casting doubt on the efficacy of this technique to infer site quality.

Storie and Wieslander (Storie and Wieslander, 1949) related a site's timber quality to soil parameters by studying conifer stands in California. The soil factors chosen were: the depth of appropriately textured soil; permeability; chemical toxicity (e.g., degrees of salinity, alkalinity); drainage class; and climate. Kiniry et al. (1983) developed a Productivity Index (PI) for Missouri farmland, which was then tailored for use in the Missouri Ozarks by Scrivner (Scrivner et al., 1985). This model used "sufficiencies" (or optimal values) for available water capacity, pH and bulk density as input into the model. Gale et al. (Gale et al., 1991) also applied the Productivity Index to white spruce plantations. The confounding factors of multicollinearity between variables and

inadequately accounting for soil parameter interaction caused it to meet with limited success.

These early efforts in developing forest soil quality measures were plagued by poor selection of soil parameters (including an overreliance on physical properties), poor understanding of soil property interactions and multicollinearity effects amongst the chosen parameters. These techniques produced models that appear to work well on training areas, but tend not to translate well to even adjacent plots (McQuilkin, 1976; Gale et al., 1991). As a result, for most of the twentieth century, forest researchers have relied primarily on forest site index as a measurement of site quality (Gale et al., 1991).

Use of larger number of soil characteristics and the integration of chemical parameters to assess soil quality became more commonplace towards the end of the twentieth century. Burger and Kelting (1999) suggested developing new models using parameters more specifically tuned to the impacts that different land use practices might have on a forested site. They stated that the goal should be to measure deviation of the parameters from a known baseline to deduce impact from a particular activity. Kelting et al. (1999) suggested that forest site index was a poor indicator of management-induced productivity change and proposed using the more rigorous agricultural indices as a basis. Kelting et al. (1999) used a small subset of parameters (i.e., bulk density, depth to water table, net nitrogen mineralization, soil aeration and volumetric water content) to evaluate soil health conditions at three loblolly pine plantations. The plantations were treated to a variety of bed preparation and harvest practices. They found that winter water table depth, net N-mineralization and aeration depth during the growing season were the best

predictors of site quality; whereas, bulk density and volumetric water content showed little correlation with tree productivity.

Page-Dumroese et al. (2000), working with three regions of the U.S. Forest Service (Regions 1, 4 and 6, located in the Pacific Northwest) recognized that the soil properties of forests can be highly variable. They proposed a larger subset of soil parameters to assess soil health conditions that are related directly to harvest and burn impacts as follows: soil displacement; compaction; rutting and puddling; surficial erosion; soil cover; organic matter; and burned condition. They further recognized that impacts were very site specific, having different limiting soil factors to future productivity after impact. They also noted that the soil parameters evaluated were merely a starting point, and other variables needed to be identified. Schoenholtz et al. (2000), in an overview of extant forest soil quality measures, suggested that agricultural-based indices based on inherent physical, chemical and biological parameters are inappropriate for forest ecosystems due to their inability to adequately account for soil-plant interactions and nutrient cycling. They propose that indices of soil quality which incorporate soil chemical and physical properties will only be adopted if they are:

1. Sensitive to management-induced change
2. Easily measured
3. Relevant across sites and/or time
4. Inexpensive
5. Closely linked to measurement of desired values (e.g., productivity, biodiversity)
6. Adaptable to specific ecosystems

Rodrigue and Burger (2004) studied a variety of forest soils impacted by coal mining in Appalachia and the Midwest. They found that base saturation, percent coarse fragments, total available water, porosity of the C horizon and electrical conductivity provided the best estimates of site quality.

Pietrzykowski (2014) created a mine soil quality index (MSQI), to measure productivity on reclaimed mine land forested with Scots Pine. The study used soil texture, pH, base cations (Ca, Mg, K, Na, P) and carbon to nitrogen ratio as indicators of soil health. The resulting index performed reasonably well, but found significant variation based on parent material (i.e., the underlying geology) and soil texture. The study suggested that estimations based on texture and parent material alone were insufficient and nutrient availability, in particular phosphorous availability, plays a large part in determining productivity on reclaimed land.

The proposal of biological indicators for forest soil quality indices is a relatively new phenomenon. At the turn of the 21st century, the USDA recognized that to effectively determine soil quality, all three components of soil properties: physical; chemical; and biological, must be assessed (Ditzler and Tugel, 2002). Accordingly, about this time, integration of biological parameters became more commonplace.

De la Paz Jimenez et al. (2002) undertook research in Argentinian forest soils that had undergone a variety of treatments ranging from pristine to deforested and having undergone 40 years of tillage to determine a minimum set of parameters for establishing a soil quality index. They tested organic carbon, total nitrogen, extractable phosphorous, aggregate stability, respiration carbon, dehydrogenase, urease, protease, acid phosphomonoesterase, β -glucosidase, and arylsulphatase activities. They found that a

minimum data set of arylsulphatase, acid phosphomonoesterase, β -glucosidase and dehydrogenase activities did nearly as well at predicting soil quality as a model containing all of the parameters. It was also noted that these three enzyme activities adequately evaluated potential changes to the sulfur, phosphorous and carbon cycles.

Amacher et al. (2007) outlines the Forest Inventory and Analysis (FIA) soil quality index, which utilizes nineteen chemical and physical properties to monitor soil health. These parameters are: bulk density; percentage of coarse fragments; pH; total organic carbon; total nitrogen; exchangeable Na; total K, Mg, Ca, Al, Mn, Fe, Ni, Cu, Zn, Cd, Pb, and S; Bray P; and Olsen P. The FIA program was further expanded into a more holistic forest health monitoring, which included non-soil qualitative biological parameters (i.e., tree crown condition, lichen communities, vegetation diversity and down woody material) (Woodall et al., 2011).

Dinesh and Chaudhuri (2013) focused solely on biochemical and microbial indices as indicators of change related to land use practices in mangrove forests of the South Andaman Islands of India. They specifically used dissolved organic carbon, dissolved organic nitrogen, soil microbial biomass carbon, soil microbial biomass nitrogen, soil microbial biomass phosphorous, soil respiration, adenosine monophosphate, diphosphate, and triphosphate adenylate energy charge, and ergosterol (a fungal biomass indicator). They found that microbial biomass, soil respiration and metabolic quotient (a ratio of respiration to microbial biomass) were robust and sensitive indicators of variations in soil quality.

Högberg et al (2013) studied spruce forests in southern Sweden and found good correlation between fungi-bacteria composition ratios (as determined by PLFA profiles) and the concentrations of nitrogen and aluminum leachates below the rooting zone.

Vasconcellos et al. (2013) studied a suite of physical, chemical and microbiological indicators in a riparian forest recovery area in Brazil. They measured soil moisture, bulk density macro- and microporosity, texture, pH, available P, total carbon, total nitrogen, NH_4^+ , NO_3^- , microbial biomass carbon, microbial biomass nitrogen, microbial metabolic quotient ($q\text{CO}_2$), dehydrogenase, urease, cellulose, acid phosphatase, and terminal restriction fragment length polymorphism (a genetic assay technique). They found that dehydrogenase, when measured in summer, was a good predictor of the age of the recovery zone and was highly correlated with most soil properties. Urease was found to be a good predictor of recovery age independent of soil properties and seasonality, although the results influenced by soil clay content.

1.6 Summary of Literature Review and Relationship to Current Study

The site-specific nature of the effects of forest harvest on soil nutrient status represents a challenge to forest managers. Although forest harvest has been widely studied, more recent research has shifted focus from temperate sites such as the Missouri Ozarks to the Northern U.S. tier states and Canada. This has the unfortunate side effect of reducing opportunities for more modern analysis techniques to be applied to temperate forests. By applying soil enzyme activities, phospholipid fatty acid analysis and integrating these analyses with more traditional soil solution and solid phase chemistry, we hope to further illuminate the processes controlling nutrient cycling in the Central United States. Research featuring the impact of forest management on soil enzyme

activity appear to be limited to two studies in the Ozarks (Ponder and Eivazi, 2008; Hatch, 2014). Phospholipid fatty acid analysis to examine forest harvest impact on microbial groups appears to be limited to a single study (Ponder and Tadros, 2002). None of these studies incorporated integration of this data with other soil properties. Furthermore, several of these studies featured composited samples both with respect to areal distribution and depth which may impact the utility of the findings. Literature review yielded no research in the Ozarks examining all three aspects (soil physical, chemical and biological) of soil properties. Furthermore, a similar lack of an integrative approach to harvest effects on soil properties was discovered in the literature review, regardless of region. As a result, part of the novelty of this research was not in the analyses themselves, but in the evaluation of many different soil health indices at different time periods after harvest.

1.7 Research Objectives

Primary Research Objective

To quantify the influence of anthropogenic activities (selection harvesting, clearcutting and organic residue removal) on selected physical, chemical and biological parameters in the soils at the Missouri Ozark Forest Ecosystem Project and Long-Term Soil Productivity sites, and to determine whether the influenced parameters referenced above change over time.

Hypotheses

1. Soil health indicators deteriorate over time with increasing levels of anthropogenic activity.

2. The soil parameters indicative of reduced soil health at the MOFEP site (i.e., 5 years after harvest) are not the same as the parameters indicative of reduced soil quality at the LTSP site (i.e., 25 years after harvest).

Secondary Objective

To enhance the understanding of soil nutrient dynamics within soil solution at the MOFEP site.

Specific Research Objectives

1. To quantify the influence of anthropogenic activities (selection harvesting versus clearcutting) on the nutrient pools in soil solution over time at the MOFEP site.
2. To determine if a “steady state” of soil nutrient levels (concentrations at a stable, sustainable level) will occur after harvest.
3. To determine the amount of time for the soil nutrient concentrations to reach this steady state.
4. To determine if the “steady state” of soil nutrient status is significantly above, at, or below initial pre-harvest concentration levels.

Hypothesis

Soil nutrient concentrations in solution initially rise post-harvest and then decline to a stable, sustainable level (i.e., steady state) at or near initial (pre-harvest) concentration levels over time.

Alternate Hypothesis

Soil nutrient concentration within soil solution initially rise post-harvest, and then decline to a stable level significantly below the initial (pre-harvest) concentration levels, thus indicating potentially permanent alteration to the soil nutrient levels as a result of harvest management.

1.8 Potential Benefits of this Research

The potential benefits of the research presented in this document align with three directions of inquiry. First, this research represents the culmination of over 10 years of soil geochemical research into nutrient cycling in the Missouri Ozarks. Examination of soil liquid phases at the throughfall and the 15 and 40 cm depths combined with soil solid phases at 10 cm intervals to a total depth of 40 cm over a period of 5 years post-harvest should provide unique insight into the changes in nutrient concentrations from the effects of clearcut and single-tree selection harvests in the Missouri Ozarks. Specifically, it is hoped that this research will illuminate whether harvest management effects on soil geochemical concentrations persist over time, thus permanently altering nutrient cycling over time or whether the nutrient concentrations return to pre-harvest levels prior to a subsequent harvest rotation. Second, through the synthesis of soil physical, chemical and biological properties it is intended to gain further insight into the changes in nutrient concentrations, and cycling and changes in microbial community structure triggered by forest management in the Missouri Ozarks. Finally, this research is intended to identify potential soil health indicators to be used in the creation of a forest soil health index. This index would be used to monitor changes in soil health wrought by forest management,

with the goal of providing a tool to forest managers to assess the level of impact of harvests on the soil environment.

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**CHAPTER 2: SOIL CHEMICAL RESPONSE TO CLEARCUT AND
SINGLE-TREE SELECTION HARVESTS IN THE MISSOURI
OZARKS**

A.W. Taylor^{a*}, K.W. Goyne^a, J.M. Kabrick^b,

^a School of Natural Resources, University of Missouri, 103 ABNR Bldg., Columbia, MO
65211, USA

^b USDA Forest Service, Northern Research Station, 203 ABNR Bldg., Columbia, MO
65211, USA

* *Corresponding author*: School of Natural Resources, University of Missouri, 103
ABNR Bldg., Columbia, MO 65211, USA; email: awtkvf@mail.missouri.edu (A.W.
Taylor)

Abstract

Forest harvest activities have been shown to affect nutrient cycling; however, the specific impact and duration of the impact vary with climate and forest composition. The objective of this study was to evaluate the effects of clearcut, single-tree selection and no harvest (control) treatments on soil solution and whole soil chemistry from pre-harvest to post-harvest in mixed oak-hickory (*Quercus spp.*-*Carya spp.*) forest growing in a temperate climate. To achieve our objective, sampling sites (n=18) were identified at the Missouri Ozark Forest Ecosystem Project (MOFEP) site in southern Missouri, U.S.A., prior to harvest. Sampling sites were instrumented with canopy throughfall samplers, standard rain gauges, and zero-tension soil solution samplers installed at depths of 15 and 40 cm. Bulk soil samples were collected within 20 m of the instrumentation on a yearly basis. Solution and soil samples were analyzed to determine nutrients concentrations and to monitor other soil chemical parameters from one year pre-harvest to five years post-harvest. Evidence for a large nutrient flush was observed in soil solution following clearcut harvest. In particular, concentrations of the following chemical constituents increased post-harvest and then declined with time: Ca^{2+} ; Mg^{2+} ; K^{+} ; NH_4^{+} ; NO_3^{-} ; PO_4^{3-} ; dissolved organic carbon (DOC); and total nitrogen (TN). Concentrations in solution increased between 2 and 15 months post-harvest and remained elevated for 17 to 31 months. A nutrient flush was not observed in single-tree selection and control treatments. At five years post-harvest, base cation concentrations in soil collected from clearcut treatment were either elevated above pre-harvest levels (Ca^{2+} and K^{+}) or unchanged (Mg^{2+} and Na^{+}). Concentrations of total sulfur and nitrogen within soil under clearcut treatments increased; however, Bray-1 phosphorous concentration were observed to

decrease. Single-tree selection harvest minimally affected soil chemical properties relative to unharvested control sites.

Highlights

- Clearcut forest harvesting increased nutrient concentrations in soil solution for up to 2.5 years.
- At five years post-harvest, clearcutting had no substantive effect or it increased nutrient concentrations in whole soil with exception for available phosphorus which declined in concentration post-treatment.
- Soil solution and whole soil sample chemistry within single-tree selection treatments were largely indistinguishable from control sites.
- Evidence of a significant PO_4^{3-} nutrient flush paired with lowered post-harvest Bray-1 phosphorus concentrations within clearcuts suggest that altered P-cycling may be a concern in the Missouri Ozarks.

2.1 Introduction

Nutrient removals from forested ecosystems via harvesting is largely undisputed. Grigal (2000) in his landmark paper on forest management effects and soil productivity considered this statement as axiomatic. Long-term, repeated harvesting activities can be considered unsustainable when the ratio of input to losses is less than 0.9, and if the stock of nutrients contained within live trees, the forest floor and the soil is insufficient for the next ten harvest rotations (Vangansbeke et al., 2015). Studies have shown that the long term effects of harvest on forest soils may be site-dependent, thus necessitating careful consideration of harvest methods on a case by case basis (Hartmann et al., 2012; Ponder et al., 2012; Vangansbeke et al., 2015). Studies have also shown that the various regeneration methods (even- and uneven-aged) and slash management (removal, piling, dispersal) have differing effects and site-dependent effects on nutrient status of soil (Achat et al., 2015; Johnson et al., 2016). This study was performed at the Missouri Ozark Forest Ecosystem Project (MOFEP) site in southern Missouri, USA. This long-term project administered by the Missouri Department of Conservation is intended to inform the development of best management practices to protect the sustainability of Missouri forests and woodlands by experimentally testing the long-term effects of forest management on the flora and fauna at landscape scales. It is estimated that over fourteen million acres of Missouri is forested, comprising nearly a third of the landcover. The harvesting and processing of timber is significant to the economy of Missouri, generating \$3.2 billion dollars in 2016 (“Missouri Forest Facts,”) making the long-term sustainability of forest harvest an important component of Missouri’s economic outlook.

2.1.1 Objectives

The overall objective of this study was to provide insight into changes in nutrient dynamics as a result of forest harvest activity. Soil solution and solid phase were testing for the following soil chemical properties (concentrations and related soil chemical properties):

- Soil solution: Al_T (total aluminum); Br^- ; Ca^{2+} ; Cl^- ; DOC (dissolved organic carbon); EC (electrical conductivity); F^- ; K^+ ; Mg^{2+} ; Na^+ ; NH_4^+ ; NO_2^- ; NO_3^- ; pH; PO_4^{3-} SO_4^{2-} ; and TN (total nitrogen).
- Soil solid phase: Al_T ; exchangeable Ca^{2+} , K^+ , Mg^{2+} and Na^+ ; pH (via KCl and water extraction); TN; TOC (total organic carbon); sum of bases; base saturation; and cation exchange capacity.

Four specific research objectives were proposed. Objective 1 was to identify whether there was an observable flush of nutrients post-harvest within soil solution. Objective 2 was to identify whether there was an observable nutrient flush on a seasonal basis, unrelated to forest harvest activities. Objective 3 was to examine the effect of forest canopy on throughfall samples. Finally, Objective 4 was to determine whether post nutrient flush values were above, at or below pre-harvest values in soil solution and solid phases.

Soil and soil solution samples were analyzed for nutrient concentrations and other geochemical properties beginning on April 2010 (approximately one year prior to harvest) and continued until the final solution sampling ended in July 2015 and the final soil sample collection in the spring of 2016.

2.2 Materials and Methods

2.2.1 Site Description

The Missouri Forest Ecosystem Project (MOFEP) is located in south-central Missouri, USA, within the counties of Shannon, Reynolds and Carter (37° 08'N, 91° 00'W). The MOFEP experiment was initiated in 1989 by the Missouri Department of Conservation as a long-term, landscape-scale study examining the effects of even-aged and uneven-aged forest management methods, applied in an operational manner, on the flora and fauna of the Ozark Highlands. The experimental site is located within the Current River Oak-Pine Woodland Hills and Current River Forest Breaks land type associations of the Ozark Highlands (Shifley and Kabrick, 2002). Soils at the MOFEP site are primarily derived from parent materials of Ordovician-aged Roubidoux and Gasconade formations and the Cambrian-aged Eminence formation. These marine sedimentary rocks are primarily composed of chert, dolomite and quartz sandstones and they strongly influence the physical and chemical properties of the overlying soils. Soils in the region range from highly weathered very gravelly pedisements classified as Ultisols (Typic Paleudult and Typic Hapludult) to gravel-free clayey pedisements and residuum classified as Alfisols (Typic Paleudalf and Typic Hapludalf) (Kabrick et al., 2008).

Figure 2.1. Missouri Ozark Forest Ecosystem Project (MOFEP) study sites and treatments (Sork et al., 2005).

The Current River Oak-Pine Woodland Hills have broad ridges and less than 90 m of relief. In contrast, the Current River Oak Forest Breaks have narrow ridges and steep relief that ranges from 90 to 140 m (Meinert et al., Kabrick et al., 2000). The forests at MOFEP are characterized by mixed hardwood and hardwood-softwood forests. Four oak species predominate the area making up 71% of the basal area: white oak (*Quercus alba*); black oak (*Quercus velutina*); scarlet oak (*Quercus coccinea*); and post oak

(*Quercus stellata*). Shortleaf Pine (*Pinus echinata*), various species of hickory (*Carya spp.*), flowering dogwood (*Cornus florida*), blackgum (*Nyssa sylvatica*) and various other oaks (*Quercus spp.*) comprise the remainder of tree species in the forest (Kabrick et al., 2008).]

2.2.2 Missouri Ozark Forest Ecosystem Project

The MOFEP site is a randomized complete block design encompassing nine compartments ranging in size from 314 to 516 hectares (Figure 2.1). Each of the three blocks comprise three compartments assigned by their spatial proximity. In each block, three forest management treatments were randomly assigned to a compartment: no-harvest (control); uneven-aged management (with group or single-tree selection harvest); and even-aged management (with clearcutting). Approximately 10 percent of each site is left as old growth. The old growth reserve will be preserved from harvest activities on a permanent basis. Harvest entries in compartments occur at 10 to 15 year intervals. Even-aged and uneven-aged management was practiced according to MDC Forest Land Management Guidelines. Even-aged management consisted of a rotation of 80-100 years resulting in a regulated harvest of 10-12.5% of the area per entry period (Shifley and Kabrick, 2002). Nearly all non-merchantable timber was felled during a slash operation after the removal of merchantable boles at clearcut sites (Sheriff, 2002). Some snag or den trees were preserved to provide shelter for wildlife. Additionally, some shortleaf pine was preserved to provide seed for pine regeneration. Uneven-aged management utilized single-tree and group selection harvest, with a target tree size class distribution equivalent to the composite size class distribution across the even-aged management sites (Shifley and Kabrick, 2002).

2.2.4 Experimental Design

A detailed soil survey that was previously conducted at the MOFEP study sites (Meinert et al., 1997) provided a means for examining harvested stands having different levels of soil nutrient availability. Prior to the planned second harvest entry in 2011, Geographic Information System (GIS) data layers were used to identify stands in the nine forest compartments that contained low and medium nutrient status soils and were scheduled to be harvested. Nutrient status was determined on the basis of base cation saturation (BS) in their taxonomic diagnostic subsurface horizon. Low (<20% BS) nutrient status soils are primarily found on the upper backslope landscape position and formed from weathered hillslope sediments; the Clarksville soil series is often representative of these conditions. Clarksville soil (loamy-skeletal, siliceous, semiactive, mesic Typic Paleudults) is very deep and somewhat excessively well-drained (Simmons et al., 2006). In contrast, medium (20-50% BS) nutrient status soils are usually found on the lower backslope landscape position, and they are formed in weathered hillslope sediments over clayey residuum. The Alred soil series (loamy-skeletal over clayey, siliceous semiactive, mesic Typic Paleudalfs) is very deep, well-drained, and typifies medium nutrient status soils of the study area (Simmons et al., 2006).

After the identification of suitable forest stands, professional foresters and soil scientists evaluated candidate sites to determine if the forest and soils were representative of the area. Subsequently, two long-term monitoring sites were established within each of the nine compartments on low and medium nutrient status soils (18 sampling sites total). Each of the eighteen sites were excavated to a depth of 1m either by manual excavation or backhoe. Care was taken to ensure that the sidewalls, upslope and lateral positions to

the pit remained undisturbed. Excavated soil was piled in the downslope position. Pre-harvest samples were taken in 10 cm increments and analyzed to confirm nutrient status.

2.2.5 Solution Sampling Network

In 2010, a network of solution samplers was installed at the Missouri Ozark Forest Ecosystem Project study site prior to a scheduled forest harvest in 2011. Zero Tension samplers (ZTS) were chosen over other collection methods due to the tendency of others samplers to introduce chemical artifacts (Goyne et al., 2000). Although, ZTS primarily capture solution transiting via macropore flow, effectively bypassing solution and nutrients residing in micropores within the soil architecture (Goyne et al., 2000), the ease of construction, installation and usage associated with ZTS outweighed any potential benefits of other sampler types.

The ZTS units were constructed from high density polyethylene HDPE plastic with a sampling cross-sectional area of 0.086 m². Each sampler was acid washed and then filled with acid-washed sand, prior to installation at depths of 15 and 40 cm. Cavities were excavated in the side of each pit at appropriate depths, and samplers were placed in hydraulic contact with the upper surface of the excavation using wedges made of pine blocks and shims. Each sampler was connected to a 25 liter HDPE bottle using a nylon-braided PVC hose, from which soil solution samples could be collected. A trough-style throughfall sampler, consisting of a longitudinally sectioned 10 cm PVC pipe of 1 meter length, was installed to catch throughfall and a rain gauge was also included to measure rainfall in the intervening periods between collections. Two sampling sites were installed per MOFEP compartment – one soil considered to be a low nutrient status (Clarksville or similar soils) and the other within medium nutrient status soil (Alred or similar soils) -

resulting in a total of 36 ZTS units and 18 throughfall samplers. A schematic of the solution samplers is shown in Figure 2.2.

Figure 2.2 Schematic of solution sampling site including throughfall collector, standard rain gauge, and zero tension samplers and Ion Exchange Resin emplacement (after Singh, 2013)

2.2.6 Solution Sampling

Solution sampling began late March 2010, prior to harvests scheduled for the 2011 calendar year (Gaddie, 2012). The sample collection continued post-harvest until a break from March to September 2013 (Singh, 2013). Sampling resumed in September 2013 and concluded in July 2015.

After a sufficient rainfall event (~2.5 cm in a single event, or 4.0 cm cumulative rainfall within 24 hours) samples were collected at each of the 18 solution sampler sites. From each throughfall or soil solution collection bottle, up to 1 L of solution was

collected and stored in an acid-washed 1L HPDE bottle. In cases where throughfall samplers were compromised (e.g., clogged, frozen, or damaged), throughfall samples were collected from an adjacent standard, plastic rain gauge. All samples were labeled in the field and transferred to a cooler with ice for storage and transport to the laboratory. Samples were stored at 4° C for no more than 24 hours prior to filtration, and they were vacuum-filtered through a 0.45µm MF-Millipore™ membrane filter (MilliporeSigma, Burlington, MA). Each filtrate sample was subdivided into three subsamples and stored in labeled acid-washed 60ml HDPE bottles. The three subsamples included: unacidified solution (pH and anion analysis); acidified with HNO₃ to pH 2 (cation analysis); or acidified with H₃PO₄ or H₂SO₄ to pH 2 (dissolved organic carbon and total nitrogen analysis). Samples were stored at 4°C until analyzed.

2.2.7 Soil Sampling

Soil samples were collected within 20 m of solution sampling locations before (2011) and after harvest (2013, 2015 and 2016). Triplicate subsamples were collected at each of the 18 sampling sites in 10 cm increments from a depth of 0 to 40 cm. The subsamples were air-dried and sieved to less than 2 mm. The subsamples were then composited for subsequent analysis.

2.2.8 Solution and Soil Analyses

Approximately 100ml of unfiltered solution was divided into 50 ml portions to determine pH and electrical conductivity (EC) after solution collection. The portions were placed into two separate 50ml glass beakers for analysis and were rinsed thoroughly with de-ionized water between samples. Solution pH was measured with a FisherBrand

accumet™ XL600 portable pH meter (ThermoFisher Scientific, Waltham, MA) equipped with an accumet Probe model # 13-620-183A. Electrical conductivity was determined using a FisherBrand accumet™ AP75 portable conductivity meter.

Unacidified filtrate was analyzed to measure concentrations of NH_4^+ and anions (Br^- ; Cl^- ; F^- ; NO_2^- ; NO_3^- ; SO_4^{2-} PO_4^{3-}). Ammonium concentration was determined using a Lachat Quikchem Flow Injection Analysis System and colorimetric method 10-107-06-1-K (15 March 2001 revision; Lachat Instruments; Hach Co., Loveland, CO). Anion concentrations were quantified using a Dionex ICS-1000 ion chromatography system equipped with an IonPac™ AG14A 4mm guard column (4x50mm) and an IonPac™ AS14A analytical column (4x250mm) (Dionex Corporation, Sunnydale, CA). Dionex AS14A eluent (8.0M sodium carbonate and 1.0M sodium bicarbonate in deionized water) with a flow rate of 1.0 ml min^{-1} was used as the mobile phase for all anion analyses.

Solution samples acidified to pH 2.0 with HNO_3 were analyzed for the following cation concentrations using an inductively coupled plasma (ICP) atomic emission spectrophotometer (AES) (Varian Liberty RL, Australia): Al^+ ; Ca^{2+} ; Mg^{2+} ; K^+ ; and Na^+ . Sodium concentration in solutions collected after January 2015 were quantified with a Thermo Scientific iCE 3000 series flame absorption spectrometer (Thermo Fisher Scientific, Waltham, MA).

Solution samples acidified to pH 2.0 with either H_3PO_4 or H_2SO_4 were analyzed for non-purgeable organic carbon [hereafter referred to as dissolved organic carbon (DOC)] and total nitrogen (TN) on a Shimadzu TOC-V™ liquid carbon analyzer equipped with a TNM-1™ nitrogen analyzer, ASI autosampler and sparging needle

(Shimadzu Corporation, Kyoto, Japan). Analysis of TN ceased in November 2013.

Detection limits for the analytes can be seen in Supplemental Materials.

Soil analyses were performed using procedures outlined in Burt (2004) for the following properties: pH was measured in water and a 0.01M CaCl₂ slurry (1:1 and 1:2 w/v, respectively); exchangeable concentrations of Ca²⁺, K⁺, Mg²⁺ and Na⁺ (NH₄Cl extraction technique); cation exchange capacity by the summation of BaCl₂-triethanolamine released extractable acidity and NH₄Cl exchangeable bases; extractable Al (1M KCL extract with ICP-AES analysis); total organic carbon, total nitrogen and total sulfur by the dry combustion method and Bray-1 extractable phosphorus. TOC and TN were measured using a LECO TruMac CN combustion analyzer model 630-300-400 and total sulfur content was quantified using a LECO Sulfur-Carbon analyzer model SC-144DR (LECO Corporation; St. Joseph, MI).

2.2.9 Data Analysis

Because MOFEP is an operational experimental forest and of considerable size, harvesting occurred over a period of one year. This extended harvest period resulted in sites being treated at different times and, consequently, a unique approach was required to analyze changes in soil solution through time. To facilitate data analysis, soil solution samples from each sampling location were split into discrete phases over time: pre-harvest; lag; nutrient flush; and post-flush. These phases align with widely-published nutrient dynamics observed post-harvest in previous studies (Jerabkova et al., 2011; Knoepp et al., 2014; Achat et al., 2015), with the exception of the lag phase. We could find little evidence in the literature of research into the period between harvest and the onset of the nutrient flush. We used NO₃⁻ relative concentrations in clear-cut sites at the

15 cm solution sampling depth as a reference for assigning phases to the data set (Figure 2.3). Nitrate was used as the reference ion due to its high mobility in soil solution and large magnitude of change post-harvest. Descriptions of each phase is as follows:

- Pre-harvest phase: First to the last solution sample collected pre-harvest.
- Lag phase: First sample collected post-harvest to the last sample collected before NO_3^- increased above pre-harvest background concentration.
- Flush phase: First sample with NO_3^- concentration greater than pre-harvest background values to return to steady state (described below).
- Post-Flush: Post-harvest samples containing NO_3^- concentrations with relatively low

variance and values comparable to pre-harvest values.

Figure 2.3. Plot illustrating nitrate (NO_3^-) dynamics in soil solution pre- and post-harvest, and the assignment of different phases used to evaluate the data.

Graphical plots of other anions and cations collected at individual sites were examined to ensure the onset of elevated concentrations (if observed) had timing similar to NO_3^- ; no appreciable differences were noted. The timing of phases for single-tree selection sites was chosen using clearcut sites of the same nutrient status that were harvested near the same date. Preliminary data exploration indicated that the onset of flush appeared to be related to the date of harvest (e.g., a late season harvest may delay the onset of flush until the next growing season). The timing of the phases for no-harvest control plots was identified using the clearcut site which was physically the closest and with the same soil nutrient status.

Capturing non-anthropogenic seasonal variation in nutrient concentrations unrelated to harvest was critical to understanding post-harvest changes directly related to harvest activities. Therefore, the timing determined from analysis of the clear-cut plots was applied to the no-harvest control plots. It was theorized that the flush timing would occur at the same seasonal intervals, regardless of the source – (be it natural or harvest-derived); however, the magnitude of the nutrient concentrations would differ by the source of the nutrients (i.e., higher concentrations for harvest-derived). Example comparisons of the timing for various phases and analytes can be observed in the *Supplemental Materials*.

2.2.10 Statistical Analysis

A two-way analysis of variance (ANOVA) using a linear mixed model (Proc Mixed) in SAS 9.4 (SAS Institute; Cary, NC) was used to evaluate effects of treatment (clearcut, single tree selection, and control), nutrient status (low and medium), phase (pre-harvest,

lag, flush and post-flush) and depth (throughfall, 15cm and 40 cm depth intervals) on solution and solid phase chemistry.

The experimental design of MOFEP allows for the examination of treatments, soil nutrient status, and their interactions by analyzing the data using a statistical model for a split-plot analysis with different error terms (see Brookshire and Shifley, 1997). To test for treatment effects (i.e, the whole plot effects), the appropriate error term is the treatment x block interaction. To test for effects of soil nutrient status (i.e., the split plot effects) and its interaction with treatments, the three-way interaction of treatment x soil nutrient status x block is used.

The data were tested for normality using the Shapiro-Wilk test. Those data which did not pass the test for normality were transformed using a square root transformation. Least squares differences were computed to determine marginally significant differences ($p < 0.10$) and significant differences ($p < 0.05$ and $p < 0.01$) between treatments, nutrient status, phase and depth.

Several independent statistical analyses were performed to answer specific questions. For each question, a limited subset of data and independent variables were used to simplify analysis and eliminate data not germane to a specific objective. An outline of the method used to answer each question posed previously is as follows:

- Question 1 (Observable Harvest Flush): Independent variables used were treatment, nutrient status and phase (using only the pre-harvest and flush phases). Each depth interval was analyzed independently to simplify analysis.

- Question 2: (Seasonal Flush) Independent variables used were nutrient status and season. Analysis was only performed on the no-harvest control samples.
- Question 3: (Depth Effect) Independent variables used were treatment, nutrient status and depth. Only the flush phase data for dependent variables found to have experienced a flush were analyzed.
- Question 4: (Post-flush Concentrations) Independent variables used were treatment, nutrient status and phase (only pre-harvest and post-flush). Each depth was analyzed independently to simplify analysis.

2.3 Results

2.3.1 Soil Solution Seasonal Nutrient Flush

Analysis of nutrient concentrations within solution on the control sites indicated significant ($p < 0.01$) seasonal changes in concentration for the following solutes collected at the 15 cm depth (Table 2.1): Al_T ; Br^- ; Cl^- ; NO_3^- ; SO_4^{2-} . The timing of the concentration maxima varied by each analyte measured. There was a marginally significant change ($p < 0.1$) in TN. At the 40 cm depth interval, there were significant ($p < 0.05$) seasonal changes for the following variables: Cl^- ; F^- ; pH; SO_4^{2-} ; and TN. There was marginally significant ($p < 0.1$) seasonal changes in K^+ concentration. Timing of the seasonal flush for the analytes were as follows: summer for Al_T and NO_3^- ; fall for Br^- , F^- and TN; and winter for SO_4^{2-} , Cl^- and K^+ .

Table 2.1 Type III test of seasonal variation and mean values of soil solution analytes at control sites. Significant differences between seasons (SPR) spring, (SUM) summer, (FALL) fall, and (WIN) winter are indicated by * $p < 0.10$ (marginal significance), **

p<0.05 and *** p<0.01. Significance values at p<0.05 are in bold. Means within a row followed by different letters are significantly different at the p<0.10 level.

<u>Variable</u>	<u>Depth</u>	<u>Type 3 Test</u>	<u>SPR</u>	<u>Season</u>		
				<u>SUM</u>	<u>FALL</u>	<u>WIN</u>
Al _T	15	0.0016***	59.6a	115.0b	48.7ac	32.6c
(μmol L ⁻¹)	40	0.8662	54.0a	70.5a	49.0a	49.7a
Br ⁻	15	0.0002***	2.40a	bd	8.20b	2.90a
(μmol L ⁻¹)	40	0.4943	2.90a	1.70a	3.20a	bd
Ca ²⁺	15	0.3784	55.4a	67.0a	5.19a	56.9a
(μmol L ⁻¹)	40	0.6832	81.4a	86.0a	63.7a	89.2a
Cl ⁻	15	0.0205**	24.8a	33.3ab	40.0b	44.5b
(μmol L ⁻¹)	40	0.028**	28.9a	26.8ab	42.5ab	70.1b
DOC	15	0.2663	1390.0a	1410.0a	1680.0a	1500.0a
(μmol L ⁻¹)	40	0.3849	1290.0a	2220.0a	1320.0a	1600.0a
EC	15	0.2344	54.6a	55.1ab	63.4b	58.1ab
(μS cm ⁻¹)	40	0.5691	69.2a	69.9a	79.8a	82.1a
F ⁻	15	0.3286	3.19a	4.34a	3.60a	3.18a
(μmol L ⁻¹)	40	0.0036***	3.50a	3.90ab	6.10c	4.40b
K ⁺	15	0.7992	64.2a	70.9a	64.9a	87.0a
(μmol L ⁻¹)	40	0.0505*	118.0a	62.5ab	53.2a	226.0b
Mg ²⁺	15	0.2682	43.5a	54.2a	39.7a	46.0a
(μmol L ⁻¹)	40	0.7483	72.7a	71.0a	52.9a	75.6a
Na ⁺	15	0.5963	27.1a	28.9a	35.5a	27.0a
(μmol L ⁻¹)	40	0.6005	23.9a	21.3a	31.4a	26.2a
NH ₄ ⁺	15	0.1013	4.52a	45.7b	16.5a	15.0a
(μmol L ⁻¹)	40	0.5189	21.6a	75.3a	27.9a	36.4a
NO ₂ ⁻	15	0.6653	4.60a	11.4a	5.50a	15.7a
(μmol L ⁻¹)	40	0.1451	6.60a	6.5ab	7.50a	5.00b
NO ₃ ⁻	15	0.0108**	10.7a	24.2b	22.7b	11.0a
(μmol L ⁻¹)	40	0.1183	10.9a	83.3b	29.8ab	39.6b
pH	15	0.1349	5.85a	5.84ab	5.70b	5.79ab
	40	0.0001***	6.00a	5.9ab	5.40c	5.80b
PO ₄ ³⁻	15	0.6022	7.88a	11.7a	3.45a	9.63a
(μmol L ⁻¹)	40	0.396	5.80ab	9.60a	3.80b	6.50ab
SO ₄ ²⁻	15	<.0001***	30.7a	22.6b	24.8b	33.6a
(μmol L ⁻¹)	40	0.0036***	38.2ab	29.9b	46.6bc	47.9c
TN	15	0.0887*	27.8a	77.6ab	98.3b	48.5ab
(μmol L ⁻¹)	40	0.0337**	47.9a	269.0b	94.1a	105.0a

Key to abbreviations: DOC – dissolved organic carbon; EC – electrical conductivity; bd indicates the values were below the limits of detectability of the instrument.

2.3.2 Soil Solution Nutrient Flush due to Harvest

Analysis of the soil solution sampling in the treatments during the flush phase allowed us to quantify the timing and magnitude of these flushes. Timing of the flush period in our experiment varied across the replicated clearcut treatments. In the first clear-cut compartment (site 3, see Figure 2.1), low and medium nutrient status soils experienced a 14 month “lag” in onset of increased nutrient concentrations post-harvest (between October 2011 harvest and onset of nutrient flush in December 2012). Whereas, in the second compartment (Site 5), the low nutrient status site experienced a 5 month lag (harvested in May 2011 and flush starting in October 2011); whereas, low nutrient status site exhibited a 4 month lag (harvested in October 2011 and an onset of flush in February 2012). In the third compartment (Site 9), the low and medium nutrient status sites were harvested simultaneously in August 2011 and were exhibiting their flush two months later in October. As mentioned earlier, the end of the flush period was identified as when the concentration values had returned to a “steady state” (near a 0 slope). The flush period was observed to last anywhere from 17 to 31 months. The shortest flush was observed in the third compartment – also the site of the shortest lag period. This potentially indicates that a summer harvest (August), rather than late fall, allowed for more timely breakdown of harvest residues. By November 2013, all replicates had nutrient concentrations reduced to a lesser concentration, relative to their flush phase values (Figure 2.4).

Figure 2.4 Plot of NO_3^- concentration over time illustrating the onset and cessation of the flush phase. Vertical line indicates first sample date with reduced post-harvest concentrations.

The following solutes in soil solution collected at the 15 cm depth within clearcuts were significantly greater ($p < 0.05$) during the flush phase relative to pre-harvest phase (Table 2.2): Ca^{2+} (130%); K^+ (162%); Mg^{2+} (195%); NH_4^+ (3024%); NO_3^- (2475%); DOC (47%); PO_4^{3-} (274%); and TN (1826%). Electrical conductivity ($p < 0.01$; 139%) and pH ($p < 0.01$; -3.3%) also changed significantly. Marginally significant changes were noted for the concentration of Al_T (92%). At the 40 cm depth within clearcut treatments, significant increases ($p < 0.05$) in soil solution analyte concentrations were observed during the flush phase relative to pre-harvest for the following solutes: Br^- (141%); Ca^{2+} (92%); F^- (84%); K^+ (118%); Mg^{2+} (178%); NO_3^- (1692%); SO_4^{2-} (-40%);

and TN (540%). Consequent to greater ions in solution, electrical conductivity also increased significantly (122%).

In contrast to clearcut sites, soil solution chemistry was minimally impacted by single tree selection harvest. At the 15 cm depth level, the following variables changed significantly in soil solution mean concentrations during the flush phase relative to pre-harvest within single-tree selection treatments: F^- (85%); TN (926%) and pH (-4%). At the 40cm depth level, significant changes in mean concentrations in soil solution during the flush phase relative to pre-harvest within single tree selection treatments were found for the following variables: Al_T (100%); NO_2^- (148%); and pH (-4%). Concentrations of PO_4^{3-} were marginally significantly changed (-42%).

Within control treatments, there were changes in concentrations between pre-harvest and flush period were seen at the 15cm depth level for Al_T (164%). At the 40 cm depth level, there were changes in mean concentrations between pre-harvest and flush phase for the following variables: Al_T (215%); K^+ (147%); Mg^{2+} (65%); PO_4^{3-} (-51%); and pH (-4%).

Table 2.2 Differences in mean values for soil chemical properties in soil solution between pre-harvest and flush phase for the clearcut (CC), single tree selection (SEL) and control (CON) treatments. Significant differences between pre-harvest and flush phases within a treatment are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$. Means within a row followed by different letters are significantly different within a treatment at the $p < 0.10$ level.

Variable	Depth (cm)	CON		SEL		CC	
		Pre-Harvest	Flush	Pre-Harvest	Flush	Pre-Harvest	Flush
Al _T ($\mu\text{mol L}^{-1}$)	15	21.6a	56.9b***	26.6a	43.5a	17.3a	33.2b*
	40	18.7a	59.0b***	20.2a	40.3b***	8.40a	17.1a
Br ⁻ ($\mu\text{mol L}^{-1}$)	15	1.60a	3.50a	4.70a	3.80a	2.07a	4.70a
	40	2.10a	2.60a	1.10a	3.20a	1.50a	3.70b**
Ca ²⁺ ($\mu\text{mol L}^{-1}$)	15	58.3a	54.6a	52.5a	66.0a	60.1a	138.0b***
	40	65.0a	86.9a	43.0a	30.2a	63.0a	120.0b***
Cl ⁻ ($\mu\text{mol L}^{-1}$)	15	28.7a	42.0a	38.7a	45.2a	92.5a	132.0a
	40	30.3a	53.7a	30.6a	86.7a	60a*	83.1b
DOC ($\mu\text{mol L}^{-1}$)	15	1480a	1650a	1230a	1340a	1120a	1640b***
	40	1380a	1360a	886.0a	839.0a	563.0a	574.0b
EC ($\mu\text{S cm}^{-1}$)	15	67.5a	61.6a	71.7a	92.4a	92.3a	220.0b***
	40	75.5a	89.9a	61.6a	64.0a	79.7a	177.0b***
F ⁻ ($\mu\text{mol L}^{-1}$)	15	2.80a	3.10a	3.90a	7.20b***	4.70a	4.00a
	40	2.50a	4.30a	2.80a	3.40a	2.30a	4.20b**
K ⁺ ($\mu\text{mol L}^{-1}$)	15	74.4a	79.1a	81.7a	105.0a	110.0a	288.0b***
	40	87.3a	216.0b***	62.4a	102.0a	95.9a	209.0b***
Mg ²⁺ ($\mu\text{mol L}^{-1}$)	15	46.1a	39.9a	47.8a	61.6a	50.7a	150.0b***
	40	52.0a	85.9b**	38.5a	28.3a	59.5a	165.0b***
Na ⁺ ($\mu\text{mol L}^{-1}$)	15	34.0a	32.5a	46.1a	41.8a	71.7a	78.6a
	40	33.6a	29.5a	38.6a	40.8a	41.3a	54.0a
NH ₄ ⁺ ($\mu\text{mol L}^{-1}$)	15	18.6a	15.7a	24.1a	36.7a	17.3a	540.0b***
	40	21.1a	20.8a	19.8a	21.2a	13.9a	13.5a
NO ₂ ⁻ ($\mu\text{mol L}^{-1}$)	15	5.50a	5.40a	4.00a	8.50a	3.80a*	7.90b
	40	3.40a	5.70a	2.40a	6.10b**	3.30a	8.50b***
NO ₃ ⁻ ($\mu\text{mol L}^{-1}$)	15	13.3a	18.4a	13.6a	48.0a	18.0a	463.0b***
	40	20.1a	25.9a	8.40a	20.3a	24.3a	435.0b***
pH	15	5.90a	5.80b*	5.90a	5.60b**	6.10a	5.9b***
	40	6.0a	5.80b***	5.90a	5.70b***	6.20a	6.00a
PO ₄ ³⁻	15	7.10a	10.6a	7.30a	9.60a	8.20a	30.6b***

($\mu\text{mol L}^{-1}$)	40	9.10a	4.50a**	7.30a	4.30b*	9.20a	6.80a
SO_4^{2-}	15	30.5a	30.8a	30.1a	31.6a	33.5a	33.5a
($\mu\text{mol L}^{-1}$)	40	41.9a	46.3a	42.1a	45.5a	44.2a	26.3b***
TN	15	44.9a	74.1a	55.8a	573.0b**	48.1a	925.0b***
($\mu\text{mol L}^{-1}$)	40	61.5a	102.5a	30.8a	134.1a	76.8a	491.0b***

Key to abbreviations: DOC – dissolved organic carbon; EC – electrical conductivity; TN – Total Nitrogen

2.3.3 Canopy Effects on Solution Chemistry

Analysis of throughfall data was performed to better understand the effect that the forest canopy was having on soil solution concentration. Comparison of throughfall solution chemistry pre- and post-harvest within clearcut treatments yielded the following significant ($p < 0.05$) changes (Table 2.3): Al_T (397%); Ca^{2+} (-47%); EC (-52%); K^+ (-51%); Mg^{2+} (-76%); DOC (-84%); pH (p-8.4%); PO_4^{3-} (-45%); SO_4^{2-} (-43%); and TN (-94%). Within single-tree selection harvests, there were significant changes in throughfall sampler chemistry post-harvest, as compared to pre-harvest for: Al_T (186%); Ca^{2+} (-31%); K^+ (60%); Mg^{2+} (-41%); NO_2^- (71%); DOC (-54%); pH (-3.8%); PO_4^{3-} (-34%); SO_4^{2-} (-26%); and TN (-103%). Within the control treatment, there were significant changes between pre- and post-harvest solution chemistry for: Al_T (312%); Ca^{2+} (-15%); K^+ (69%); Mg^{2+} (-19%); NO_2^- (32%); NO_3^- (27%); DOC (-29%); pH (-3.6%); and SO_4^{2-} (-21%). There were also marginally significant throughfall differences pre- and postharvest chemistry for EC (-23%) and F^- (36%).

Table 2.3. Differences in mean values for throughfall solution sampler chemical properties between pre-harvest and post-harvest after imposing clearcut (CC), single tree selection (SEL) and control (CON) treatments. Significant differences between pre-harvest and post-harvest within a treatment are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$. Means within a row followed by different letters are significantly different at the $p < 0.10$ level within each treatment. Values in bold are significantly different between treatment at $p < 0.05$.

<u>Variables</u>	CON		SEL		CC	
	Pre-Harvest	Post-Harvest	Pre-Harvest	Post-Harvest	Pre-Harvest	Post-Harvest
Al ³⁺ ($\mu\text{mol L}^{-1}$)	0.67a	2.75b**	0.98a	2.80b**	0.70a	3.48b***
Br ⁻ ($\mu\text{mol L}^{-1}$)	2.90a	3.63a	3.68a	3.80a	2.62a	3.90a
Ca ²⁺ ($\mu\text{mol L}^{-1}$)	37.3a	31.7b**	38.1a	26.1b***	47.0a	24.9b***
Cl ⁻ ($\mu\text{mol L}^{-1}$)	45.1a	29.2a	30.0a	34.0a	43.5a	43.4a
DOC ($\mu\text{mol L}^{-1}$)	1140.0a	804.0b**	976.0a	449.0b***	1530.0a	239.0b***
EC ($\mu\text{S cm}^{-1}$)	51.9a	40.0b*	56.6a	47.1a	69.1a	33.4b***
F ⁻ ($\mu\text{mol L}^{-1}$)	1.74a	2.37b*	1.60a	2.17a	2.54a	2.17a
K ⁺ ($\mu\text{mol L}^{-1}$)	39.8a	67.0b***	36.6a	58.7b**	53.3a	26.2b***
Mg ²⁺ ($\mu\text{mol L}^{-1}$)	18.7a	15.0b**	18.4a	11.0b***	25.6a	6.02b***
Na ⁺ ($\mu\text{mol L}^{-1}$)	11.6a	8.64a	10.5a	7.30a	12.7a	9.65a
NH ₄ ⁺ ($\mu\text{mol L}^{-1}$)	27.1a	29.1a	29.9a	22.3b*	27.1a	25.3a
NO ₂ ⁻ ($\mu\text{mol L}^{-1}$)	5.21a	6.91b**	3.58a	6.10b***	6.14a	6.32a
NO ₃ ⁻ ($\mu\text{mol L}^{-1}$)	26.7a	33.7b***	31.0a	31.7a	29.9a	28.7a
pH	5.75a	5.54b***	5.65a	5.44b***	5.69a	5.21b***
PO ₄ ³⁻ ($\mu\text{mol L}^{-1}$)	7.26a	6.22a	8.20a	5.42b**	8.67a	4.79b***
SO ₄ ²⁻ ($\mu\text{mol L}^{-1}$)	19.2a	15.0b***	20.8a	15.3b***	24.4a	14.0b***
TN ($\mu\text{mol L}^{-1}$)	62.4a	69.7a	72.0a	35.5b***	71.7a	36.9b**

Key to abbreviations: DOC – dissolved organic carbon; EC – electrical conductivity; TN – Total Nitrogen

2.3.4 Post-Flush Soil Solution Nutrient Concentrations

Soil solution concentration of nutrients after the flush period has ended allowed us to determine the potential for lasting effects on soil concentrations. Post-flush clearcut concentrations of a nutrient above pre-harvest levels may indicate a short-term increase of the potential of leaching or mobilization of nutrient into lower soil horizons. Within our study, particular solutes in soil solution collected post-flush and at a depth of 15 cm

within clearcut treatments were significantly different ($p < 0.05$) compared to pre-harvest concentrations (Table 2.5): Ca^{2+} (-36%); EC (-53%); Mg^{2+} (-37%); Na^+ (-78%); NO_3^- (326%); DOC (-60%); and pH (-6%). Soil solution concentrations were marginally significantly changed for Cl^- (-71%) and K^+ (-49%). At the 40 cm depth within clear-cut treatments, post-flush soil solution concentrations were significantly different ($p < 0.05$) for EC (-21%), F^- (73%), Na^+ (-64%) and pH (-7%) when compared to pre-harvest values. Post-flush concentrations of Cl^- (-61%) and SO_4^{2-} (-10%) were marginally significantly ($p < 0.1$) decreased relative to pre-flush values.

Within single tree selection harvest treatments at the 15cm depth interval, the following variables were significantly changed in post-flush as compared to pre-harvest values: Ca^{2+} (-35%); EC (-42%); Mg^{2+} (-23%); Na^+ (-64%); pH (-7%); and SO_4^{2-} (-15%). The concentration of DOC in solution was marginally significantly ($p < 0.1$) changed (-29%). At the 40 cm depth interval, single tree selection harvest treatments appeared to significantly change the following soil solution properties: Al_T (361%); F^- (46%); K^+ (91%); Na^+ (-23%); NO_2^- (298%); and pH (-4%). The concentration of PO_4^{3-} was marginally significantly increased (74%).

Within control harvest treatments at the 15 cm depth interval, the following variables were significantly changed post-flush as compared to pre-harvest values: Al_T (430%); EC (-36%); Mg^{2+} (25%); Na^+ (-41%); and DOC (24%). Concentrations of Ca^{2+} (16%); and F^- (51%) were marginally significantly changed in post-harvest control soil solution relative to pre-harvest. At the 40 cm depth interval, single tree selection harvest treatments appeared to significantly affect the following soil solution properties: Al_T

(301%); F⁻ (92%); Mg²⁺ (22%); Na⁺ (-49%); NH₄ (481%); DOC (62%); pH (-6%) and SO₄²⁻ (-15%).

Table 2.4 Differences between mean values of analytes measured in soil solution samples collected during pre-harvest and post-flush phases within clearcut (CC), single tree selection (SEL) and control (CON) treatments. Significant differences between pre-harvest and flush phases are indicated by * p<0.10 (marginal significance), ** p<0.05 and *** p<0.01. Means within a row followed by different letters are significantly different at the p<0.10 level.

<u>Variable</u>	<u>Depth</u> <u>(cm)</u>	<u>CON</u>		<u>SEL</u>		<u>CC</u>	
		<u>Pre-harvest</u>	<u>Post-flush</u>	<u>Pre-harvest</u>	<u>Post-flush</u>	<u>Pre-harvest</u>	<u>Post-flush</u>
Al _T	15	21.6a	114.0b***	26.6a	54.7a	17.3a	20.5a
(μmol L ⁻¹)	40	18.7a	75.1b***	20.2a	93.0b***	8.40a	21.7a
Br ⁻	15	4.70a	5.80a	1.60a	3.90a	2.30a	3.50a
(μmol L ⁻¹)	40	2.1a	3.00a	1.10a	4.00a	1.50a	3.20a
Ca ²⁺	15	58.3a	67.7b*	52.5a	34.2b***	60.1a	38.6b***
(μmol L ⁻¹)	40	65.0a	95.9a	43.0a	34.8a	63.0a	60.4a
Cl ⁻	15	28.7a	34.3a	38.7a	49.2a	92.5a	26.8b*
(μmol L ⁻¹)	40	30.3a	52.7a	30.6a	38.8a	60.0a	23.2b*
DOC	15	1480.0a	1840.0b**	1230.0a	875.0b*	1120.0a	447.0b***
(μmol L ⁻¹)	40	1380.0a	2240.0b***	886.0a	809.0a	563.0a	416.5a
EC	15	67.5a	43.4b***	71.7a	41.8b***	92.3a	43.0b***
(μS cm ⁻¹)	40	75.5a	61.8a	61.6a	58.7a	79.7a	63.0b**
F ⁻	15	2.80a	4.30b*	3.90a	4.00a	4.70a	3.90a
(μmol L ⁻¹)	40	2.50a	4.70b***	2.80a	4.10b***	2.30a	4.00b***
K ⁺	15	74.4a	50.2a	81.7a	129.0a	110.0a	56.2b*
(μmol L ⁻¹)	40	87.3a	107.0a	62.4a	119.0b**	95.9a	110.0a
Mg ²⁺	15	46.1a	57.6b**	47.8a	37.1b***	50.7a	31.9b***
(μmol L ⁻¹)	40	52.0a	74.8b**	38.5a	36.6a	59.5a	52.8a
Na ⁺	15	34.0a	20.0b**	46.1a	16.4b***	71.7a	15.6b***
(μmol L ⁻¹)	40	33.6a	17.1a***	38.6a	29.6b**	41.3a	14.9b***
NH ₄ ⁺	15	18.6a	7.50a	24.1a	15.8a	17.3a	12.6a
(μmol L ⁻¹)	40	21.1a	123.0b***	19.8a	18.1a	13.9a	15.2a
NO ₂ ⁻	15	5.50a	21.0a	4.00a	14.4a	3.80a	6.80a
(μmol L ⁻¹)	40	3.40a	7.10a	2.40a	9.70b***	3.30a	7.00a
NO ₃ ⁻	15	13.3a	11.8a	13.6a	16.9a	18.0a	76.5b**
(μmol L ⁻¹)	40	20.1a	44.3a	8.40a	45.2a	24.3a	74.3a
pH	15	5.90a	5.60b***	5.90a	5.50b***	6.10a	5.70b***
	40	6.00a	5.70b***	5.90a	5.70b***	6.20a	5.80b***
PO ₄ ³⁻	15	7.10a	8.20a	7.30a	6.40a	8.20a	5.20a
(μmol L ⁻¹)	40	9.10a	6.90a	7.30a	12.7b*	9.20a	6.00a
SO ₄ ²⁻	15	30.5a	25.9b**	30.1a	25.6b***	33.5a	30.0a
(μmol L ⁻¹)	40	41.9a	35.5b**	42.1a	46.0a	44.2a	40.0b*

TN	15	44.9a	3.60a	55.8a	-	48.1a	72.6a
($\mu\text{mol L}^{-1}$)	40	61.5a	164.0a	30.8a	31.7a	76.8a	329.0a

Key to abbreviations: DOC – dissolved organic carbon; EC – electrical conductivity; TN – Total Nitrogen

2.3.5 Analysis of pre-harvest soil chemical properties

Pre-harvest soil chemical properties were analyzed to determine if there were any important differences in the treatment sites prior to the start of the experiment that would complicate data analysis. During the 2011 collection, we found the following variables to have significantly different values in clearcut treatments as compared to control treatments: exchangeable K^+ at the 0 to 10 cm and 10 to 20 cm depth intervals (100% and 62.6%, respectively); base saturation at the 0 to 10 cm depth interval (31.4%); and C:N ratio at the 20 to 30 cm and 30 to 40 cm depth interval (-48.5% and -30.0%, respectively); and pH(water) at the 10 to 20 cm and 20 to 30 cm depth intervals (7.3% and 2.7%, respectively). Marginally significant differences ($p < 0.1$) between clearcut and control from the 2011 sample collection were observed for pH(salt) at the 0 to 10 cm and 10 to 20 cm depth intervals (8.7% and 7.5%, respectively) and pH(water) at the 0 to 10 cm depth intervals (8.3%).

Within single-tree selection treatments the following variables were observed to have significantly different values from control: Al_T at the 20 to 30 cm and 30 to 40 cm depth intervals (-12.7% and 52.3%, respectively) and TOC at the 0 to 10 cm interval (-21%); C:N ratio at the 20 to 30 cm and 30 to 40 cm depth interval (-30.8% and -28.5%, respectively).

2.3.6 Comparison of pre-harvest and pooled post-harvest soil chemical properties

We compared soil chemical properties for the pre-harvest (2011) and pooled post-harvest (2012-2016) to determine what the nutrient availability (for exchangeable ions) and chemical status. Within clearcut treatments, significant increases ($p < 0.05$) were observed in post-harvest values, relative to pre-harvest values, at the 0 to 10 cm depth (table in supplemental material) for exchangeable Ca^{2+} (95%), CEC (34%), and sum of exchangeable bases (69%). Total nitrogen was also significantly greater ($p < 0.05$) in clear-cut treatments at the 10 to 20 cm, 20 to 30 cm and 30 to 40 cm depth intervals (36%, 76% and 119%, respectively). Total organic carbon was significantly greater ($p < 0.05$) post-harvest at the 20 to 30 cm and 30 to 40 cm depth intervals (84%, 111%, respectively). Marginally significant increases ($p < 0.10$) post-harvest, relative to pre-harvest, were found within clearcut treatments for exchangeable Ca^{2+} at the 10 to 20 cm depth (103%) and TOC at the 10 to 20 cm depth (40%).

Within single tree selection treatments, there were significant differences between pre- and post-harvest for the following variables: Al_T at the 20 to 30 and 30 to 40 cm depth intervals (-54% and -60%, respectively); TN at the 10 to 20cm, 20 to 30cm and 30 to 40 cm depth intervals ($p < 0.01$; 95%, 109% and 141%, respectively) and TOC at the 10 to 20 and 30 to 40 depth intervals (58% and 112%, respectively). There were marginally significant differences between pre- and post-harvest in: exchangeable Ca^{2+} at the 0-10 cm depth interval (146%); exchangeable Mg^{2+} at the 0 to 10cm depth interval (56%); sum of bases at the 0 to 10cm depth interval (105%); TOC at the 0 to 10 cm and 20 to 30 cm depth intervals (49% and 69%, respectively).

Within control treatments, significant differences between pre- and post-harvest were observed for the following variables: TN at the 10 to 20 cm, 20 to 30 cm, and 30 to

40 cm depth intervals ($p < 0.1$; 103%, 159% and 200%, respectively) and TOC at the 10 to 20 cm, 20 to 30 cm and 30 to 40 cm depth intervals (63%, 80%, and 117%, respectively). A marginally significant difference between pre- and post-harvest was observed in exchangeable Mg^{2+} at the 0 to 10cm depth interval (-52%).

2.3.7 Post-harvest pooled pH data

Due to the changes observed in all treatments of the comparison of pre-harvest and pooled post-harvest data, further analysis of pH was performed on the just the post-harvest data. At the 0-10 cm depth interval, the post-harvest clear-cut pH (4.88) was significantly greater ($p < 0.01$) than the post-harvest control (4.38) and single-tree selection (4.48) (Figure 2.5).

Figure 2.5. Comparison of post-harvest soil pH at the 0-10 cm depth interval for clearcut (CC), single-tree selection (SEL), and control (CON) treatments. Means with different lower-case letters are significantly different at $p < 0.10$. Significant differences between treatments are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$.

2.3.8 Year-by-year Trends in soil chemical properties

Significant changes in soil solid phase chemistry on a year-by-year basis were observed for a large number of variables (table in supplemental material). For clearcut treatments significant differences ($p < 0.05$) through time were noted for: base saturation at the 0-10 cm depth interval; cation exchange capacity at the 0 to 10 cm interval. C:N ratio at the 10 to 20cm, 20 to 30cm and 30 to 40cm depth intervals; pH at the 20 to 30 cm and 30 to 40 cm depth intervals; and TN; TOC and pH(water) at all depth intervals. Marginally significant ($p < 0.10$) changes were seen for the following properties: base saturation at the 20 to 30 cm depth interval; CEC at the 10 to 20 cm depth interval; and exchangeable K^+ at the 0-10 cm depth interval.

Within single-tree selection treatments significant changes were seen in base saturation at the 0 to 10cm depth interval; C:N ratio at the 10 to 20cm, 20 to 30cm and 30 to 40cm depth intervals; exchangeable K^+ at the 0 to 10cm depth interval; pH(salt) at the 30 to 40cm depth intervals; and TN, TOC and pH(water) for all depth intervals. Marginally significant ($p < 0.1$) changes were observed for the following soil properties: base saturation at the 10 to 20 cm depth interval and sum of bases at the 0 to 10 cm depth interval.

Within control treatments significant changes were observed in: Al_T at the 0 to 10 cm depth interval; base saturation at the 0 to 10cm depth interval; base saturation at the 0 to 10 cm depth interval; exchangeable Ca^{2+} at the 0 to 10 cm depth interval; CEC at the 0 to 10 cm depth interval; C:N ratio at all depth intervals; exchangeable K^+ at the 0 to 10 cm and 10 to 20 cm depth intervals; K^+ at the 0 to 10 cm, 10 to 20 cm and 20 to 30 cm depth intervals; ph(salt) at all depth intervals; sum of bases at the 0-10 cm depth interval; TN at

the 10-20, 20-30, and 30 to 40 cm depth intervals; TOC at the 20 to 30 cm and 30 to 40 cm depth intervals; and pH(water) at all depth intervals. Marginally significant ($p < 0.1$) changes were observed for TOC at the 10 to 20cm depth interval and exchangeable K^+ at the 30 to 40 cm depth interval.

2.4 Discussion

2.4.1 Seasonal Flush

Analysis of control site soil solution data revealed seasonal variation in concentrations unrelated to forest harvest activities for several soil nutrients (Table 2.1). Concentrations of Al_T , Br^- , Cl^- , NO_3^- and SO_4^{2-} at the 15cm depth interval and Cl^- , F^- , pH, SO_4^{2-} and TN at the 40 cm depth interval changed significantly on a seasonal basis. Concentrations were at a maximum during the summer months for Al_T and NO_3^- , during the fall for Br^- , F^- and TN; and during the winter for SO_4^{2-} and Cl^- . Soil solution pH reached a minimum value during the fall.

Johnson and Swank (1973) limited their research to cations, but found that there was seasonal variation in soil solution concentration for Ca^{2+} , Mg^{2+} , K^+ and Na^+ in undisturbed hardwood and softwood forests in southern Appalachia. Concentrations were observed to be greatest in the summer and least in the winter. Mulholland and Hill (1997) observed seasonal variability in NO_3^- , PO_4^{3-} and DOC concentrations in streamwater runoff from forests in Tennessee. Nitrate and PO_4^{3-} were found to have a maximum concentration in the summer; whereas DOC was found to have a maximum value in the fall.

The amount of lag between the harvest and the onset of the flush phase varied considerably between our clearcut harvest sites (2 to 14 months). We could find no evidence in the literature attempting to quantify the timing of the flush; however, most studies suggest that the flush typically ceases within three years post-harvest (Hynes and Germida, 2013; Knoepp et al., 2014). This is consistent with our study, as we found the cessation of the flush interval in our study ranged from 17-31 months post-harvest. We attribute differences in timing of post-harvest nutrient flush to the season during which harvest occurred. Replicate treatments with harvests occurring earlier in the year appeared to exhibit more immediate flushes of nutrients and other solutes; whereas, the harvests occurring in late fall experienced a delayed onset of solute movement. This may be tied to the seasonality of the microbial populations which perform the decomposition of the litter from which the nutrient are being derived. Temperature is well-documented to increase the rate of soil organic matter decomposition (von Lützow and Kögel-Knabner, 2009) assuming sufficient soil moisture (Allison and Treseder, 2008); therefore, the warmer summer and early fall months would likely give microbial populations a “head-start” on litter breakdown. Whereas, late season harvests, when microbial activity is already declining due to lower temperatures may slow litter breakdown until the following summer. Single-tree selection harvest were not observed to experience a nutrient flush due to harvest activity. We could find no evidence in the literature indicating that single-tree selection experienced nutrient flushes specifically related to harvest, although the possibility does exist that localized changes do occur at the site of the downed tree.

2.4.2 pH

Post-harvest canopy throughfall pH was decreased across all treatments, relative to pre-harvest levels; however, the decrease within clearcut treatments was greater (8.4%) than that observed at the control (3.6%) and single-tree selection (3.8%) sites (Table 2.3). Consistent with our finding reduced pH of throughfall solution in clearcut treatments post-harvest (i.e., no canopy present), tree canopies have been documented to increase the pH of incident rainfall (Mahendrappa, 1989; McBride, 1994; Piirainen et al., 2004).

The pH of soil solution collected post-harvest at the 15 cm depth within clearcut treatments was greater relative to control; however, pre-harvest clearcut pH values were also slightly greater than control sites. Reduction of the pH in soil solution have been widely recorded post-harvest (Adams et al., 2000); however, it should be noted that we observed decreases in pH in all treatments, including the control. Therefore, we suspect that the reduction of pH within soil solution during the flush phase was not directly related to harvest activity. Environmental or seasonal fluctuations may be contributing factor to the observed changes in pH.

Post-harvest soil pH (CaCl_2) in clearcut treatments at the 0-10 cm depth interval was nominally greater (4.88) than pre-harvest clearcut (4.60) and significantly greater than post-harvest control (4.38) treatments. At lower depth intervals (10-20 cm, 20-30 cm and 30-40 cm), soil pH values were unchanged within the treatments before and after harvest. However, examination of just the post-harvest data yields that the post-harvest clear-cut soil pH at the 0-10 cm depth interval was significantly greater than the post-harvest control or single-tree selection; whereas there was no statistical difference between pH values within the treatments during pre-harvest. A similar increase in post-

harvest clear-cut pH, relative to post-harvest single-tree selection and control treatments was also seen at the 10-20 cm depth interval. This suggests that there was a statistically significant effect on pH (an increase) at the 0-10 and 10-20 cm depth intervals due to clear-cut harvest. Clear-cut harvests have been documented to alternately increase (Marschner and Noble, 2000; Jerabkova et al., 2011; Hynes and Germida, 2013) and decrease (Johnson et al., 1991) soil pH. Increases in pH are primarily attributed to the addition of significant amounts of slash material (Marschner and Noble, 2000). More recent studies have begun to propose that forest litter addition may function to neutralize soil pH rather than simply creating acidic or basic conditions (Xu et al., 2006; Hong et al., 2018).

2.4.3 Base Cations in Throughfall

Forest canopies have been well documented to have effects on nutrient concentrations in throughfall solution (Parker et al., 1995; Levia and Frost, 2003). Changes in base cation concentration to unaltered precipitation via canopy interaction have been shown to be due to two processes: either interaction with leaves, generally referred to as throughfall, or alternatively precipitation runs down the stems to the forest floor, referred to as stemflow (Parker et al., 1995). The throughfall samplers at our sites capture primarily the throughfall portion of incident precipitation, which makes up to 85% of the precipitation reaching the forest floor (Parker et al., 1995). The processes by which ions are added or reduced in concentration via canopy interaction are complex, vary due to geographic location and tree species composition and not always well understood; but range from additions, such as simple dissolution by precipitation of the results of dry deposition of ions on the canopy surface and leaching of ions from leaves;

to losses, such as active uptake by trees (Lovett et al., 1996) and nutrient scavenging by epiphytes (Coxson and Nadkarni, 1995).

In our study, throughfall concentrations of Ca^{2+} and Mg^{2+} were found to be reduced in all treatments post-harvest relative to pre-harvest; however, the magnitude of concentration decline was significantly greater in clearcuts (Ca^{2+} : -47%; Mg^{2+} : -76%) relative to control (Ca^{2+} : -15%; Mg^{2+} : -19%) (Table 2.3). The concentration of K^+ in throughfall increased in control sites post-harvest (41%), relative to pre-harvest levels; whereas, in the clearcut treatment, the K^+ concentration post-harvest was reduced (-103%) relative to pre-harvest. Several studies have documented changes in base cation concentrations in throughfall chemistry within clear-cut forests. Robertson, et al. (2000) found that total ionic concentration in throughfall was reduced in clearcuts relative to control under conifer canopy in the U.K., but found increases in throughfall total ionic concentration underneath broadleaf clearcuts. Conversely, Piirainen et al., (2004), studying Podzolic soils in eastern Finland observed flux reductions in annual depositional loads delivered to the forest floor in clear-cut mixed boreal forests for Ca, Mg and K. Likens et al. (Likens et al., 1998) noted that at the Hubbard-Brook Experimental Forest site in New Hampshire, U.S.A, throughfall concentrations of calcium were considerably greater (nearly 10x) than that found within bulk precipitation. They suggested that the additional sources of calcium within the unaltered plots were from washoff of dry-deposited particles and from leaching of calcium from sources on and within the canopy. Our results are largely consistent with the findings of Piirainen et al. and Likens, et al. Whereas, the changes in throughfall concentration for the base cations in clear-cut as compared to control sites at post-harvest in our study were not of the level of magnitude

observed at Hubbard-Brook (Ca^{2+} : -21%; K^+ : -60%; Mg^{2+} : -61%; Na^+ : 12%), the reductions were still significant ($p < 0.05$) for Ca^{2+} and Mg^{2+} . The differences between control and clearcut treatments post-harvest were not significant for throughfall concentrations of Na^+ and K^+ .

2.4.4 Base Cations in Solution

In our study, the base cations Ca^{2+} , Mg^{2+} , and K^+ in solution increased during the post-harvest flush phase within clearcut treatments (Figure 2.6). The observed increases in Mg^{2+} within soil solution were greater than that observed for Ca^{2+} particularly at the 40 cm sampling depth during the flush phase. This is consistent with the widely reported finding that Ca^{2+} is preferentially adsorbed onto exchange sites as compared to Mg^{2+} (Udo, 1978; Sposito et al., 1983; Selim et al., 1987). Post-flush soil solution values for Ca^{2+} , Mg^{2+} and K^+ were significantly reduced as compared to their pre-harvest levels. Concentrations of Na^+ were statistically unchanged during flush and post-flush phases as compared to pre-harvest levels. Evidence of post-harvest increases of base cations within soil solution are common in the literature. Piirainen et al. (2004) observed a significant increase in the concentration of K^+ in soil percolate collected from below the O horizon immediately after a clear-cut harvest. Occasional increases in the concentration of Ca^{2+} and Mg^{2+} post clear-cut harvest were also observed. Knoepp, et al. (2014) found increased concentrations of Ca^{2+} in soil solution in Ultisols and Inceptisols in a commercial sawlog harvest at the Coweeta Hydrologic Laboratory in North Carolina. On a year-by-year basis, Knoepp et al., (2014) also found intermittent increases in K^+ and Mg^{2+} ; however, these levels did not remain elevated throughout the study. Increases in the concentration of base cations in solution and soil exchangeable base cations have

been widely interpreted to be the result of the decomposition and subsequent mineralization of slash material (Robertson et al., 2000; Piirainen et al., 2004)

2.4.5 Base Cation nutrient response in Soil

Soil exchangeable concentrations of Ca^{2+} and K^{+} at the 0-10 cm depth increased in 2013, reached a maximum in 2015, and apparently began to decline in 2016; however, the Ca^{2+} and K^{+} concentrations were still significantly greater than clearcut pre-harvest (2011) and 2016 control and single-tree selection treatments (Figure 2.7). In contrast, exchangeable Mg^{2+} concentrations within the soil did not change by treatment on a yearly basis. Concentrations of Na^{+} within the soil were below the limits of detectability throughout the course of the experiment. Within soil, exchangeable base cation concentrations have been reported to increase post-harvest (Liechty et al., 2002; Knoepp et al., 2014), decrease post-harvest (Pennock and Kessel, 1997; Likens et al., 1998; Huntington et al., 2000) or experience no statistical change (Johnson and Todd, 1998; Liechty et al., 2002). Knoepp et al., (2014) studying pine-hardwood and oak-hickory forests in the Blue Ridge Mountains of North Carolina found initial increases in exchangeable Ca^{2+} , Mg^{2+} and K^{+} concentrations for 3 years post-harvest. After this initial increase in concentration, exchangeable Ca^{2+} and K^{+} concentrations were observed to reach pre-harvest levels; whereas the concentration of Mg^{2+} remained elevated for 30 years post-harvest.

Concentrations of exchangeable Ca^{2+} at lower depth intervals tended to follow the same yearly trend within clear-cut treatments as the 0-10 cm depth, but to a lesser degree of change, and with less statistical significance. Concentrations Mg^{2+} and K^{+} were unchanged on a yearly basis at lower depth intervals.

Figure 2.6 Boxplots of calcium (A), magnesium (B), and potassium (C) concentrations in soil solution collected at a depth of 15 cm from control (CON), single-tree selection (SEL) and clearcut (CC) treatments during different phases, and calcium (D), magnesium (E), and potassium (F) concentrations in soil solution collected at a depth 40 from control (CON), single-tree selection (SEL) and clearcut (CC) treatments during different phases

Means with different lower-case letters are significantly different at $p < 0.10$ within each phase. Significant differences between treatments within each phase are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$.

Figure 2.7 Boxplots of soil exchangeable calcium (A), magnesium (B), and potassium (C) concentrations in soil samples collected at 0 to 10 cm depth within treatments as a function of time. Means with different lower-case letters are significantly different at $p < 0.10$. Significant differences between time intervals are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$.

2.4.6 Nitrogen

Increased nitrogen concentrations in soil solution after harvest, particularly NO_3^- , are well-documented in the literature (Likens et al., 1969; Piirainen et al., 2002; Jerabkova et al., 2011; Johnson et al., 2016). In our study there were no observed changes in throughfall NO_3^- concentration as a result of clearcut or single-tree selection harvest treatment (Table 2.3); however clearcut harvesting did dramatically change NO_3^- concentration in soil solution during the post-harvest flush phase (Table 2.2; Figure 2.8). At the 15 cm solution sampling interval during the flush phase, mean NO_3^- concentration increased by 24-fold within clearcuts. Similarly, at the 40cm solution sampling depth within clearcuts, NO_3^- concentration increased by nearly 17-fold (Figure 2.8). Likens et al. (1969) documented increased NO_3^- concentration in stream water runoff from deforested sites at the Hubbard-Brook Experimental Forest site in New Hampshire, USA. Jerabkova et al. (2011) in a meta-analysis of the effects of clear-cut and selection harvest on soil nitrogen found that clearcut harvest significantly increased soil and soil solution nitrogen concentrations.

Ammonium also increased quite substantially in soil solution collected at the 15cm depth from clear-cut treatments during the flush phase (30-fold); however, no change was observed at the 40 cm depth interval. Post-flush NH_4^+ concentration in

solution within clearcuts returned to pre-harvest levels at the 15 cm and 40 cm depths. Piirainen et al (2002) studying an N-limited spruce-dominated forest in Finland observed that NH_4^+ in soil solution moderately increased, but concentrations were low compared to other studies in N-sufficient forests.

In our study, post-flush NO_3^- concentrations within clear-cut harvests treatments remained elevated above pre-harvest levels at the 15 cm and 40 cm depths by 326% and 206%, respectively (Table 2.5). The closest long-term soil solution analog to the MOFEP site is the Coweeta Hydrologic Laboratory site in North Carolina. Knoepp, et al. (2014) reported increases in nitrogen (primarily NO_3^-) concentrations in soil solution for 20 years post-harvest at the Coweeta. They attributed these changes due to the large input of logging residue introduced by the harvest and changes in forest species composition. Prior to harvest, the Coweeta sites were dominated by oak (*Quercus* spp.) and hickory (*Carya* spp.); whereas, post-harvest, tulip tree (*Liriodendron* spp.) locust (*Robinia* spp.) and maple (*Acer* spp.) were dominant. Similar changes in species composition has been reported to affect nitrogen uptake by resident plant species (Hendrickson, 1988).

The reductions in clear-cut flush and post-harvest concentration for NO_3^- and NH_4^+ in solution at the 40 cm depth interval as compared to the 15 cm depth interval is primarily attributed to be due to plant uptake of nitrogen by successive species (Weis et al., 2001); however, nitrogen immobilization by microbial populations may have also contributed to the decreases in concentration with depth (Qualls et al., 1991).

Total nitrogen concentration in soil sampled from the 0 to 10 cm depth within clearcut treatments increased by 28.6% during years 2013 and 2015 relative to pre-harvest levels, but was reduced to pre-harvest levels by 2016 (Table 2.A.3). Similar

trends were noted for soil sampled at deeper depths as well (10-20 cm, 20-30 cm and 30-40 cm). Although control and single-tree selection treatments varied on a year-to-year basis within the treatments, in most cases the 2016 TN concentration for these treatments were significantly above 2011 levels. This appears to indicate that some factor other than harvesting was altering soil TN concentrations within the control and single-tree selection treatment. One potential explanation is increased anthropogenic nitrogen deposition during 2016. Although levels of deposition of regulated nitrogen forms (NO_x) has been decreasing over the last decade, deposition of unregulated nitrogen forms (e.g., NH_3) in the atmosphere have been increasing over the same period (Ibáñez et al., 2018). Recent publications have noted a shift from NO_3^- to NH_4^+ additions to soil (Hůnová et al., 2017; Ibáñez et al., 2018) sourced from atmospheric deposition.

Single-tree selection treatments had no observable effect on NO_3^- , NH_4^+ or TN concentrations in soil solution. This finding is consistent with the meta-analysis of Jerabkova et al. (2011), which found that single-tree selection had a lesser impact on post-harvest nitrogen concentrations than clearcut or group selection harvests.

Figure 2.8 Boxplots of nitrate concentration in soil solution by phase for samples collected at the (A) 15 cm depth and (B) 40 cm depth within control (CON), single-tree selection (SEL) and clearcut (CC) treatments. Means with different lower-case letters are significantly different at $p < 0.10$. Significant differences between pre-harvest and flush phases within treatments are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$.

2.4.7 Sulfur

All treatments were observed to have significantly reduced SO_4^{2-} concentration in throughfall post-harvest, as compared to pre-harvest sampling; however, the reduction in the clearcut treatments was greater (-43%) as compared to control (-21%) and single-tree selection (-26%). During the flush phase, SO_4^{2-} concentrations in soil solution were unchanged from pre-harvest levels for all treatments at the 15 cm depth. However, at the 40 cm depth, SO_4^{2-} concentration was significantly reduced in the clear-cut treatment (-40%) from pre-harvest levels, while control and single-tree selection harvests remained unchanged during the flush phase, as compared to pre-harvest. Comparison of pre-harvest and post-flush SO_4^{2-} concentration at the 15 cm depth led to the observation that concentration was reduced (15%) in the control and single-tree selection treatments post-flush, relative to control, but unchanged in the clearcut treatment. At the 40 cm depth soil solution sampling depth SO_4^{2-} soil solution concentration was reduced post-flush, relative to pre-harvest in the control and clearcut treatments but SO_4^{2-} concentrations were statistically unchanged in the single-tree selection treatment.

Within the solid phase, total sulfur concentrations were greater (30%) within clearcut treatments as compared to control treatments at the 0-10 cm depth but no changes were noted deeper within the soil profile.

Our results are consistent with the findings of Likens et al. (2002). Over thirty years of accumulated throughfall, soil solution and soil sampling at the Hubbard Brook Experimental Forest have resulted in the findings that: a) concentrations of atmospheric depositional sulfur have diminished over time; b) forest vegetation harvest or removal results in increased SO_4^{2-} adsorption onto mineral soil and; c) reduced SO_4^{2-} concentration within soil solution and reduced losses to stream water. Knoepp et al. (2014) found increased SO_4^{2-} leaching from slash material after a clear-cut forest harvest, as compared to canopy leaching from a control treatment. The combination of increased SO_4^{2-} leaching from decomposing slash material and increased adsorption of SO_4^{2-} onto the mineral soil explains the low SO_4^{2-} concentrations we observed at the 15 cm depth (well below the forest floor source) and increased total sulfur observed within the top 10 cm of soil within the clear-cut treatments. Another potential sink for the sulfur decomposed from the decomposing slash material within clear-cuts includes incorporation into soil organic matter. Although not measured in this study, organic forms of sulfur predominate within the organic and mineral horizons of forest soils (David et al., 1982; Mitchell et al., 1989).

Conversely, our findings are not consistent with the findings of Spratt (1998). Spratt (1998), studying sulfur at MOFEP and the nearby Deer Run State Forest, found that clearcut harvests reduced both total and organic sulfur in the A horizon. However, Spratt (1998) observed that the O horizons were reduced in thickness within clearcut

treatments; whereas, we observed thicker O horizons in our clear-cut treatments.

Interpretation of remotely-sensed imagery sourced from Google Earth Pro (Google LLC; Mountain View, CA) collected two years post-harvest (May 13, 2013) reveals a broad and random dispersion of slash and unmerchantable timber in the clear-cut harvest plots, potentially providing additional source material for sulfur.

2.4.8 Phosphorus

Clearcut and single-tree selection harvests were observed to have reduced PO_4^{3-} concentration in throughfall post-harvest, relative to pre-harvest levels (Table 2.3); whereas, concentrations of PO_4^{3-} in throughfall were unchanged in control sites over time.

At the 15 cm depth, soil solution concentrations of PO_4^{3-} within clearcut treatments increased in concentration (273%) during the flush phase; whereas, single-tree selection and control sites were unchanged during the same period (Table 2.2). This is consistent with Qualls et al. (2014), who observed increases in both inorganic and organic dissolved P fluxes in leachates below slash material and the forest floor within clear-cut harvests at the Coweeta site in the Blue Ridge Mountains of North Carolina, USA.

No changes during the flush phase, relative to pre-harvest concentrations, were noted at the 40cm depth in clearcut treatments; however, there were slight reductions in control and single-tree selection concentrations during the flush phase, relative to pre-harvest levels. No significant differences ($p < 0.05$) were observed in post-flush soil solution PO_4^{3-} concentrations relative to pre-harvest levels at either depth in any of the treatments. Qualls noted solution concentrations of P were abruptly reduced in clear-cut treatments in the A horizon of the soil (as compared to O horizon leachate P concentrations), which

they attributed to strong adsorption of phosphorous (PO_4^{3-} and organic phosphate esters) to Al and Fe hydroxides within the A horizon.

This is consistent with the significantly ($p < 0.05$) decreased Bray-1 P concentrations within clearcuts reported by our study, relative to control and single-tree selection treatments at the 0-10 depth interval. Singh et al. (2015) conducted extensive analysis into soil P within the Ozark Highlands. Total and available (Mehlich-3 and Bray-1) mean concentrations of phosphorus were found to be low (total P:116.2; Mehlich-3:7.87; and Bray-1:5.81 mg kg^{-1}) and reported that the Missouri Ozarks may be P-limited. Singh et al. also suggest that P sorbs to manganese via weaker outer-sphere complexes and stronger inner-sphere complexes, particularly at pH values less than 6. Extractions of P using the Bray-1 method can have difficulty measuring P strongly sorbed to metal oxides, indicating that the P present in the system may have reduced bioavailability. Olander and Vitousek (2005) examining P availability and retention in P-limited Oxisols found that the uptake of phosphorus was extremely rapid – with 70% being sorbed or immobilized in the non-labile fraction within 30 minutes and 95% sorbed or immobilized with 48 hours. This is also consistent with our finding that the Bray-1 P concentrations in soil collected from lower depth intervals were statistically inseparable in the clear-cut treatment. Any additional P added to the system via the PO_4^{3-} flush was likely immobilized within the upper 10 cm and not transported deeper within the soil profile.

2.5 Conclusion

Our research suggests that nutrient cycling at the MOFEP experimental site was significantly altered as a result of clearcut harvest treatments. Concentrations of carbon, nitrogen, calcium, magnesium, potassium and phosphorous dramatically increased within

soil solution within 2 to 14 months after clearcutting. The concentrations remained elevated throughout the flush phase, lasting from 17 to 31 months. Concentrations of nutrients experiencing a flush were significantly reduced post-flush, although NO_3^- did remain above pre-harvest levels. In general, although losses of soil nutrients probably did occur, concentrations within the soil solid phase were either elevated or remained unchanged, with the sole exception of available phosphorus. Reductions in available phosphorous present a potential scenario of concern for the long-term prognosis for phosphorus cycling in the area with repeated forest harvest activities.

2.6 References

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2.A Supplemental Material

Table 2.A.1. Detection limits for instruments used for ionic analysis (after Singh, 2013)

Instrument	Analyte	Detection Limit ($\mu\text{mol L}^{-1}$)
ICP-AES	Aluminum (AL_T)	0.95
	Calcium (Ca^{2+})	0.751
	Magnesium (Mg^{2+})	0.084
	Potassium (K^+)	37.431
	Sodium (Na^+)	2.433
Dionex ICS-1000	Fluoride (F^-)	2.915
	Bromide (Br^-)	0.354
	Chloride (Cl^-)	0.421
	Nitrite (NO_2^-)	0.508
	Nitrate (NO_3^-)	0.386
	Sulfate (SO_4^{2-})	0.328
	Phosphate (PO_4^{3-})	0.785
Shimadzu TOC-V	Non-purgeable organic carbon (NPOC)	13.436
	Total nitrogen (TN)	7.192
Thermo Fisher iCE3300	Sodium (Na^+)	0.1609

Figure 2.A.1 Concentration of NO_3^- for control sites 3 low nutrient status and 9 medium nutrient status illustrating the increased lag of the onset of the flush phase due to late season harvest of site 3.

Figure 2.A.2 Timing of the onset of the flush phase for NO_3^- , Mg^{+2} and Na^+ ions for control treatments at site 9 medium nutrient status soils at the 15 centimeter depth interval.

Figure 2.A.3. Concentrations of NO_3^- for single-tree select sites 8 (low nutrient status) and clear-cut site 9 (medium nutrient status), with the NO_3^- -derived phase timing superimposed on the graph. A seasonal flush effect can be noted in the site 8 response.

Table 2.A.2. Differences in mean values for soil chemical properties in solid phase between pre-harvest (2011) and post-harvest (2012-2016) after imposing clearcut (CC), single tree selection (SEL) and control (CON) treatments. Significant differences between pre-harvest and post-harvest are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$. Means within a row followed by different letters are significantly different at the $p < 0.10$ level.

<u>Variable</u>	<u>Depth (cm)</u>	<u>CON</u>		<u>SEL</u>		<u>CC</u>	
		<u>Pre</u>	<u>Post</u>	<u>Pre</u>	<u>Post</u>	<u>Pre</u>	<u>Post</u>
Exch. Al _T (cmol _c kg ⁻¹)	0-10	1.00a	0.64a	0.75a	0.56a	0.38a	0.84a
	10-20	1.27a	0.86a	1.15a	0.73a	0.58a	1.54a
	20-30	0.92a	0.74a	2.17a***	0.99b	0.80a	0.75a
	30-40	0.73a	0.76a	3.02a***	1.19b	1.12a	0.85a
Exch. Ca ²⁺ (cmol _c kg ⁻¹)	0-10	1.40a	2.26a	1.05a*	2.58b	2.20a**	4.28b
	10-20	0.98a	0.89a	0.78a	1.24a	0.88a*	1.79b
	20-30	1.67a	1.02a	1.25a	1.21a	0.48a	1.36a
	30-40	2.10a	1.54a	1.53a	1.31a	0.55a	1.24a
Exch. K ⁺ (cmol _c kg ⁻¹)	0-10	0.18a	0.20a	0.18a	0.22a	0.25a	0.29a
	10-20	0.15a	0.13a	0.13a	0.16a	0.30a***	0.19b
	20-30	0.13a	0.14a	0.15a	0.17a	0.22a	0.18a
	30-40	0.15a	0.13a	0.17a	0.16a	0.22a	0.19a
Exch. Mg ²⁺ (cmol _c kg ⁻¹)	0-10	0.47a	0.63a	0.52a*	0.81b	0.68a	0.81a
	10-20	0.98a*	0.47b	0.75a	0.62a	0.50a	0.57a
	20-30	1.52a	0.75a	1.35a	0.82a	0.65a	0.63a
	30-40	2.05a	1.24a	1.47a	1.00a	0.85a	0.75a
Exch. Na ⁺ (cmol _c kg ⁻¹)	0-10	0.03a	0.01a	0.03a	0.01a	bd	0.01a
	10-20	bd	0.01	bd	0.01	bd	bd
	20-30	bd	0.02	bd	bd	bd	bd
	30-40	bd	0.01	bd	bd	bd	bd
pH (salt)	0-10	4.23a	4.38a	4.25a	4.48a	4.60a	4.88a
	10-20	4.20a	4.34a	4.27a	4.38a	4.52a	4.59a
	20-30	4.22a	4.32a	4.15a	4.34a	4.33a	4.47a
	30-40	4.23a	4.42a	4.13a	4.38a	4.25a	4.43a
pH (water)	0-10	5.00a	4.80a	5.05a	4.97a	5.42a	5.32a
	10-20	5.00a	4.84a	5.05a	4.93a	5.37a	5.14a
	20-30	4.92a	4.94a	5.00a	5.02a	5.20a	5.09a
	30-40	5.00a	5.00a	5.00a	5.01a	5.13a	5.12a
TN	0-10	0.14a	0.15a	0.09a	0.13a	0.14a	0.16a

(g kg ⁻¹)	10-20	0.04a***	0.07b	0.04a***	0.08b	0.06a**	0.09b
	20-30	0.02a***	0.04b	0.02a***	0.05b	0.03a***	0.06b
	30-40	0.01a***	0.04b	0.02a***	0.04b	0.02a***	0.05b
	0-10	2.38a	2.45a	1.43a*	2.14b	1.88a	2.30a
TOC (g kg ⁻¹)	10-20	0.77a**	1.24b	0.73a**	1.16b	0.90a*	1.26b
	20-30	0.40a**	0.72b	0.40a*	0.67b	0.45a**	0.83b
	30-40	0.27a***	0.58b	0.28a***	0.60b	0.30a***	0.63b
	0-10	2.10a	3.13a	1.77a*	3.62b	3.18a**	5.38b
sumbases	10-20	2.15a	1.52a	1.68a	2.03a	1.73a	2.57a
	20-30	3.32a	1.92a	2.78a	2.18a	1.35a	2.17a
	30-40	4.32a	2.91a	3.18a	2.46a	1.65a	2.17a
	0-10	5.43a	6.09a	5.20a	6.50a	5.90a**	7.91b
ECEC	10-20	4.83a	4.67a	4.75a	4.82a	4.67a	5.72a
	20-30	6.82a	4.73a	6.72a	5.43a	5.05a	5.76a
	30-40	6.93a	5.66a	7.63a	6.34a	5.37a	5.66a
	0-10	37.7a	40.1a	29.8a	43.7a	49.5a	56.2a
Basesat (%)	10-20	29.3a	24.9a	29.8a	33.2a	36.0a	38.5a
	20-30	23.5a	25.2a	31.0a	31.3a	27.7a	33.3a
	30-40	32.3a	25.6a	36.3a	30.6a	29.5a	32.2a

Key to abbreviations: TN – Total Nitrogen; TOC – Total Organic Carbon; sumbases – sum of bases;

ECEC Effective Cation Exchange Capacity; base sat = Base Saturation. bd indicates that the analyte was below the limits of detectability of the instrument.

Table 2.A.3. Differences in mean values for soil chemical properties in solid phase by year after imposing clearcut (CC), single tree selection (SEL) and control (CON) treatments. Significant differences between pre-harvest and post-harvest are indicated by * p<0.10 (marginal significance), ** p<0.05 and *** p<0.01. Means within a row followed by different letters are significantly different at the p<0.10 level.

Variable	CON				SEL				CC			
	2011	2013	2015	2016	2011	2013	2015	2016	2011	2013	2015	2016
<u>Al_t</u>	1.00a	0.64b	0.63b	nodata	0.75a	0.57a	0.55a	nodata	0.38a	1.64a	0.17a	nodata
<u>basesat</u>	37.7a	27.5a	56.8b	36.0a	29.8a	32.2a	48.1b	50.8b	49.5a	35.2b	70.9c	62.5ac
<u>Exch. Ca²⁺</u>	1.40ac	2.27a	3.38b	1.13c	1.05a	3.17b	2.47b	2.12ab	2.2a	4.28b	5.08b	3.48ab
<u>CEC</u>	5.43ac	6.25ab	7.45b	4.57c	5.2a	7.57b	6.48ab	5.45a	5.9a	8.65b	8.7b	6.37a
<u>C:N</u>	18.9ab	21.0a	14.4b	17.5c	17.2a	17.6a	16.9a	14.6a	14.0ab	16.2a	13.2b	14.0ab
<u>Exch. K⁺</u>	0.18ab	0.23a	0.25a	0.12ab	0.18a	0.30b	0.19a	0.18a	0.25a	0.27a	0.37b	0.23a
<u>Exch. Mg²⁺</u>	0.47ac	0.75b	0.73b	0.42c	0.52a	0.88b	0.83ab	0.70ab	0.68a	0.87a	0.88a	0.67a
<u>Exch. Na⁺</u>	0.03	0.03	bd	bd	0.03	0.02	bd	bd	bd	0.03	bd	bd
<u>pH(salt)</u>	4.23ac	4.63b	4.40a	4.10c	4.25a	4.58b	4.52ab	4.35ab	4.60a	4.88ab	5.07b	4.70ab
<u>sumbase</u>	2.10ac	3.35ab	4.37b	1.67c	1.77a	4.38b	3.48b	3.00ab	3.18a	5.43b	6.33b	4.38ab
<u>TN</u>	0.14ab	0.13ab	0.19a	0.11b	0.093a	0.15b	0.12ac	0.13bc	0.14a	0.18b	0.18b	0.13a
<u>TOC</u>	2.38ab	2.78a	2.67ab	1.91b	1.43a	2.70b	1.95a	1.78a	1.88a	2.83b	2.28a	1.78a
<u>pH(water)</u>	5.00a	5.35b	4.73c	4.31d	5.05a	5.48b	4.85ac	4.58c	5.42ab	5.68a	5.33b	4.94c
<u>10-20</u>												
<u>Variable</u>	CON				SEL				CC			
<u>Al_t</u>	2011	2013	2015	2016	2011	2013	2015	2016	2011	2013	2015	2016
<u>basesat</u>	1.27a	0.66a	1.02a	nodata	1.15a	0.73a	0.72a	nodata	0.58a	2.86a	0.43a	nodata
	29.33a	25.0a	28.40a	21.17a	29.83ab	23.67a	38.03b	37.83b	36	27.5	47.38	40.5

<u>Exch. Ca²⁺</u>	0.98a	1.47a	0.72a	0.48a	0.78a	1.48b	1.3ab	0.95ab	0.88a	2.07b	1.92b	1.38ab
<u>CEC</u>	4.83a	5.02a	4.27a	4.72a	4.75a	5.08a	4.92a	4.47a	4.67a	6.32b	5.73bc	5.10ac
<u>C:N</u>	22.34a	22.31a	14.0b	17.43b	19.99a	19.72a	12.02b	15.57ab	14.36a	17.19b	11.75c	14.44a
<u>Exch. K⁺</u>	0.15a	0.2b	0.1c	0.1c	0.13a	0.22b	0.13a	0.13a	0.3a	0.2ab	0.23ab	0.15b
<u>Exch. Mg²⁺</u>	0.98a	0.75a	0.4a	0.25a	0.75a	0.63a	0.65a	0.57a	0.5a	0.57a	0.58a	0.57a
<u>Exch. Na⁺</u>	bd	0.033	bd	bd	bd	0.017	bd	bd	bd	bd	bd	bd
<u>pH(salt)</u>	4.2a	4.65b	4.2a	4.17a	4.27a	4.48b	4.35ab	4.30a	4.52ab	4.72a	4.65a	4.39b
<u>sumbase</u>	2.15a	2.5a	1.22a	0.83a	1.68a	2.35a	2.08a	1.65a	1.73a	2.88b	2.73ab	2.1ab
<u>IN</u>	0.035a	0.07bc	0.086b	0.056ac	0.039a	0.085b	0.077bc	0.066c	0.064a	0.097b	0.103b	0.063a
<u>TOC</u>	0.77a	1.5b	1.25bc	0.97ac	0.73a	1.58b	0.9a	0.99a	0.9a	1.65b	1.22c	0.93a
<u>pH(water)</u>	5.0a	5.42b	4.62c	4.48c	5.05a	5.35b	4.75c	4.7c	5.37a	5.5a	5.02b	4.89b

20-30

20-30

20-30

Variable	CON				SEL				CC			
	2011	2013	2015	2016	2011	2013	2015	2016	2011	2013	2015	2016
<u>Al_T</u>	0.92a	0.66ab	0.80ab	nodata	2.17a	0.95a	1.03b	nodata	0.80a	0.74a	0.75a	nodata
<u>basesat</u>	23.5a	27.5a	24.78a	23.33a	31.0ab	23.17a	33.52ab	37.17b	27.67a	24.67a	32.63ab	42.5b
<u>Exch. Ca²⁺</u>	1.67a	1.98a	0.52a	0.55a	1.25a	1.27a	1.32a	1.03a	0.483a	1.35b	1.217b	1.517b
<u>CEC</u>	6.82a	6.42a	3.73a	4.03a	6.72a	5.53a	5.63a	5.13a	5.05a	5.02a	6.23a	6.02a
<u>C:N</u>	27.47a	20.92b	12.79c	15.63bc	19.02a	18.38a	11.82b	14.55b	14.15a	17.45b	10.01c	13.08a
<u>Exch. K⁺</u>	0.13a	0.22b	0.1a	0.1a	0.15a	0.22b	0.13a	0.15a	0.22a	0.2a	0.18a	0.17a
<u>Exch. Mg²⁺</u>	1.52a	1.48a	0.4a	0.37a	1.35a	0.85a	0.9a	0.7a	0.65a	0.52a	0.58a	0.78a
<u>Exch. Na⁺</u>	bd	0.05	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
<u>pH(salt)</u>	4.22a	4.6b	4.2a	4.17a	4.15a	4.42b	4.3ab	4.32ab	4.33a	4.67b	4.367a	4.373a
<u>sumbase</u>	3.32a	3.72a	1.02a	1.02a	2.78a	2.32a	2.35a	1.88a	1.35a	2.07ab	1.98ab	2.47b
<u>IN</u>	0.017a	0.048b	0.049b	0.036b	0.022a	0.056b	0.048c	0.036d	0.034a	0.068b	0.069b	0.043a
<u>TOC</u>	0.4a	0.95b	0.65a	0.56a	0.4a	0.97b	0.55c	0.51ab	0.45a	1.2b	0.7c	0.59bc
<u>pH(water)</u>	4.92a	5.37b	4.72a	4.73a	5	5.383	4.817	4.868	5.2a	5.45b	4.8c	5.01d

30-40		CON				SEL				CC			
30-40		2011	2013	2015	2016	2011	2013	2015	2016	2011	2013	2015	2016
Variable		0.73a	0.64a	0.85a	nodata	3.02a	1.15a	1.23b	nodata	1.12a	0.84a	0.85a	nodata
Al _r													
basesat		32.33a	31.33a	21.69a	23.69a	36.33a	24.67b	33.27ab	33.83ab	29.5ab	24.17a	33.61b	38.83ab
Exch. Ca ²⁺		2.1a	2.63a	1.38a	1.38a	1.53a	1.22a	1.6a	1.12a	0.55a	1.23b	1.12ab	1.38b
CEC		6.93a	6.42a	6.32a	6.32a	7.63a	5.8a	7.02a	6.22a	5.37ab	4.45a	5.82ab	6.72b
C:N		25.41a	21.34a	14.56b	14.56b	18.18a	18.69a	12.61b	13.02b	17.76a	17.15a	10.86b	13.12ab
Exch. K ⁺		0.15ab	0.2a	0.08b	0.08b	0.17a	0.18a	0.13a	0.15a	0.22a	0.2a	0.17a	0.2a
Exch. Mg ²⁺		2.05a	2.17a	1.13a	1.13a	1.47a	0.98a	1.15a	0.87a	0.85a	0.55a	0.68a	1.02a
Exch. Na ⁺		bd	0.033	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
pH(salt)		4.23a	4.93b	4.25a	4.25a	4.13a	4.72b	4.25a	4.16a	4.25a	4.67b	4.35a	4.29a
sumbase		4.32a	5.0a	2.6a	2.6a	3.18a	2.37a	2.88a	2.13a	1.65a	1.93a	1.97a	2.6a
TN		0.012a	0.035bc	0.045b	0.045c	0.017a	0.041bc	0.049b	0.034c	0.021a	0.055b	0.051b	0.032a
TOC		0.27a	0.7b	0.65b	0.65a	0.28a	0.75b	0.62b	0.44a	0.3a	0.92b	0.57c	0.42bc
pH(water)		5.0a	5.45b	4.8a	4.8a	5.0a	5.27b	4.9a	4.85a	5.13a	5.43b	4.97a	4.97a

Key to abbreviations: TN – Total Nitrogen; TOC – Total Organic Carbon; sumbases – sum of bases; ECEC Effective Cation Exchange Capacity; [basesat](#); [base](#)

Saturation. [bd](#) indicates that the analyte was below the limits of detectability of the instrument.

CHAPTER 3: SOIL MICROBIAL COMMUNITY AND
GEOCHEMICAL RESPONSE TO CLEARCUT AND SINGLE-TREE
SELECTION HARVESTS FIVE YEARS POST-HARVEST

A.W. Taylor^{a*}, K.W. Goyne^a, J.M. Kabrick^b, K.S. Veum^c, and R.J. Kremer^a

^a School of Natural Resources, University of Missouri, 103 ABNR Bldg., Columbia, MO
65211, USA

^b USDA Forest Service, Northern Research Station, 203 ABNR Bldg., Columbia, MO
65211, USA

^c USDA-Agricultural Research Service, Cropping Systems and Water Quality Research
Unit, 269 Ag. Eng. Bldg. Columbia, MO 65211, USA

* *Corresponding author*: School of Natural Resources, University of Missouri, 103
ABNR Bldg., Columbia, MO 65211, USA; email: awtkvf@mail.missouri.edu (A.W.
Taylor)

Abstract

The importance of robust and diverse microbial communities for supporting healthy forest ecosystems is increasingly being recognized. Forest harvesting has been shown to affect soil microbial communities; however, the impact is variable based upon climate, forest composition, and harvest level. This study was conducted to identify changes to soil microbial community structure, soil microbial function and geochemical soil properties related to microbial populations from timber harvesting in the Central Hardwoods Region of the United States. The effects of clearcutting with reserves, single-tree selection and no-harvest (control) management on soil properties were evaluated five years post-harvest at the Missouri Ozarks Forest Ecosystem Project site in southern Missouri, U.S.A. Soil microbial characteristics were evaluated via Phospholipid Fatty Acid (PLFA) analysis (microbial community structure and biomass), activities of four soil enzymes (microbial community function), and soil chemical analyses (e.g., pH, total organic C, total N, active C, etc.). A significant increase in soil pH (from pH 4.30 to 5.47) and an improvement in microbial substrate quality, as measured by the ratios of soil total organic carbon to total nitrogen and total organic carbon to active carbon were found in the clearcut harvest treatment relative to control management plots. These changes contributed to significant shifts in the following microbial functional groups within clearcuts relative to control treatment: saprophytic fungi (25.2% decrease); arbuscular mycorrhizal fungi (86.1% increase); and eukaryotes (14.4% increase). Two PLFA-derived ratios, fungal:bacterial (38.2% decrease) and actinobacterial:fungal (41.9% increase), also changed significantly within clearcuts compared to control treatment, indicating a shift in the microbial community toward single-celled decomposers.

Although most geochemical properties showed nominal to significant increases within clearcut treatments relative to control treatment, Bray-1 extractable phosphorus concentration and acid phosphomonoesterase (AcdP) activity declined significantly (32.9 and 30.8%, respectively). Single-tree selection harvest exhibited little change from control treatments. With the notable exception of available phosphorous levels and AcdP activity, abundant and dispersed slash remaining onsite following a bole-only clearcut harvest provided the microbial communities with improved pH and substrate quality conditions.

Highlights

- Robust and diverse soil microbial communities are essential to healthy forest ecosystems
- The impact of clearcut with slash retention and single-tree selection on soil microbial functional groups, soil enzyme activities and soil geochemical properties were examined
- Increases in pH and substrate quality found within clearcuts
- Decreases in fungal:bacterial ratio and increases in actinobacterial:fungal ratio indicate potential population shifts within clearcuts
- Substantial reduction in concentrations of available phosphorus (P) within clearcuts highlight potential risk in the P-limited soils of the Missouri Ozarks

Single-tree selection harvest effects on microbial communities were indistinguishable from control treatments.

3.0 Introduction

The important role of robust and diverse microbial communities for supporting healthy forest ecosystems has been increasingly recognized (Hartmann et al., 2012; Holden and Treseder, 2013; Lewandowski et al., 2015; Lladó et al., 2017). Soil microbial communities regulate organic matter decomposition, nutrient cycling and nutrient uptake by plants. However, the site-specific nature of soil microbial community composition and the exact role and contribution of microbial communities to forest ecosystem processes are still not well understood (Hartmann et al., 2012; Lladó et al., 2017). Previous research suggests that soil microbial communities and enzyme activity levels are altered as a result of harvest activities (Marshall, 2000; Hartmann et al., 2012; Holden and Treseder, 2013; Fichtner et al., 2014). These effects were found to be enhanced by clearcutting compared to partial harvesting methods (Holden and Treseder, 2013). The effects of harvest activity on fungal communities appear to be greater than on bacterial populations (Siira-Pietikäinen et al., 2001; Hartmann et al., 2012; Holden and Treseder, 2013), leading to the proposition that a fungal:bacterial ratio might be a suitable indicator of soil disturbance (Frostegård and Bååth, 1996). However, the variability of shifts in microbial community structure observed in other studies indicate that an element of site-specificity is associated with microbial change (Hartmann et al., 2012; Lewandowski et al., 2015). The causal phenomena for shifts in microbial communities has been attributed to microclimate factors (e.g., air and soil temperature and moisture), availability and quality of soil organic matter (Malik et al., 2016), and soil chemical properties (e.g., soil solution nutrient concentrations and pH) (Högberg et al., 2013).

3.1 Objectives

The objectives of this study were to examine the effects of clearcutting and single-tree selection harvesting on soil microbial communities and related soil biogeochemical properties in the Central Hardwoods region of the United States. To do this we used soil geochemical properties, soil enzymatic analysis, and an assessment of microbial community structure to gain greater insight into nutrient cycling processes active at our research site.

The geochemical properties selected for this study are those regarded to be closely linked to microbial activity: total organic carbon (TOC); active carbon (AC); total nitrogen (TN) and potentially mineralizable nitrogen (PMN); total sulfur (TS); Bray-1 extractable phosphorus (Bray1P) and pH determined in 0.01M CaCl₂. The soil enzyme analysis focused on four enzyme assays well-documented to be sensitive to changes in soil management: β -glucosidase (β -glu) – involved in the breakdown of cellulose; N-acetyl- β -D-glucosaminidase (NAG) - an important enzyme in the breakdown of chitin; arylsulfatase (Aryl) – a common enzyme involved in the conversion of organic sulfur-bearing compounds to available inorganic S forms; and acid phosphomonoesterase (AcdP) – an assay for determining the breakdown of organic phosphorous-bearing compounds in acidic soil environments. Soil microbial community structure and biomass, including: total microbial; total bacterial; saprophytic fungal; actinobacterial; arbuscular mycorrhizal fungal; eukaryotes; gram positive and gram negative bacterial biomarkers were analyzed via the use of Phospholipid Fatty Acids (PLFA).

3.2 Materials and Methods

3.2.1 Site Description

The Missouri Forest Ecosystem Project (MOFEP) is located in south-central Missouri, USA, within the counties of Shannon, Reynolds, and Carter (37° 08'N, 91° 00'W). The MOFEP experiment was initiated in 1989 by the Missouri Department of Conservation as a long-term, landscape-scale study examining the effects of even-aged and uneven-aged forest management methods applied in an operational manner on the flora and fauna of the Ozark Highlands. The experiment site is located within the Current River Oak-Pine Woodland Hills and Current River Forest Breaks land type associations of the Ozark Highlands (Shifley and Kabrick, 2002). Soils at the MOFEP site are primarily derived from parent materials of Ordovician-aged Roubidoux and the Gasconade formations and the Cambrian-aged Eminence formation. These marine sedimentary rocks are primarily composed of chert, dolomite and quartz sandstones and strongly influence the physical and chemical properties of the overlying soils. The soils in the region range from highly weathered very gravelly pedisements classed as Ultisols (Typic Paleudult and Typic Hapludult) to gravel-free clayey pedisements and residuum classified as Alfisols (Typic Paleudalf and Typic Hapludalf) (Kabrick et al., 2008).

The Current River Oak-Pine Woodland Hills have broad ridges and less than 90 m of relief. In contrast, the Current River Oak Forest Breaks have narrow ridges and steep relief that ranges from 90 to 140m.(Meinert et al., 1997).The forests at MOFEP are characterized by mixed hardwood and hardwood-softwood forests. Four oak species predominate the area making up 71% of the basal area: white oak (*Quercus alba*); black oak (*Quercus velutina*); scarlet oak (*Quercus coccinea*); and post oak (*Quercus stellata*). Shortleaf Pine (*Pinus echinata*), various species of hickory (*Carya spp.*), flowering

dogwood (*Cornus florida*), blackgum (*Nyssa sylvatica*) and various other oak species (*Quercus spp.*) make up the remainder of tree species in the forest (Kabrick et al., 2008).]

3.2.2 Experimental Design

The MOFEP site is a randomized complete block design encompassing nine compartments ranging in size from 314 to 516 hectares (Figure 2.1). Each of the three blocks comprise three compartments assigned by their spatial proximity. In each block, three forest management treatments were randomly assigned to a compartment: no-harvest (control); uneven-aged management (with group or single-tree selection harvest); and even-aged management (with clearcutting). Approximately 10 percent of each site is left as old growth. The old growth reserve will be preserved from harvest activities on a permanent basis. Harvests entries in compartments occur at 10 to 15 year intervals. Even-aged and uneven-aged management was practiced according to MDC Forest Land Management Guidelines. Even-aged management consisted of a rotation of 80-100 years resulting in a regulated harvest of 10-12.5% of the area per entry period (Shifley and Kabrick, 2002). Nearly all non-merchantable timber was felled during a slash operation after the removal of merchantable boles at clearcut sites (Sheriff, 2002). Some snag or den trees were preserved to provide shelter for wildlife. Additionally, some shortleaf pine was preserved to provide seed for pine regeneration. Uneven-aged management utilized single-tree and group selection harvest, with a target tree size class distribution equivalent to the composite size class distribution across the even-aged management sites (Shifley and Kabrick, 2002).

3.2.3 Our Study

A detailed soil survey that was previously conducted at the MOFEP study sites (Meinert et al., 1997) provided a means for examining harvested stands having different levels of soil nutrient availability. Prior to the planned second harvest entry in 2011, the nine harvest sites were surveyed to determine nutrient status. Nutrient status was determined on the basis of the base cation saturation (BS) in their taxonomic diagnostic subsurface horizon. Low (<20% BS) nutrient status soils are primarily found on the upper backslope landscape position, formed in weathered from hillslope sediments and included the Clarksville or similar soil series. In contrast, medium (20-50% BS) nutrient status soils are usually found on the lower backslope landscape position, are formed in weathered hillslope sediments over clayey residuum, and included the Alred or similar soil series. After the identification of suitable forest stands, professional foresters and soil scientists evaluated candidate sites to determine if the forest and soils were representative of the area. Subsequently, long-term monitoring sites were established on low and medium nutrient status soils within each compartment (18 sampling sites total). Each of the eighteen sites were excavated to a depth of 1m either by manual excavation or backhoe. Care was taken to ensure that the sidewalls, upslope and lateral positions to the pit remained undisturbed. Excavated soil was piled in the downslope position. Pre-harvest samples were taken in 10 cm increments and analyzed to confirm nutrient status.

The representative soil series of our study area are Clarksville (loamy-skeletal, siliceous, semiactive, mesic Typic Paleudults) and Alred (loamy-skeletal over clayey, siliceous semiactive, mesic Typic Paleudalfs). Clarksville is very deep, somewhat excessively well-drained, and formed in hillslope sediments overlying clayey residuum

derived from cherty limestones and dolomites. Alred is very deep, well-drained, and formed in cherty hillslope sediments overlying clayey residuum (Simmons et al., 2006).

3.2.4 Soil Sampling

Three distinct bulk soil sampling campaigns were performed over the course of the study. The first collection, intended for geochemical analysis, consisted of three subsamples collected from top 10 cm of the mineral soil at each of the treatment-nutrient status sites (3 subsamples x 2 nutrient status x 3 treatments x 3 replicates) in the winter of 2016 (approximately five years post-harvest). Similarly, a second and third set of samples were collected in May 2016 and June 2017, respectively, for analysis of microbial and enzymatic properties. To focus on the zone of greatest potential biological change (Lladó et al., 2017), the top 5 cm of the mineral soil was sampled for microbial analyses.

Samples were transported to the University of Missouri under ambient temperature and field moist conditions. The geochemical subsamples were air-dried and sieved to ≤ 2 mm, composited and stored for subsequent analysis. Samples for biological analysis were moist sieved to ≤ 2 mm and subdivided. One portion was lyophilized and stored at -20°C for analysis of microbial community structure. The other portion was stored at -4°C for analysis of soil microbial function and pH. Soil moisture content was determined by drying five grams of field moist soil in a Fisher Scientific IsoTemp drying oven (Fisher Scientific; Waltham MA) for 48 hours at 105°C , and weighing the oven-dry soil on a Mettler Toledo PB3002-S analytical balance (Mettler Toledo; Columbus, OH).

3.2.5 Analysis of Soil Microbial Community Structure

Phospholipid fatty acid (PLFA) analysis was performed by the using a high throughput method, allowing for up to 96 samples to be processed simultaneously (Buyer and Sasser, 2012). Soil samples were lyophilized in a centrifugal evaporator after which lipid extraction was performed using the Bligh-Dyer method (Bligh and Dyer, 1959) and the high-throughput extraction, separation, and transesterification protocol of Buyer and Sasser (2012). The final extract is dissolved in hexane and analyzed via gas chromatography for the relative amounts of fatty acid methyl esters present. The fatty acid biomarkers were assigned to microbial groups using MIDI Sherlock™ microbial identification system PLFAD1 software version 1.10 (MIDI, Inc.; Newark, DE). A list of the specific fatty acids assigned to each microbial group can be found in the *Supplemental Materials*. The three subsamples collected from each site for microbial analyses were analyzed separately and the results were averaged prior to data analysis.

3.2.6 Analysis of Soil Microbial Community Function

Soils were analyzed for the activities of four microbial enzymes: β -glucosidase (β -glu); N-acetyl- β -D-glucosaminidase (NAG); arylsulfatase (Aryl); and acid phosphomonoesterase (AcdP). Enzyme activities were determined using procedures outlined in Dick et al. (2011). The only modification introduced to the procedures was elimination of toluene to improve safety. All enzyme procedures involved introducing 1g of field moist soil into a 50ml flask and adding 1 ml of the appropriate substrate to 4 ml of acidic buffer. Samples were capped and incubated in the dark for 1 hour at 37°C. After incubation, 4 ml of a basic buffering solution and 1 ml of 0.5M CaCl₂ were added to the flask, and samples were gently mixed to terminate the reaction. The solution was

vacuum-filtered through Fisher P4 cellulose fiber filter paper with a particle retention of 4-8 μm . Approximately 3.5 ml of the filtrate was transferred to a cuvette and absorbance was measured at 405 nm using a Spectronics Genesys 8 spectrophotometer (Spectronics Camspec Ltd; Garforth, England)). Standards of p-nitrophenol (PNP) solution in THAM at 0, 2.5, 5, 10, 15, and 25 $\mu\text{g PNP ml}^{-1}$ were prepared and analyzed. All measurements were expressed as $\mu\text{g PNP ml}^{-1} \text{ gram}^{-1} \text{ soil hour}^{-1}$ adjusted to a dry-mass basis. The three subsamples collected from each site for microbial analyses were analyzed separately, and the results were averaged prior to data analysis.

3.2.7 Geochemical Analysis

Total organic carbon (TOC) and total nitrogen (TN) content were measured using a LECO TruMac CN combustion analyzer model 630-300-400 and total sulfur content was quantified using a LECO Sulfur-Carbon analyzer model SC-144DR (LECO Corporation; St. Joseph, MI). Potentially mineralizable nitrogen (PMN) content was determined using a modification of the 7-day anaerobic incubation method described by Anderson et al. (2010). The modification involved reduction of soil mass from 20g to 8g for improved throughput. Following incubation, 2M KCl was used to extract ammonium, which was quantified using the colorimetric method of Nelson (1983), and absorbance of the solution was measured at 550 nm on a spectrophotometer to determine $\text{NH}_4^+\text{-N}$ concentration. Active carbon content was determined using the method employed by the USDA NRCS (Weil et al., 2003). In short, five grams of soil was added to a 0.02M KMnO_4 solution. The suspension was shaken for 2 minutes and then allowed to settle for 5 to 10 minutes. One-half milliliter of supernatant was extracted and added to de-ionized

water to attain a total volume of 50ml. Absorbance at 550nm was read on a spectrophotometer to determine active carbon concentration. Available phosphorus was determined using the Bray P-1 method and soil pH was determined using a 1:2 slurry of soil and 0.01M CaCl₂ solution (Burt, 2004). Samples were shaken for 1 hour, allowed to settle for 5 minutes and pH was measured on an Accumet AR-60 pH meter equipped with an Accumet 13-620-285 electrode (Fisher Scientific; Waltham, MA).

3.2.8 Statistical Analysis

A two-way analysis of variance (ANOVA) using a linear mixed model (Proc Mixed) in SAS 9.4 (SAS Institute; Cary, NC) was used to evaluate effects of treatment (clearcut, single tree selection, and control), nutrient status (low and medium) on solid phase chemistry and microbial community variables.

The experimental design of MOFEP allows for the examination of treatments, soil nutrient status, and their interactions by analyzing the data using a statistical model for a split-plot analysis with different error terms (see Brookshire and Shifley, 1997). To test for treatment effects (i.e., the whole plot effects), the appropriate error term is the treatment x block interaction. To test for effects of soil nutrient status (i.e., the split plot effects) and its interaction with treatments, the three-way interaction of treatment x soil nutrient status x block is used. The data were tested for normality using the Shapiro-Wilk test. Those data which did not pass the test for normality were transformed using a square root transformation. Least squares differences were computed to determine marginally

significant differences ($p < 0.10$) and significant differences ($p < 0.05$ and $p < 0.01$) between treatment and nutrient status.

A single significant outlier was observed in the total sulfur and arylsulfatase activity at treatment site 4 (single tree selection), a medium nutrient status plot. This single outlier was removed and the dataset was re-analyzed. Pearson's correlation coefficients were computed in SAS 9.4 to identify relationships between the dependent variables ($p < 0.05$) within the 0 to 10 cm geochemical dataset and separately for the 0 to 5 cm biological dataset.

3.3 Results

3.3.1 Soil Microbial Community Structure

There were no significant changes in total microbial biomass (as estimated by PLFA biomarkers) as a result of harvest activity. However, there were significant changes in the biomass of individual microbial groups (Table 1). Saprophytic fungi exhibited a significant decrease (25%; $p < 0.01$) in clearcut treatments relative to the control treatment. However, the arbuscular mycorrhizal fungi increased by 86% ($p < 0.05$). The eukaryote group marginally increased in within clearcuts by 14% ($p < 0.10$), relative to the control treatment. Nominal increases were observed within clearcuts for all other microbial functional groups.

Due to differences in the estimated biomass of several microbial groups, differences in the relative abundance (i.e. proportion) of microbial groups relative to total microbial biomass were calculated and analyzed. In comparison to the control treatment, biomarkers for saprophytic fungi in clear-cuts significantly decreased ($p < 0.01$) from 5% to 3% of the total biomass (Figure 1). In contrast, actinobacteria marginally significantly increased ($p < 0.10$) from 13% to 14%, and arbuscular mycorrhizal fungi significantly increased ($p < 0.01$) from 4% to 5%, relative to the control treatment. Within single-tree selection treatments, saprophytic fungi significantly ($p < 0.01$) decreased from 5.1% to 4.5%, relative to the control treatment.

Table 3.1. Differences in biological and geochemical soil properties five years after imposing clearcut, single-tree selection (SEL), and Control (CON) treatments. Significant differences from control are indicated by * $p < 0.10$ (marginal significance), ** $p < 0.05$ and *** $p < 0.01$. Within rows, Means with a different letter are significantly different at the $p < 0.10$ level.

<u>Property</u>	<u>Treatment</u>		
	<u>Control</u>	<u>Selection</u>	<u>Clearcut</u>
<u>Microbial Functional Groups</u>			
TotPLFA (pmol g dry soil ⁻¹)	170600a	169700a	181600a
Bact (pmol g dry soil ⁻¹)	83590a	84840a	93610a
Act (pmol g dry soil ⁻¹)	15600a	16100a	18450a
AMF (pmol g dry soil ⁻¹)	5044a	5311a	7012b**
Euk (pmol g dry soil ⁻¹)	3460a	3128a	3959b*
Fung (pmol g dry soil ⁻¹)	5970a	5287a	4467b***
G(+) (pmol g dry soil ⁻¹)	29786a	30319a	32382a
G(-) (pmol g dry soil ⁻¹)	59368a	59971a	67068a
Act:Fung	3.51a	3.19a	4.98b***
Fung:Bact	0.076a	0.065b**	0.047c***
<u>Geochemical</u>			
Active C (mg C kg ⁻¹)	198.48a	261.6a	308.4a
Bray1P (mg P kg ⁻¹)	7.87a	8.3a	5.28b*
pH	4.3a	4.23a	5.47b***
PMN (mg N kg ⁻¹)	49.5a	59.5a	56.83a
TN (g N kg ⁻¹)	1.55a	1.47a	2.09b*
TOC (g C kg ⁻¹)	31.52a	34.51a	37.26a
TS (g S kg ⁻¹)	0.10a	0.10a	0.13b***
C:N	23.15a	22.37a	17.68b***
TOC:AC	97.28a	69.91b**	63.51b**
<u>Enzyme Activities</u>			
AcdP (µg PNP g ⁻¹ hr ⁻¹)	651.3a	551.9b*	450.8c***
Aryl (µg PNP g ⁻¹ hr ⁻¹)	154.92a	125.95a	265.8b*
β-glu (µg PNP g ⁻¹ hr ⁻¹)	63.52a	59.72a	77.28a
NAG (µg PNP g ⁻¹ hr ⁻¹)	80.95a	79.03a	87.97a

Key to abbreviations: TotPLFA – total microbial biomass via PLFA; Bact – total bacteria; Act – actinobacteria; AMF – arbuscular mycorrhizal fungi; Euk – eukaryotes; Fung – fungal biomass; G(+) – gram positive bacteria; G(-) – gram negative bacteria; Act:Fung – actinobacterial to fungal ratio; Fung:Bact – fungal to bacterial ratio; Active C – active carbon; Bray1P – Bray-1 extractable phosphorus; PMN – potentially mineralizable nitrogen; TN – total nitrogen; TOC – total organic carbon; TS – total sulfur; C:N – total organic carbon to total nitrogen ratio; TOC:AC – total organic carbon to active carbon ratio; PNP - p-nitrophenol; AcdP – acid phosphomonoesterase activity; Aryl – arylsulfatase activity; B-glu – β-glucosidase activity; NAG – N-Acetyl-β-D-glucosaminidase activity.

Figure 3.1. Relative distribution of important soil microbial functional groups within (A) clearcut, (B) single-tree selection (STS), and (C) control treatments. Asterisks denote significant ($p < 0.10$) changes relative to the NH treatment.

The two derivative PLFA measures, the fungal to bacterial ratio and actinobacterial to fungal ratio both experienced significant changes. The fungal:bacterial ratio and actinobacterial:fungal ratio were found to alternately significantly decrease (38%; $p < 0.01$) and increase (42%; $p < 0.01$) in the clearcut treatment relative to control treatment. Within single-tree selection treatments, the fungal:bacterial ratio was found to have decreased significantly (14.5%; $p < 0.05$).

3.3.2 Geochemical Properties – Clearcut Treatment

There were nominal increases in total organic carbon (18%) and active carbon (55%) and a marginally significant increase in total nitrogen (35%; $p < 0.10$), significant changes were observed for the C:N and TOC:AC ratios of the clearcut treatments when compared to control treatment (Figure 2.). The C:N ratio and TOC:AC ratio significantly decreased (24 and 35%, respectively; $p < 0.05$) in clearcuts relative to control treatments. Active carbon displayed an increase in standard deviation from 40.7 to 157.4 mg C kg⁻¹ dry soil in the clearcuts treatments relative to control, potentially masking statistically significant changes. Total sulfur significantly increased by 29% ($p < 0.01$), Bray-1 extractable phosphorus marginally decreased (32.9%; $p < 0.10$) in clearcuts compared to control treatment, and pH significantly increased (27.2%; $p < 0.01$) from a highly acidic pH of 4.3 to a more moderate value of pH 5.5 in the clear-cut treatment compared to the control. The single-tree selection treatment appeared to only affect the TOC:AC ratio which demonstrated an increase relative to the control treatment (28%; $p < 0.05$). Figures for

selected geochemical responses to harvest treatments can be found in *Supplemental Materials*.

Figure 3.2. Boxplots of (A) carbon to nitrogen ratio; (B) total organic carbon to active carbon ratio; and (c) pH for control (CON), single-tree selection (SEL) and clearcut (CC) harvest treatments: Means with different lower-case letters are significantly different at $p < 0.10$.

3.3.3 Soil Enzyme Activities

Activities of the β -glu and NAG soil enzymes were not affected by treatment, although the variability of NAG did increase in the clearcut treatments relative to the control. A marginally significant increase in arylsulfatase activity (30%; $p < 0.10$) was noted in the clearcut treatments when compared to the control treatment. A statistically significant decrease (30.8%; $p < 0.01$) in acid phosphomonoesterase activity was observed in the clearcut treatments relative to the control. Single-tree selection harvest marginally decreased AcdP activity (15.5%; $p < 0.1$) relative to the control treatments.

3.3.4 Soil Nutrient Status Effects and Interactions

Although soil nutrient status (low and medium) significantly affected biological and chemical properties at the control sites (Table 2), evaluation of data from all treatments yielded few significant differences between the soils studied. The TOC:AC ratio in medium nutrient status soil was marginally reduced (11.7%; $p < 0.10$) relative to the low nutrient status sites. Significant interactions between harvest treatment and soil nutrient status were not observed.

Table 3.2. Response of measured soil properties by soil nutrient status for the control treatments, highlighting the effect that base saturation has on soil properties in undisturbed ecosystems. Significant differences in nutrient status control sites are indicated by * $p < 0.10$, ** $p < 0.05$ and *** $p < 0.01$. Within rows, means with different lower-case letters are significantly different at $p < 0.10$.

<u>Property</u>	<u>Medium Control</u>	<u>Low Control</u>
<u>Microbial Functional Groups</u>		
TotPLFA (pmol g dry soil ⁻¹)	199700a**	141500b
Bact (pmol g dry soil ⁻¹)	99810a**	67380b
Act (pmol g dry soil ⁻¹)	18320a*	12890b
AMF (pmol g dry soil ⁻¹)	6116a*	3972b
Euk (pmol g dry soil ⁻¹)	3913a	3005a
Fung (pmol g dry soil ⁻¹)	6494a	5446a
GPos (pmol g dry soil ⁻¹)	35330a**	24240b
GNeg (pmol g dry soil ⁻¹)	71350a*	47390b
Act:Fung	3.926a	3.094a
Fung:Bact	0.069a	0.083a
<u>Geochemical</u>		
ActiveC (mg C kg ⁻¹)	182.6a	214.3a
Bray1P (mg P kg ⁻¹)	7.70a	8.03a
pH	4.37a	4.22a
PMN (mg N kg ⁻¹)	50.8a	48.2a
TN (g N kg ⁻¹)	2.01a**	1.08b
TOC (g C kg ⁻¹)	42.9a***	26.1b
TS (g S kg ⁻¹)	0.10a	0.10a
C:N	21.8a	24.5a
TOC:AC	93.9a	100.7a
<u>Enzyme Activities</u>		
AcidP (µg PNP g ⁻¹ hr ⁻¹)	786.1a**	516.5b
Aryl (µg PNP g ⁻¹ hr ⁻¹)	212.6a*	97.2b
β-glu (µg PNP g ⁻¹ hr ⁻¹)	81.9a**	45.1b
NAG (µg PNP g ⁻¹ hr ⁻¹)	91.0a*	70.9b

Key to abbreviations: TotPLFA – total microbial biomass via PLFA; Bact – total bacterial biomass; Act – actinobacterial biomass; AMF – arbuscular mycorrhizal fungal biomass; Euk – eukaryote biomass; Fung – fungal biomass; G(+) – gram positive bacterial biomass; G(-) – gram negative bacterial biomass; Act:Fung – actinobacterial to fungal ratio; Fung:Bact – fungal to bacterial ratio; Active C – active carbon; Bray1P – Bray-1 extractable phosphorus; PMN – potentially mineralizable nitrogen; TN – total nitrogen; TOC – total organic carbon; TS – total sulfur; C:N – total organic carbon to total nitrogen ratio; TOC:AC – total organic carbon to active carbon ratio; PNP - p-nitrophenol AcidP – acid phosphomonoesterase activity; Aryl – arylsulfatase activity; B-glu – β-glucosidase activity; NAG – N-Acetyl-β-D-glucosaminidase activity.

3.4 Discussion

3.4.1 Microbial Activity and Population Shifts

A recent meta-analysis of microbial community responses to harvest disruption across multiple biomes indicates that, across studies, decreases in microbial biomass may be the norm. However, the authors noted that changes in bacterial biomass in temperate climates (comparable to our study area) appeared to be non-significant (Holden and Treseder, 2013). This finding is in direct contrast to our reported results, which found only a significant decrease in saprophytic fungi in clearcut treatments, and either no increase to significant increases in other functional groups. The underlying causes of our reported findings may be rooted in the changes in soil geochemistry observed in the harvested treatments. A number of studies have suggested that two of the most important drivers altering soil microbial community composition are changes in soil pH (Rousk et al., 2009; Lauber et al., 2009; Lladó et al., 2017) and the quality of substrate (Högberg et al., 2006; Rousk and Baath, 2007; Colombo et al., 2016; Cyle et al., 2016; Lladó et al., 2017).

3.4.2 Soil pH

In our study, we found a significant increase in mean pH (4.30 to 5.47) in the clearcut treatment relative to control. Clearcut harvests have been documented to alternately increase (Marschner and Noble, 2000; Jerabkova et al., 2011; Hynes and Germida, 2013) and decrease (Johnson et al., 1991) soil pH. Increases in pH are primarily tied to the addition of significant amounts of slash material. It is important to note that pH increases following slash additions do not always occur. More recent studies have begun to propose

that forest litter addition may function to neutralize soil pH rather than simply creating acidic or basic conditions (Xu et al., 2006; Hong et al., 2018). In our study, pH was positively correlated with a number of the PLFA microbial groups including total bacteria, gram negative bacteria, actinobacteria, arbuscular mycorrhizal fungi and the actinobacterial:fungal ratio (Table 3.). We also found that pH was negatively correlated with the fungal:bacterial and carbon:nitrogen ratios. Although pH was not correlated with TOC, pH was positively correlated with AC, and negatively correlated with the TOC:AC ratio. These correlations indicate a linkage between pH and labile carbon stocks. A similar linkage was observed between pH and TN and PMN. Therefore, we hypothesize that the generous additions of slash material likely increased labile carbon and nitrogen stocks and increased pH, creating an atmosphere more conducive to activity and growth of bacterial populations.

Table 3.3. Pearson's correlation coefficients between various soil properties and pH.

Values highlighted with * are significant at the $p < 0.05$ level. Values in bold were found to be strongly correlated ($|r| > 0.7$).

<u>Property</u>	<u>Pearson's r</u>
<u>Microbial Functional Groups</u>	
TotPLFA (pmol g dry soil ⁻¹)	0.49
Bact (pmol g dry soil ⁻¹)	0.57*
Act (pmol g dry soil ⁻¹)	0.66*
AMF (pmol g dry soil ⁻¹)	0.81*
Euk (pmol g dry soil ⁻¹)	0.48
Fung (pmol g dry soil ⁻¹)	-0.21
G(+) (pmol g dry soil ⁻¹)	0.42
G(-) (pmol g dry soil ⁻¹)	0.59*
Act:Fung	0.51*
Fung:Bact	-0.58*
<u>Geochemical</u>	

Active C (mg C kg ⁻¹)	0.72*
Bray1P (mg P kg ⁻¹)	-0.15
pH	1
PMN (mg N kg ⁻¹)	0.66*
TN (g N kg ⁻¹)	0.66*
TOC (g C kg ⁻¹)	0.26
TS (g S kg ⁻¹)	0.5*
C:N	-0.79*
TOC:AC	-0.64*
<u>Enzyme Activities</u>	
AcidP (μg PNP g ⁻¹ hr ⁻¹)	-0.37
Aryl (μg PNP g ⁻¹ hr ⁻¹)	0.84*
β-glu (μg PNP g ⁻¹ hr ⁻¹)	0.36
NAG (μg PNP g ⁻¹ hr ⁻¹)	0.27

Key to abbreviations: TotPLFA – total microbial biomass via PLFA; Bact – total bacterial biomass; Act – actinobacterial biomass; AMF – arbuscular mycorrhizal fungal biomass; Euk – eukaryote biomass; Fung – fungal biomass; G(+) – gram positive bacterial biomass; G(-) – gram negative bacterial biomass; Act:Fung – actinobacterial to fungal ratio; Fung:Bact – fungal to bacterial ratio; Active C – active carbon; Bray1P – Bray-1 extractable phosphorus; PMN – potentially mineralizable nitrogen; TN – total nitrogen; TOC – total organic carbon; TS – total sulfur; C:N – total organic carbon to total nitrogen ratio; TOC:AC – total organic carbon to active carbon ratio; PNP - p-nitrophenol AcidP – acid phosphomonoesterase activity; Aryl – arylsulfatase activity; B-glu – β-glucosidase activity; NAG – N-Acetyl-β-D-glucosaminidase activity.

3.4.3 Substrate Quality and Volume

Two potential indicators of changes in substrate quality, the C:N ratio and the TOC:AC ratio, significantly decreased in the clearcut treatments relative to the control, and lower values indicate better substrate quality (S. Enríquez et al., 1993; Blair et al., 2001). Interpretation of remotely-sensed imagery sourced from Google Earth Pro (Google LLC; Mountain View, CA) collected two years post-harvest (May 13, 2013) reveal a broad and random dispersion of slash and unmerchantable timber in the clearcut harvest plots. A number of studies have identified significant increases in the coarse woody debris content in clearcuts, relative to control sites (Li et al., 2007). Increases in the total volume of available substrate and in the quality of the substrate at the 5-year post-harvest interval likely contributed to greater bacterial populations observed in

clearcut sites. In contrast, slash distribution in single-tree selection treatments would be more geographically localized, generally found towards the top of the position of the felled tree, thus increasing the potential for being missed during randomized soil sampling. Physical examination of the clearcut plots on a yearly basis shows vigorous re-growth of successional species. Indeed, by the summer of 2017, the clearcut plots were so overgrown and dense that access to the collection sites was difficult.

3.4.4 Roles of Microbial Functional Groups

It is important to reiterate that PLFA does not give information about specific microbial species. Using PLFA analysis, it is difficult to determine whether microbial populations are shifting in species makeup within microbial groupings, or are simply adapting to the changing pH and substrate availability within the clearcut environment. Regardless, it is important to consider the roles these communities have in the functioning of the forest ecosystem. Plant matter is primarily composed of three molecular structures - cellulose, hemicellulose and lignin. Although many different species of microbes are capable of breaking down cellulose and hemicellulose, the complex molecular structure of lignin renders it more recalcitrant. Only a few species of fungi (e.g., white rot fungi) are capable of breaking down lignin, ostensibly to gain access to occluded cellulose. More recent research has identified actinobacteria as an additional group of lignin decomposers (Eisenlord and Zak, 2010). Lewandowski et al. (2015) reported results that supported the potential for competitive interaction between actinobacterial and fungal communities within group selection harvest treatments. Although PLFA analysis cannot specify that the species of actinobacteria present in our samples are the lignin decomposers, future research with microbial genomic DNA could

be used to determine if lignin-decomposing actinobacteria are increasing in the clearcuts and occupying the ecological niche formerly filled by lignin-degrading fungi within the clearcuts. Regardless, an environment more conducive to actinobacteria, in particular the elevated pH, allowed this microbial group to increase within the clearcut harvest treatments.

Another PLFA group that increased in the clearcuts were the arbuscular mycorrhizal fungi. The mycorrhizal fungi form symbiotic relationships with plant species and have been shown to enhance the uptake of nitrogen and phosphorus by increasing the root surface area and by mineralizing organic nitrogen and phosphorous sources (Sylvia et al., 2005). In general, ectomycorrhizal fungi are associated with tree species (Lladó et al., 2017); whereas, arbuscular mycorrhizal fungi can be associated with many of the successional species found in our field area (Kormanik et al., 1982). Although there is no reliable way to determine ectomycorrhizal fungi via PLFA (Landeweert et al., 2003), it might be inferred that a shift to successional species within the canopy gaps following clearcutting allowed the arbuscular mycorrhizal fungi to gain a foothold.

3.4.5 Soil Enzyme Activities

Previous work at MOFEP found that β -glu and NAG enzyme activities initially decreased in the clearcut treatments relative to the control; however, at the two year post-harvest interval values within clearcuts increased over control sites (Hatch, 2014). Ponder and Eivazi (Ponder and Eivazi, 2008) examined AcdP, Aryl, β -glu, and NAG soil enzyme activities at a whole tree harvest site relative to control eight years post-harvest at a nearby U.S. Forest Service Long Term Soil Productivity site in Southern Missouri. They found significant differences only in β -glu activity. Although our study was performed

five years post-harvest, there is still evidence of nominal to significant increases in three of the soil enzyme activities tested (the exception being acid AcdP). Our approach to methodology diverged from Ponder and Eivazi (2008) study in three significant ways. First, they collected samples from the top 10 centimeters of the soil horizon, whereas we sampled from the top 5 cm, following the suggestion posited by Llado, et al. (Lladó et al., 2017) where the greatest changes in microbial activity will occur. Second, the authors included the data from the varying levels of organic matter removal (bole-only, whole tree harvest and whole-tree harvest+forest floor removal) as a single harvest treatment. The MOFEP study includes only bole-only harvest treatments. Lastly, Ponder and Eivazi (2008) composited the five subsamples prior to analysis; whereas we analyzed each subsample separately.

We speculate that a difference in sample collection methodology led to a difference in reported results between Ponder and Eivazi and this study. We also speculate that the aforementioned changes in substrate quality and pH created a more suitable environment for the many microbial producers of soil enzymes, resulting in an increase in enzyme activities. Although our study was performed five years post-harvest, there is still evidence of nominal to significant increases in three of the soil enzymes tested indicating the potential for elevated soil enzyme activity to persist for several years post-harvest (the exception being AcdP, which is discussed below).

3.4.6 The “Disturbance Effect”

One of the initial expectations of this study was a strong nutrient status-treatment interaction effect. Prior studies have shown that soils with greater base saturation respond differently to forest harvest than those with lesser base saturation (Johnson and Todd,

1998). In our study, nutrient status effects across all treatments were only observed for one dependent variables (i.e., total organic carbon:active carbon ratio). However, examination of the (non-significant) interactions between treatment and nutrient status of many of the dependent variables illuminated an interesting trend. Those dependent variables found to not have significantly changed, the control harvest medium nutrient status values were the highest mean values recorded and the control harvest low nutrient status values were the lowest mean values recorded. This indicates that soil nutrient status has a strong effect on many soil properties within unharvested forest. However, any treatment, regardless of whether it was selection or clearcut harvest convoluted this high-low relationship; the ranking of clearcut and single-tree harvest treatments were somewhat randomly distributed and statistically inseparable with respect to value. Response value figures for selected variables exhibiting this effect can be seen in *Supplemental Materials*. We have tentatively named this as the “disturbance effect”. Previous studies have not documented this change and it warrants further study.

3.4.7 Phosphorus

The observed decreases in available phosphorous and AcdP activity in this study are similar to results reported by other research scientists working in the region. Ponder and Eivazi (2008) examined Bray-1 P and AcdP activity eight years post clearcut harvest at the adjacent U.S. Forest Service Long Term Soil Productivity (LTSP) site and found decreases in Bray-1 phosphorous and in AcdP. It should be noted they also observed an increase in alkaline phosphomonoesterase concomitant with a slight increase of pH (5.6 to 5.9). The Bray-1 phosphorous method primarily targets weakly-held inorganic P complexes, such as an Al-P complex; whereas, acid phosphomonoesterase is an indicator

of the potential for breakdown of organic P forms available in the soil (Sparks et al., 1996).

Determining causal mechanisms for the decrease in phosphorous in the clearcut plots is difficult based on the soil properties we characterized. However, several previous studies at other locations may provide insight into processes that may be occurring. Lang et al. (2016) reviewed forest phosphorus cycling from an ecosystem perspective. They noted that the P-cycling process was distinctively different in P-limited compared to P-sufficient environments. Phosphorus cycling in P-sufficient environments is an open system, tuned towards acquiring phosphorus from primary minerals; whereas, P-limited sites are closed systems geared towards recycling phosphorus efficiently. They suggest microbial populations and changes in vegetative root system architecture effectively scavenge phosphorus in P-limited ecosystems. Olander and Vitousek (2005) examined P availability and retention in P-limited Oxisols. The experiment involved increasing P concentration in P-limited Oxisols of varying age via the addition of $^{32}\text{PO}_4^{3-}$ tracer. Phosphorous concentration, enzyme activities and soil microbial biomass phosphorous were measured in the labile and non-labile fractions of the organic and mineral horizons. Pre-treatment phosphorus concentrations and acid phosphatase activities were found to be significantly greater (as much as an order of magnitude) in the O horizon than in the first mineral horizon. The authors also found that the uptake of the phosphorus was extremely rapid – with 70% being sorbed or immobilized in the non-labile fraction within 30 minutes and 95% sorbed or immobilized with 48 hours. They concluded that when a pulse of available P that exceeds immediate biological demand enters the soil solution,

the proportion not consumed by biota is quickly sorbed by soil minerals and becomes unavailable for biological processes.

Singh et al. (2015) conducted extensive analysis into soil P within the Ozark Highlands. Total and available (Mehlich-3 and Bray-1) mean concentrations of phosphorus were found to be low (116.2, 7.87 and 5.81 mg kg⁻¹, respectively). The authors also examined explanatory variables for the low concentrations of total and available phosphorous in the MOFEP study area. The authors found that extractable manganese was the most significant explanatory variable for total (33%) and available phosphorus (32%). Lesser significant variables were total organic carbon and pH for total P and exchangeable calcium lithology and depth to A and B horizons for Bray-1 P. The authors suggest that P sorbs to manganese via weaker outer-sphere complexes and stronger inner-sphere complexes, particularly at pH values less than 6.

We hypothesize that the addition of slash material in clearcut harvest within the P-limited Ozarks created a unique situation where an increase in aboveground biomass growth by successional species triggered phosphorus scavenging by soil microbial populations and the vegetative community. The available P which was not scavenged likely became strongly sorbed to metal oxides (e.g., Mn and Fe). These two factors are hypothesized to have contributed to the observed reduction in the available phosphorus in the mineral horizons of the clearcut plots. We also hypothesize that microbial use of acid phosphomonoesterase may not have been an effective strategy in reclaiming phosphorus from the strongly-sorbed phosphorus present, thus keeping this soil enzyme activity low in the soil.

3.4.8 Sulfur

Significant variation in total S concentration within soil post clearcut harvest has been reported (Mitchell et al., 1989; Spratt Jr, 1997; Likens et al., 2002; Piirainen et al., 2004). Studies at Hubbard Brook Experimental Forest (New Hampshire, USA) have shown greater S concentrations in E and B horizons after a whole tree harvest. Mass balance calculations have shown that 88% of the organic forms of sulfur can be accounted for in the E and B horizons (Mitchell et al., 1989). In contrast, other sites have shown losses in S after harvesting activities (Merino et al., 1998; Piirainen et al., 2004). Prior studies at MOFEP (Spratt, 2002) and the nearby Deer Run State forest (Spratt Jr, 1997) indicated post-harvest losses of total and organic S although a large amount of yearly variation in S concentrations was observed. Our reported results in total sulfur and Aryl activity run counter to the decreased response reported by Spratt (1997, 2002). Increases in sulfur concentrations in soil post-harvest at Hubbard-Brooks (Mitchell et al., 1989) was attributed to decreased soil pH increasing sulfate adsorption in the E and B horizons. In the upper organic (O) horizons they report little change in sulfur concentration in plots post-harvest. Mitchell et al and Likens, et al. (Mitchell et al., 1989; Likens et al., 2002) document increased organic matter decomposition as contributing to the pool of available sulfur. We found that concentrations of total sulfur in the upper 10 cm of soil was positively correlated with total organic carbon, active carbon, total nitrogen pH and active carbon (see *Supplemental Materials*). We hypothesize that the increase in substrate quality and increased pH within the clearcut harvest treatments ultimately increased the amount of the organic matter to be decomposed with the upper soil horizons, thus

increasing the amount of sulfur present in the system. Microbial populations responded to the increased availability by producing enzymes, increasing Aryl activity within the soil.

Generally, it is presumed that atmospheric deposition is sufficient to replace S losses that might occur through bole-only harvesting activities (Grigal, 2000). However, reduced annual S deposition during the past 25 years due to reduced sulfur dioxide emissions by many industries to meet clean air regulations (Dick et al., 2008) may affect S replenishment in forests.

3.4.9 Single-Tree Selection vs Clearcut Harvest Treatments

Our research showed few statistically significant differences between single-tree selection harvest and control sites, and numerous significant differences between clearcut and single-tree selection harvests at the MOFEP study site. As mentioned previously, the slash in the single-tree selection treatments was not widely dispersed as in the clear-cut treatments. Therefore, the increase in substrate quality and pH values, which may have triggered increases in selected microbial populations, would be much more localized. From this we infer that disruption of nutrient cycling is minimized in stands treated with single-tree selection harvest. Longer-term studies (~50 year) at European sites report mixed results with respect to carbon and nitrogen concentration impacts on mineral soil horizons. Pötzelsberger and Hasenauer (2015) largely backs up an inference of minimal nutrient cycling disruption due to single-tree selection harvest, with respect to carbon and nitrogen stocks, and carbon to nitrogen ratio. Other long-term studies have shown a decline in carbon and nitrogen at single-tree selection sites, relative to the control. The changes are most evident in the forest floor (O) horizons, with the effects being muted, or

showing increases in carbon and nitrogen levels in the top mineral horizons (Christophel et al., 2013).

3.4.10 Other Mitigating Factors

In addition to the biogeochemical parameters studied in this paper, it is necessary to consider that a clearcut produced by forest harvest has a number of immediate effects on the microclimate of the forest. Within the clearcut, direct solar irradiation on the forest floor increases daytime soil temperature during the summer months (Dan Moore et al., 2005). Previous studies at the MOFEP and the nearby USFS Long Term Soil Productivity sites have shown greater variability in air and soil temperatures and humidity in the even-aged management (clearcut) sites as compared to control and selection harvests (Zheng et al., 2000; Ponder, 2005). A number of authors have shown a correlation between increasing soil temperature and increased microbial activity (Davidson et al., 2000; von Lützow and Kögel-Knabner, 2009); however, seasonal variation in temperature and soil moisture can complicate the interpretation of the effects of harvesting on microbial populations (Lewandowski et al., 2015).

3.5 Conclusions and Implications

Our research suggests that clearcut harvest in the Central Hardwoods region led to a microenvironment five years post-harvest that encouraged microbial activity and nominal to significant increases in soil carbon, nitrogen and sulfur in the clearcut plots. These increases, paired with a shift toward more neutral pH and improved substrate quality, as measured by C:N and TOC:AC ratios, all indicate a healthy recovering environment. We suspect that the bole-only harvest, adherence to the best management practices for

harvest, and the liberal amount of slash retained and distributed sites leads to a more favorable soil environment. The exception was the inferred reduction in phosphorous cycling in a phosphorous-limited environment. Reductions in available phosphorous and acid phosphomonoesterase activity present a potential scenario of concern for the long-term prognosis for phosphorus cycling in the area with repeated forest harvest activities. As noted earlier, reduced phosphorus cycling can be seen years after the harvest, although the cumulative effect of repeated harvests is still unclear.

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3.7 References

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Supplemental Material 3.A.: Biomarkers used by MIDI Sherlock™ microbial

identification system PLFAD1 software version 1.10

Actinobacteria (Actinomycetes):

16:0 10-methyl, 17:1 w7c 10-methyl, 17:0 10-methyl, 22:0 10-methyl, 18:1 w7c 10-methyl, 18:0 10-methyl, 19:1 w7c 10-methyl, 20:0 10-methyl

Arbuscular Mycorrhizal Fungi:

16:1w5c

Bacteria:

10:0 2OH, 10:0 3OH, 12:1 w8c, 12:1 w5c, 13:1 w5c, 13:1 w4c, 13:1 w3c, 12:0 2OH, 14:1 w9c, 14:1 w8c, 14:1 w7c, 14:1 w5c, 15:1 w9c, 15:1 w8c, 15:1 w7c, 15:1 w6c, 15:1 w5c, 14:0 2OH, 16:1 w9c, 16:1 w7c, 16:1 w6c, 16:1 w4c, 16:1 w3c, 17:1 w9c, 17:1 w8c, 17:1 w7c, 17:1 w6c, 17:0 cyclo w7c, 17:1 w5c, 17:1 w4c, 17:1 w3c, 16:0 2OH, 18:1 w9c, 18:1 w8c, 18:1 w7c, 18:1 w6c, 18:1 w5c, 18:1 w3c, 19:1 w9c, 19:1 w8c, 19:1 w7c, 19:1 w6c, 19:0 cyclo w9c, 19:0 cyclo w7c, 19:0 cyclo w6c, 19:0 cyclo 9,10 DMA, 20:1 w9c, 20:1 w8c, 20:1 w6c, 11:0 iso, 11:0 anteiso, 12:0 iso, 12:0 anteiso, 13:0 iso, 13:0 anteiso, 14:1 iso w7c, 14:0 iso, 14:0 anteiso, 15:1 iso w9c, 15:1 iso w6c, 15:1 anteiso w9c, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:1 iso w9c, 17:0 iso, 17:0 anteiso, 18:0 iso, 19:0 iso, 19:0 anteiso, 20:0 iso, 22:0 iso

Eukaryotes:

15:4 w3c, 15:3 w3c, 16:4 w3c, 16:3 w6c, 18:3 w6c, 19:4 w6c, 19:3 w6c, 19:3 w3c, 20:4 w6c, 20:5 w3c, 20:3 w6c, 20:2 w6c, 21:3 w6c, 21:3 w3c, 22:5 w6c, 22:6 w3c

Fungi:

18:2w5c, 16:1w5c

Gram Negative Bacteria:

10:0 2OH, 10:0 3OH, 12:1 w8c, 12:1 w5c, 13:1 w5c, 13:1 w4c, 13:1 w3c, 12:0 2OH, 14:1 w9c, 14:1 w8c, 14:1 w7c, 14:1 w5c, 15:1 w9c, 15:1 w8c, 15:1 w7c, 15:1 w6c, 15:1 w5c, 14:0 2OH, 16:1 w9c, 16:1 w7c, 16:1 w6c, 16:1 w4c, 16:1 w3c, 17:1 w9c, 17:1 w8c, 17:1 w7c, 17:1 w6c, 17:0 cyclo w7c, 17:1 w5c, 17:1 w4c, 17:1 w3c, 16:0 2OH, 18:0 cyclo w6c, 18:1 w8c, 18:1 w7c, 18:1 w6c, 18:1 w5c, 18:1 w3c, 19:1 w9c, 19:1 w8c, 19:1 w7c, 19:1 w6c, 19:0 cyclo w7c, 19:0 cyclo w6c, 20:1 w9c, 20:1 w8c, 20:1 w6c, 20:1 w4c, 20:0 cyclo w6c, 21:1 w9c, 21:1 w8c, 21:1 w6c, 21:1 w5c, 21:1 w4c, 21:1 w3c, 22:1 w9c, 22:1 w8c, 22:1 w6c, 22:1 w5c, 22:1 w3c, 22:0 cyclo w6c, 24:1 w9c, 24:1 w7c, 11:0 iso 3OH, 14:0 iso 3OH, 17:0 iso 3OH

Gram Positive Bacteria:

11:0 iso, 11:0 anteiso, 12:0 iso, 12:0 anteiso, 13:0 iso, 13:0 anteiso, 14:1 iso w7c, 14:0 iso, 14:0 anteiso, 15:1 iso w9c, 15:1 iso w6c, 15:1 anteiso w9c, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:1 iso w9c, 17:0 iso, 17:0 anteiso, 18:0 iso, 17:1 anteiso w9c, 17:1 iso w10c, 17:1 anteiso w7c, 18:1 w9c, 19:0 cyclo w9c, 19:0 iso, 19:0 anteiso, 20:0 iso, 22:0 iso

Supplemental 3.B: Boxplots of selected soil properties response to harvest treatment.

Means with different lower-case letters are significantly different at $p < 0.1$

Supplemental Material 3.C: Mean results of ANOVA treatment-nutrient status

interactions for selected variables shown not to have changed significantly, illustrating the “Disturbance Effect”; wherein, the maximum and minimum mean values are medium nutrient status-control and low nutrient status-control, respectively. Treatment-Nutrient status pairs are ranked in numerical order to highlight the random distribution of clearcut and single-tree selection pairs. Means with different lower-case letters are significantly different at $p < 0.1$.

Supplemental Material 3.D: Pearson's Correlation Matrices

Table C.1. Pearson's correlation coefficient matrix for soil properties from the 0-5 cm depth interval. Values highlighted with * are significant at the $p < 0.05$ level. Values in bold were found to be strongly correlated ($|r| > 0.7$).

<u>IOC</u>	<u>IOC</u>	<u>TN</u>	<u>C:N</u>	<u>β-glu</u>	<u>NAG</u>	<u>AcidP</u>	<u>Arcl</u>	<u>pH</u>	<u>TotPLFA</u>	<u>Bact</u>	<u>G(+)</u>	<u>G(-)</u>	<u>Act</u>	<u>Fung</u>	<u>AMF</u>	<u>Euk</u>	<u>Fung:Bact</u>	<u>Act:Fung</u>
0.84*	1.00																	
0.89*		0.84*	-0.09	0.86*	0.71*	0.51*	0.51*	0.26	0.73*	0.70*	0.72*	0.68*	0.54*	0.50*	0.61*	0.46	-0.07	-0.13
-0.32		1.00	-0.59*	-0.32	0.72*	0.84*	0.78*	0.66*	0.89*	0.89*	0.82*	0.89*	0.80*	0.30	0.91*	0.64*	-0.36	0.13
1.00				1.00	0.70*	0.47	0.56*	0.36	0.75*	0.73*	0.78*	0.69*	0.63*	0.34	0.66*	0.52*	0.58*	-0.50*
					1.00	0.31	0.58*	0.27	0.76*	0.70*	0.71*	0.67*	0.63*	0.60*	0.58*	0.66*	-0.22	-0.03
						1.00	-0.04	-0.37	0.33	0.28	0.39	0.23	0.11	0.53*	-0.01	0.11	0.30	-0.22
							1.00	0.84*	0.81*	0.88*	0.80*	0.84*	0.92*	0.05	0.92*	0.68*	-0.53*	-0.33
									0.49	0.57*	0.42	0.59*	0.66*	-0.20	0.81*	0.48	-0.58*	0.45
									1.00	0.97*	0.88*	0.98*	0.87*	0.43	0.87*	0.68*	-0.27	0.01
										1.00	0.94*	0.96*	0.95*	0.24	0.90*	0.61*	-0.45	0.21
											1.00	0.83*	0.92*	0.23	0.77*	0.57*	-0.40	0.25
												1.00	0.88*	0.30	0.93*	0.61*	-0.40	0.09
													1.00	0.06	0.88*	0.59*	-0.55*	0.38
														1.00	0.07	0.44	0.75*	-0.71*
															1.00	0.62*	-0.57*	0.30
																1.00	0.02	0.09
																	1.00	-0.74*
																		1.00

Table C.2. Pearson's correlation coefficient matrix for soil properties from the 0-10 cm depth interval. Values highlighted with * are significant at the $p < 0.05$ level. Values in bold were found to be strongly correlated ($|r| > 0.7$).

	<u>TOC</u>	<u>TN</u>	<u>C:N</u>	<u>pH</u>	<u>Active C</u>	<u>PMN</u>	<u>Labile C:N</u>	<u>TOC:AC</u>	<u>TN:PMN</u>	<u>TS</u>	<u>Bray1P</u>
<u>TOC</u>	1	0.87*	0.06	0.37	0.72*	0.73*	0.21	-0.01	0.04	0.66*	0.55*
<u>TN</u>		1	-0.43	0.6*	0.86*	0.86*	0.27	-0.32	0.07	0.82*	0.34
<u>C:N</u>			1	-0.55*	-0.38	-0.37	-0.15	0.59*	-0.11	-0.44	0.3
<u>pH</u>				1	0.72*	0.66*	0.31	-0.64*	-0.21	0.5*	-0.15
<u>Active C</u>					1	0.88*	0.56*	-0.67*	-0.21	0.74*	0.27
<u>PMN</u>						1	0.1	-0.54*	-0.43	0.77*	0.38
<u>Labile C:N</u>							1	-0.51*	0.26	0.22	-0.09
<u>TOC:AC</u>								1	0.47*	-0.36	0.08
<u>TN:PMN</u>									1	0.03	-0.28
<u>TS</u>										1	0.2
<u>Bray1P</u>											1

CHAPTER 4: SENSITIVITY OF POTENTIAL SOIL HEALTH
INDICATORS TO FOREST CLEARCUT HARVEST AT 5 AND 22
YEARS POST-HARVEST

A.W. Taylor^{a*}, K.W. Goyne^a, J.M. Kabrick^b and K.S. Veum^c

^a School of Natural Resources, University of Missouri, 103 ABNR Bldg., Columbia, MO
65211, USA

^b USDA Forest Service, Northern Research Station, 203 ABNR Bldg., Columbia, MO
65211, USA

^c USDA-Agricultural Research Service, Cropping Systems and Water Quality Research
Unit, 269 Ag. Eng. Bldg. Columbia, MO 65211, USA

* *Corresponding author*: School of Natural Resources, University of Missouri, 103
ABNR Bldg., Columbia, MO 65211, USA; email: awtkvf@mail.missouri.edu (A.W.
Taylor)

Abstract

The concept of soil health has primarily been applied to agroecosystems, and numerous studies have helped identify soil health indicators applicable for agroecosystems. While there is much interest for applying the soil health concept to natural and restored ecosystems, substantially less research had focused on identifying soil health indicators applicable to forest ecosystems. The objective of this research was to identify soil health indicators sufficiently sensitive for evaluating forest soil health. Soil samples were collected from 0 to 5 cm and 0 to 10 cm depth at two long-term forest experiments in the southern Missouri Ozarks, USA. Treatments sampled at the Missouri Ozark Forest Ecosystem Project (MOFEP) included control (no harvest) and clearcut harvest with stem-only removal (5 years post-harvest); treatments sampled at the Long Term Soil Productivity were control and two clearcut harvests (stem-only removal and whole-tree removal; 22 years post-harvest). Twenty-five potential soil health indicators were examined to determine indicator sensitivity to forest harvesting. The indicators examined included soil physical (four indicators), chemical (13 indicators) and biological (four indicators) properties. Of the 25 indicators measured, 15 (60%) were found to have changed significantly due to forest harvest at 5 years post-harvest: bulk density; percent coarse fragments; base saturation; Bray-1 P; C:N ratio; effective cation exchange capacity; extractable Ca^{2+} and K^{+} ; pH; total nitrogen; total organic carbon:active carbon ratio; total sulfur; arylsulfatase and acid phosphomonoesterase soil enzyme activities and the fungal:bacterial ratio. Observed changes within clearcut harvested plots for most of these parameters can be viewed as either neutral to positive developments, as they either indicate positive changes in nutrient concentrations or

nutrient availability. Indicators suggesting some decline in soil health were Bray-1 P concentration and acid phosphomonoesterase activity, which both declined, and an increase in fine-fraction bulk density. At 22 years post-harvest, only the C:N ratio and acid phosphomonoesterase varied by treatment suggesting significant progress towards recovery from forest harvest. We suggest the following list of indicators for monitoring forest soil health as affected by harvest in the Missouri Ozarks: BS; CF%; exchangeable Ca^{2+} and K^{+} ; C:N; ECEC; pH; TN; TOC:AC; T; and Bray-1 P. Although fine fraction bulk density was not found to be sensitive to harvest in this study, we would recommend it be retained as part of a soil health assessment due to the strong influence it has on limiting root growth.

Highlights

- A suite of 25 potential forest soil health indicators were evaluated to determine their applicability to studying forest soil health
- Fifteen indicators (60%) were sufficiently sensitive for detecting soil health changes 5 years post-harvest, only 2 were at 22 years
- Two indicators (8%) were sufficiently sensitive for detecting soil health changes 22 years post-harvest
- We recommend the following list of indicators for monitoring soil health in the Missouri Ozarks: BS; CF%; exchangeable Ca^{2+} and K^{+} ; C:N; ECEC; pH; TN; TOC:AC; T; Bray-1 P and fine fraction D_b

4.0 Introduction

The importance of soil health - “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans” (“Soil Health | NRCS Soils,” 2018) - has been increasingly recognized by soil scientists and the larger agronomic community. Long-term conservation of soil physical and chemical properties and maintenance of diverse and robust microbiological communities are the focus of several USDA-NRCS programs aimed at increasing the implementation of soil health-related practices at working farms (Bowman et al., 2018). However, relative to agronomic systems forest soil health is understudied (Amacher et al., 2007). In 2012, it was estimated that 33% of the U.S. was covered by forest (Oswalt et al., 2014); whereas 40% was considered farmland (NASS, 2012). A search of articles on Google Scholar performed in 2018 revealed over 504,000 articles published with a key word of soil health since 2014. Of those 184,000 also contained the keyword “agriculture”; whereas only 71,600 included the keyword “forest”, potentially indicating lowered publication of articles specific to forest soil health, relative to agricultural systems.

The connection of soil health to ecosystem protection and food security has even led some scientists to consider “soil security” as a cornerstone of global sustainability (McBratney et al., 2014). McBratney proposed a model of soil security recognizing five conceptual dimensions: soil capability; soil condition; capitalization of soil resources; connectivity of persons to the soil; and codification of proper soil management into policies and regulation. To achieve this end-goal, a unified method of soil monitoring needs to be established – one such method proposed is the use of a soil quality or health index (Karlen et al., 1997). A soil health index is a collection of soil properties that act as

indicators of changes within the soil system. Doran and Parkin (1996) recognized five features that good quantitative soil quality (health) indicators should possess:

- They should correlate well with ecosystem processes.
- They should integrate physical, chemical and biological properties and processes, and/or serve as basic input needed for estimation of soil properties or processes which are more difficult to measure.
- They should be easy to use in the field and assessable by both soil specialists and by agricultural producers.
- They should be sensitive to variation in management and climate.
- They should be components of existing soil databases, whenever possible.

At present, there is no consensus for a common set of soil indicators. Zornoza et al. (2015) noted that soil organic matter (or soil organic carbon) and pH appear to be common for most soil quality indicators, additional parameters appear to vary widely on a case-by-case basis. Nortcliff (2002) emphasized that standardization of selected indicators and the methods of analysis are critical to making assessment of soil health useful. Furthermore, Nortcliff (2002) stressed that any index of soil health must consider function. A soil useful for one purpose (e.g., supporting a healthy forest ecosystem) may be unsuitable for another (e.g., intensive agriculture). Consequently, selected soil health indicators should be chosen with a broad range of soil functions under consideration.

Mukherjee and Lal (2014) compared three soil quality index methodologies (simple additive, weighted, and principal components analysis-based) on mineral and muck soils.

They found that the minerals soils were most sensitive to indicators related to root development and nutrient storage; whereas, the muck soils were more sensitive to indicators related to water storage. The high variability of results for different ecosystems suggests that a common set of soil health indicators for all soil types and functions may be difficult to achieve and that there is an element site specificity to soil health.

Several efforts have been made to translate soil health and soil health indices to forested ecosystems. One of the most comprehensive efforts is described by Amacher et al. (2007). Amacher, et al discusses the implementation of forest soil quality data as part of the Forest Inventory and Analysis (FIA) program. A broad range of soil properties are being collected in concert with advance tree seedling regeneration, vegetation profiles, invasive plant down woody materials and tree crown conditions as part of a multiphase inventory of overall forest health (referred to as Phase 2+) (Gormanson et al., 2018).

The objective of this study was to examine the sensitivity of 25 potential soil health indicators (physical, chemical and biological) to forest harvest at two time intervals post-clearcut harvest. To accomplish this, we examined similar treatments at two adjacent forest experimental projects in southern Missouri: Missouri Forest Ecosystem Project (harvested in 2011); and the Missouri Long Term Soil Productivity sites (harvested in 1994). For this study, we focused on the top 5 or 10 centimeters of the mineral soil horizon due to the greater potential for forest management activities to influence surface soil properties

4.1 Materials and Methods

4.1.1 Site Description

The Missouri Forest Ecosystem Project (MOFEP) and Long Term Soil Productivity program sites are located in south-central Missouri, USA, within the counties of Shannon, Reynolds and Carter (37° 08'N, 91° 00'W) (Figure 1.). The MOFEP experiment was initiated in 1989 by the Missouri Department of Conservation as a long-term, landscape-scale study examining the effects of even-aged and uneven-aged forest management methods, applied in an operational manner, on the flora and fauna of the Ozark Highlands. The Missouri LTSP experiment was implemented in 1994.

The experimental sites are located within the Current River Oak-Pine Woodland Hills and Current River Forest Breaks land type associations of the Ozark Highlands (Shifley and Kabrick, 2002). Soils at the MOFEP and LTSP sites are primarily derived from parent materials of Ordovician-aged Roubidoux and Gasconade formations and the Cambrian-aged Eminence formation. These marine sedimentary rocks are primarily composed of chert, dolomite and quartz sandstones and strongly influence the physical and chemical properties of the overlying soils. The soils in the region range from highly weathered very gravelly pedisements classed as Ultisols (Typic Paleudults and Typic Hapludults) to gravel-free clayey pedisements and residuum classified as Alfisols (Typic Paleudalfs and Typic Hapludalfs) (Kabrick et al., 2008).

The Current River Oak-Pine Woodland Hills have broad ridges and less than 90 m of relief. In contrast, the Current River Oak Forest Breaks have narrow ridges and steep relief that ranges from 90 to 140 m (Meinert et al., Kabrick et al., 2000). The forests at

MOFEP and the Missouri LTSP sites are characterized by mixed hardwood and hardwood-softwood forests. Four oak species predominate the area making up 71% of the basal area: white oak (*Quercus alba*); black oak (*Quercus velutina*); scarlet oak (*Quercus coccinea*); and post oak (*Quercus stellata*). Shortleaf Pine (*Pinus echinata*), various species of hickory (*Carya spp.*), flowering dogwood (*Cornus florida*), blackgum (*Nyssa sylvatica*) and various other oak species (*Quercus spp.*) make up the remainder of tree species in the forest (Kabrick et al., 2008).

Figure 4.1. MOFEP and LTSP study sites (after (Sork et al., 2005))

4.1.2 Experimental Design

The MOFEP site is a randomized complete block design encompassing nine compartments ranging in size from 314 to 516 hectares (Figure 2.1). Each of the three blocks comprise three compartments assigned by their spatial proximity. In each block, three forest management treatments were randomly assigned to a compartment: no-harvest (control); uneven-aged management (with group or single-tree selection harvest); and even-aged management (with clearcutting). Approximately 10 percent of each site is left as old growth. The old growth reserve will be preserved from harvest activities on a permanent basis. Harvest entries in compartments occur at 10 to 15 year intervals. Even-aged and uneven-aged management was practiced according to MDC Forest Land Management Guidelines. Even-aged management consisted of a rotation of 80-100 years resulting in a regulated harvest of 10-12.5% of the area per entry period (Shifley and Kabrick, 2002). Nearly all non-merchantable timber was felled during a slash operation after the removal of merchantable boles at clearcut sites (Sheriff, 2002). Some snag or den trees were preserved to provide shelter for wildlife. Additionally, some shortleaf pine was preserved to provide seed for pine regeneration. Uneven-aged management utilized single-tree and group selection harvest, with a target tree size class distribution equivalent to the composite size class distribution across the even-aged management sites (Shifley and Kabrick, 2002).

The Missouri Long-Term Soil Productivity Research Program is a three-factor randomized split-plot design with three replications of two forest floor treatments (whole plot), two compaction treatments (whole plot) and two vegetation control treatments

(split plot) (Ponder et al., 2016). The LTSP program established in 1989, has a core set of treatments for the two main effects being studied (Table 4.2.).

Table 4.1. Description of common treatments at North American LTSP sites (after Powers et al., 2005).

<u>Main Effect</u>	<u>Symbol</u>	<u>Description of Treatment</u>
Organic Matter Modification	OM0	Tree Boles removed - retaining crowns, understory and forest floor
	OM1	All above-ground living vegetation removed - forest floor retained
	OM2	All surficial organic matter removed – bare soil exposed
Soil Porosity (Compaction)	C0	No compaction
	C1	Compaction to an intermediate bulk density
	C2	Compaction to a high bulk density

A subset of available treatments were used for this study. At the MOFEP site only the clearcut (stem-only) and control treatments were used; whereas at the LTSP site, only the whole-tree harvest (OM1C0), stem-only (OM0C0) and control treatments with no compaction or vegetative control were utilized.

Potential forest soil health indicators were identified via literature review and represent selections from both agronomic and forest soil health indices (Table 4.1).

Table 4.2. Potential soil physical, chemical and biological health indicators

<u>Soil Health Indicator</u>	<u>Reference</u>
Soil Physical Properties	
Aggregate stability (AggStb)	Andrews et al., 2004
Fine fraction bulk density (D_b)	Andrews et al., 2004
Percent coarse fragments (%CF)	Amacher, et al., 2007
Soil strength (SStr)	Idowu, et al., 2007
Soil Chemical Properties	
Active carbon (ActiveC)	Weil, et al., 2003
Base saturation (BS)	Rodrigue and Burger, 2004
Bray-1 Phosphorus (Bray1P)	Amacher, et al., 2007
Extractable Ca^{2+} concentration	Amacher, et al., 2007
Carbon:nitrogen ratio (C:N)	Gregorich, et al., 1994
Effective cation exchange capacity (ECEC)	Doran et al., 1996
Electrical conductivity (EC)	Andrews et al., 2004
Extractable K^+ concentration	Amacher, et al., 2007
Extractable Mg^{2+} concentration	Amacher, et al., 2007
Extractable Na^+ concentration	Amacher, et al., 2007
pH	Amacher, et al., 2007
Potentially mineralizable nitrogen (PMN)	Andrews et al., 2004
Total nitrogen (TN)	Amacher, et al., 2007
Total organic carbon (TOC)	Andrews, et al., 2004
Total organic carbon:active carbon ratio (TOC:AC)	No source
Total sulfur (TS)	Amacher, et al., 2007
Soil Biological Properties	
β -glucosidase activity (β -glu)	Stott et al., 2010
N-acetyl β -D-glucosaminidase activity (NAG)	Caldwell, 2005
Arylsulfatase activity (Aryl)	de la Paz Jimenez, et al., 2002
Acid phosphomonoesterase activity (AcdP)	de la Paz Jimenez, et al., 2002
Fungal:bacterial ratio (F:B)	Hogberg, et al. 2013

Although the TOC:AC ratio has not yet been suggested as a potential soil health indicator

– the use of this ratio as a measure of substrate quality for microbial communities has been documented (S. Enríquez et al., 1993; Blair et al., 2001). Total organic carbon, total nitrogen and carbon:nitrogen ratio were determined for both collection campaigns: the 0 to 10cm collection (intended for geochemical properties) and the 0 to 5cm collection

(intended for biological properties) to identify differences in substrate quality (as measured by C:N ratio) associated with the changes in biological activity between the MOFEP and LTSP sites

4.2.3 Soil sampling

Two distinct bulk soil sampling campaigns were performed over the course of the study. The first sampling, intended for geochemical analysis (Table 4.1), consisted of three subsamples collected from top 10 cm of the mineral soil at the two experimental sites (3 subsamples x 2 treatments x 3 replicates at MOFEP; 3 subsamples x 3 treatments x 3 replicates at LTSP) in the winter of 2016. The MOFEP collect represents a time interval 5 years post-harvest; whereas the LTSP collect represents 22 years post-harvest. Subsamples for soil chemical analysis were air-dried and sieved to ≤ 2 mm, composited and stored for subsequent analysis.

Similarly, a second set of samples were collected in May 2016, for analysis of microbial and enzymatic properties (Table 4.2). To focus on the zone of greatest potential biological change (Lladó et al., 2017), the top 5 cm of the mineral soil was sampled for microbial analyses. Samples were transported to the University of Missouri under ambient temperature and field moist conditions. Samples for biological analysis were moist sieved to ≤ 2 mm and subdivided. One portion was lyophilized and stored at -20°C for analysis of microbial community structure. The other portion was stored at -4°C for analysis of soil microbial function, pH, TOC and TN. Soil moisture content was determined by drying five grams of field moist soil in a Fisher Scientific IsoTemp drying oven (Fisher Scientific; Waltham MA) for 48 hours at 105°C , and weighing the oven-dry soil on a Mettler Toledo PB3002-S analytical balance (Mettler Toledo; Columbus, OH).

4.2.4 Analysis of Soil Microbial Community Structure

Phospholipid fatty acid (PLFA) analysis was performed by the using a high throughput method, allowing for up to 96 samples to be processed simultaneously (Buyer and Sasser, 2012). Soil samples were lyophilized in a centrifugal evaporator after which lipid extraction was performed using the Bligh-Dyer method (Bligh and Dyer, 1959) and the high-throughput extraction, separation, and transesterification protocol of Buyer and Sasser (2012). The final extract was dissolved in hexane and analyzed via gas chromatography for the relative amounts of fatty acid methyl esters present. The fatty acid biomarkers were assigned to microbial groups using MIDI Sherlock™ microbial identification system PLFAD1 software version 1.10 (MIDI, Inc.; Newark, DE). A list of the specific fatty acids assigned to each microbial group can be found in the *Supplemental Materials*. The three subsamples collected from each site for microbial analyses were analyzed separately and the results were averaged prior to data analysis.

4.2.5 Analysis of Soil Microbial Community Function

Soils were analyzed for the activities of four microbial enzymes: β -glucosidase (β -glu); N-acetyl- β -D-glucosaminidase (NAG); arylsulfatase (Aryl); and acid phosphomonoesterase (AcdP). Enzyme activities were determined using procedures outlined in Dick et al. (2011). The only modification introduced to the procedures was elimination of toluene to improve safety. All enzyme procedures involved introducing 1 g of field moist soil into a 50ml flask and adding 1 ml of the appropriate substrate to 4 ml of acidic buffer. Samples were capped and incubated in the dark for 1 hour at 37°C. After incubation, 4 ml of a basic buffering solution and 1 ml of 0.5M CaCl₂ were added to the flask, and samples were gently mixed to terminate the reaction. The solution was

vacuum-filtered through Fisher P4 cellulose fiber filter paper with a particle retention of 4-8 μm . Approximately 3.5 ml of the filtrate was transferred to a cuvette and absorbance was measured at 405 nm using a Spectronics Genesys 8 spectrophotometer (Spectronics Camspec Ltd; Garforth, England). Standards of p-nitrophenol (PNP) solution in THAM at 0, 2.5, 5, 10, 15, and 25 $\mu\text{g PNP ml}^{-1}$ were prepared and analyzed. All measurements were expressed as $\mu\text{g PNP ml}^{-1} \text{ gram}^{-1} \text{ soil hour}^{-1}$ adjusted to a dry-mass basis. The three subsamples collected from each site for microbial analyses were analyzed separately, and the results were averaged prior to data analysis.

4.2.6 Soil Chemical Analysis

Total organic carbon (TOC) and total nitrogen (TN) content were measured using a LECO TruMac CN combustion analyzer model 630-300-400 and total sulfur content was quantified using a LECO Sulfur-Carbon analyzer model SC-144DR (LECO Corporation; St. Joseph, MI). Potentially mineralizable nitrogen (PMN) content was determined using the 7-day anaerobic incubation method described by Anderson et al. (2010) with slight modification. The modification involved reduction of soil mass from 20 g to 8 g for improved throughput. Following incubation, 2M KCl was used to extract ammonium, which was quantified using the colorimetric method of Nelson (1983), and absorbance of the solution was measured at 550 nm on a spectrophotometer to determine $\text{NH}_4^+\text{-N}$ concentration. Active carbon content was determined using the protocol established by Weil et al. (2003) (Weil et al., 2003). In short, five grams of soil was added to a 0.02M KMnO_4 solution. The suspension was shaken for 2 minutes and then allowed to settle for 5 to 10 minutes. One-half milliliter of supernatant was extracted and added to de-ionized

water to attain a total volume of 50 ml. Absorbance at 550nm was read on a spectrophotometer to determine active carbon concentration. Available phosphorus was determined using the Bray P-1 method and soil pH was determined using a 1:2 slurry of soil and 0.01M CaCl₂ solution (Burt, 2004). Samples were shaken for 1 hour, allowed to settle for 5 minutes and pH was measured on an Accumet AR-60 pH meter equipped with an Accumet 13-620-285 electrode (Fisher Scientific; Waltham, MA). Other soil analyses were performed using procedures outline in Burt (2004) for the following properties: exchangeable concentrations of Ca²⁺, K⁺, Mg²⁺ and Na⁺ (NH₄Cl extraction technique); effective cation exchange capacity by the summation of BaCl₂-triethanolamine released extractable acidity and NH₄Cl exchangeable bases.

4.2.7 Soil Physical Analysis

Soil bulk density was determined using a variation of a water displacement method recommended in rocky forests soils outlined in Page-Dumroese et al. (1999). A level surface was created at the sample site, ensuring that a minimum amount of soil was removed during leveling, and a hole 10 cm in diameter was excavated to a depth of 10 cm. Excavated soil was carefully transferred to a storage bag, and the hole was lined with a thin plastic trash bag. A 1000 ml graduated cylinder was filled with water, and water was transferred into the hole until full. The amount of water required to fill the whole was recorded and soil samples retained during excavation were transported to the laboratory.

Soil samples were air-dried and sieved to ≤ 2 mm. The >2 mm fraction was retained to compute percent coarse fraction. The fine fraction was oven dried for 48 hours in a Fisher Scientific IsoTemp drying oven and the final mass recorded. The total volume for the

coarse fraction was computed by determining the mass of the coarse fraction and dividing by a particle density of 2.65 g cm^{-3} . Soil bulk density was computed as follows:

$$\text{Soil Bulk Density (g/cm}^3\text{)} = \frac{\text{Mass of Fine fraction (g)}}{(\text{Volume of Water})(\text{cm}^3) - (\text{Volume of Coarse fraction})(\text{cm}^3)}$$

The percentage of coarse fragments was computed on a volume basis by dividing volume of the coarse fraction by the total volume of water required to fill the excavated hole.

Aggregate stability was computed using the method outlined in Burt (2004). Soil strength was determined using a pocket penetrometer (Forestry Suppliers, Inc.; Jackson, MS.) and the method outlined in Amacher and O'Neill (Amacher and O'Neill, 2004).

4.2.8 Statistical Analysis

A two-way analysis of variance (ANOVA) using a linear mixed model (Proc Mixed) in SAS 9.4 (SAS Institute; Cary, NC) was used to evaluate effects of treatment (clearcut, single tree selection, and control), on the potential soil health indicators.

The experimental design of MOFEP allows for the examination of treatments, soil nutrient status, and their interactions by analyzing the data using a statistical model for a split-plot analysis with different error terms (see Brookshire and Shifley, 1997). To test for treatment effects (i.e., the whole plot effects), the appropriate error term is the treatment x block interaction. To test for effects of soil nutrient status (i.e., the split plot effects) and its interaction with treatments, the three-way interaction of treatment x soil nutrient status x block is used. The data were tested for normality using the Shapiro-Wilk test. Those data which did not pass the test for normality were transformed using a square

root transformation. Least squares differences were computed to determine marginally significant differences ($p < 0.10$) and significant differences ($p < 0.05$ and $p < 0.01$) between treatments. The two sites, MOFEP and LTSP, were analyzed independently.

4.3 Results

The differences between the results at the MOFEP (5 years post-harvest) and the LTSP (22 years post-harvest) sites were dramatic. At five years post-harvest, the following soil properties were significantly different ($p < 0.05$) in stem-only clearcut harvest relative to control (Table 2.): CF%; BS; exchangeable Ca^{2+} and K^{+} ; C:N (0-5cm); pH; TS; AcdP; and F:B. Marginally significant changes ($p < 0.1$) were observed for: fine fraction D_b ; Bray-1 P; C:N (0-10 cm); ECEC; TN (0-5cm); and Aryl. No significant differences ($p < 0.05$) were noted between the whole-tree and stem-only harvests, relative to the control, at 22 years post-harvest. A marginally significant ($p < 0.1$) difference between stem-only and control at 22 years post-harvest was observed for EC. Comparison of the whole-tree harvest to control, 22 years post-harvest, yielded marginally significant ($p < 0.1$) differences for C:N (0-10 cm) and AcdP.

Measurement of soil strength could not be assessed due to the high percentage of chert gravel (coarse fragments) present at the experimental sites.

Table 4.3. Differences in soil physical, chemical and biological health indicators assessed at five (MOFEP) and twenty-two years (LTSP) after imposing clearcutting with stem-only and whole-tree removal treatments. Indicator abbreviations are defined in Table 4.1. Significant differences from control are indicated by * $p < 0.10$ (marginal significance), **

p<0.05 and *** p<0.01. Within rows, lower-case letters signify means which are significantly different at the p<0.10 level at the MOFEP site and upper-case case letters signify means which are significantly different at the p<0.10 level at the LTSP site.

Indicator	MOFEP (5 years)		LTSP (22 years)		
	Control	Stem-only	Control	Stem-only	Whole-tree
<u>Soil Physical Properties</u>					
AggStb (%)	83.8a	85.4a	87.9A	88.3A	74.6A
D_b (g cm⁻³)	1.28a	1.31a	1.42A	1.31A	1.28A
D_bfine (g cm⁻³)	0.60a	0.93b*	0.98A	0.88A	0.73A
CF (%)	66.5a	39.2b***	50.1A	46.8A	58.3A
SStr	n.a.	n.a.	n.a.	n.a.	n.a.
<u>Soil Chemical Properties</u>					
ActiveC (mg C kg⁻¹)	198a	308a	315A	423A	380A
BS (%)	36.0a	62.5b***	48.7A	59.3A	64.0A
Bray1P (mg P kg⁻¹)	7.87a	5.28b*	5.23A	7.73A	4.33A
Exch. Ca²⁺ (cmol_c kg⁻¹)	1.13a	3.48b**	2.63A	4.03A	5.40A
C:N 0-5cm	23.2a	17.7b***	20.6A	19.0A	20.1A
C:N 0-10cm	17.5a	14.0b*	17.0AB	18.1A	15.7B*
ECEC (cmol_c kg⁻¹)	4.57a	6.37b*	7.17A	8.20A	9.77A
EC (dS cm⁻¹)	0.36a	0.36a	0.097A	0.25B	0.097A
Exch. K⁺ (cmol_c kg⁻¹)	0.117a	0.233b***	0.167A	0.167A	0.233A
Exch. Mg²⁺ (cmol_c kg⁻¹)	0.420a	0.670a	0.733A	0.700A	1.07A
EXch. Na⁺ (cmol_c kg⁻¹)	bd	bd	bd	bd	bd
pH	4.10a	4.70b***	4.48A	4.57A	4.85A
PMN (mg N kg⁻¹)	49.5a	56.8a	85.0A	93.0A	88.5A
TN 0-5cm (g N kg⁻¹)	1.55a	2.09b*	2.02A	2.05A	2.51A
TN 0-10cm (g N kg⁻¹)	1.10a	1.29a	1.79A	1.99A	2.01A
TOC 0-5cm (g C kg⁻¹)	31.5a	37.3a	41.8A	39.3A	48.6A
TOC 0-10cm (g C kg⁻¹)	19.1a	17.8a	30.5A	35.6A	31.6A
TOC:AC 0-10cm	97.3a	63.5b**	97.9A	84.1A	83.2A
TS (g S kg⁻¹)	0.10a	0.13b***	0.18A	0.18A	0.22A
<u>Soil Biological Properties</u>					
β-glu (μg PNP g⁻¹ hr⁻¹)	63.5a	77.3a	59.0A	64.8A	68.2A
NAG (μg PNP g⁻¹ hr⁻¹)	81.0a	88.0a	97.5A	90.4A	93.9A
Aryl (μg PNP g⁻¹ hr⁻¹)	155a	266b*	188A	247A	269A
AcdP (μg PNP g⁻¹ hr⁻¹)	651a	451b***	745A	602AB	529B*
F:B	0.076a	0.047b***	0.067A	0.046A	0.049A

Key to abbreviations: AggStb - aggregate stability; D_b – bulk density; D_bfine – bulk density of the fine fraction; CF – coarse fragment percentage; SStr – soil strength; BS – base saturation; Exch – exchangeable; C:N – carbon to nitrogen ration; ECEC – effective cation exchange capacity; EC – electrical conductivity; PMN – potentially mineralizable nitrogen; TN – total nitrogen; TS - total sulfur; β-glu – β-glucosidase; NAG – N-acetyl-D-β-glucosaminidase; Aryl – arylsulfatase; AcdP – acid phosphomonoesterase; F:B – fungal bacterial ratio; n.a. – not analyzed; bd – below limits of detectability.

4.4 Discussion

4.4.1 Soil Physical Properties

Changes in soil bulk density due to forest harvest have been well documented in the literature (Johnson et al., 1991a). There is widespread agreement in the academic community that soil compaction can occur during forest harvest, and that the severity of the effects appear to vary based upon soil texture at the site (Grigal, 2000). The primary effect that increase of bulk density has on forest regeneration is limiting root growth (Zhao et al., 2010). Generally, it is thought that soils with a predominance of silt and clay-sized particles begin to experience root-limiting behavior at soil bulk densities in excess of 1.40 g cm^{-3} (Daddow and Warrington, 1983). In our study, the bulk density of the soil fine earth fraction at five years post-harvest increased from 0.60 to 0.93 g cm^{-3} (55% increase) at the 0-10 cm depth interval in clearcut stem-only harvest relative to control sites (Table 4.3) Changes in bulk density due to forest harvest are generally associated with one of two processes: accelerated erosion of surficial layers that effectively decreases organic matter content by removing the A horizon; and compaction by the passage of heavy equipment at the site (Grigal, 2000). Missouri Department of Conservation guidelines (2014) for forest harvesting specifically require the implementation of procedures that ameliorate compaction and erosion by locating roads landings and primary skid trails on well-drained or gravelly soils, and by implementing harvest layouts which reduce the surface area being impacted and through the use of breaks to control overland water flow (source). Analysis of bulk density data suggests that these amelioration techniques were insufficient to reduce bulk density. However, inconsistencies in collection methods by successive field workers may have introduced

noise, creating the high variability evident in the data (Figure 4.2). A comparison of boxplots for the 5 and 22 year stem-only treatments suggests that there was little change over time in fine earth fraction bulk density (Figure 4.2). At 22 years post-harvest, fine earth fraction bulk density values were statistically similar between treatments with mean values ranging from 0.729 (WTH) to 0.981 g cm⁻³ (control), inferring that any potential effects of forest harvest on fine earth fraction bulk density was largely mitigated over time. Other than a few outliers, the majority of fine bulk density values seen across the treatments do not exceed the presumed root limiting value of 1.40 g cm⁻³.

Figure 4.2. Boxplot of soil fine fraction bulk density for Control and Stem only harvest at the MOFEP site and control, stem-only and whole-tree harvest at the LTSP site. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites. Means with ** are different at $p < 0.05$ and *** are different at $p < 0.01$.

4.4.2 Soil Chemical Properties

4.4.2.1 Carbon and Nitrogen

The C:N and TOC:AC ratios are indicators of changes in quality of substrates consumed by soil microbial communities. Lower values indicate better substrate quality, suggesting more nitrogen is available for the breakdown of carbon (Enríquez et al., 1993; Blair et al., 2001).

Our research indicates that the C:N and the TOC:AC ratios for stem-only harvest were significantly less than for control treatments at five years post-harvest within the 0 to 5 cm depth interval for the C:N and the 0 to 10 cm interval for the TOC:AC ratio (Table 4.3; Figure 4.3). However, TOC was not statistically different for any treatment at either the 5 or 22 year post-harvest intervals. Furthermore, TN was only marginally significantly changed at the 0 to 5 cm depth interval, at 5 years post-harvest. TN was not significantly different at the 0 to 10 cm interval nor for any treatment at 22 years post-harvest.

Figure 4.3. Mean values of (i) C:N at the 0 to 5cm interval and (ii) TOC:AC at the 0 to 10 cm interval ratios post-harvest for control, stem-only harvest at 5 years post-harvest and control, stem-only and whole-tree harvest at 22 years post-harvest. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites. Means with ** are different at $p < 0.05$ and *** are different at $p < 0.01$.

The most comparable study to our research is the Coweeta Long Term Ecological Research (LTER) site in North Carolina, USA. Knoepp et al. (2014) observed initial increases in soil TOC and TN lasting for three years following stem-only harvest at the Coweeta site. After the initial flush of nutrients, the soil concentrations of TOC and TN returned to pre-harvest levels. For the next 28 years of the study, TOC and TN changed very little in stem-only harvest treatment. However, when the stem-only harvested site was compared to the unharvested control an overall decline in carbon and nitrogen soil concentrations during the 28 year post-harvest interval in carbon and nitrogen soil concentrations. Since our study focused on a time interval at 5 years post-harvest, we likely missed the period of significantly increased TOC and TN concentration that Knoepp, et al observed at three years post-harvest. At 22 years post-harvest; however, our TOC and TN results appear to be relatively stable with respect to concentration, in contract to the results at the Coweeta site (Figure 4.4)

Figure 4.4. Mean values of mineral soil (i) TOC and (ii) TN at the 0 to 5 cm interval ratios post-harvest for control, stem-only harvest at 5 years post-harvest and control, stem-only and whole-tree harvest at 22 years post-harvest. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites.

Several meta-analyses highlight the variability in observed carbon and nitrogen responses to forest harvest. Hume et al. (2018) evaluating the impact of forest harvest intensity on carbon and nitrogen stocks and the C:N ratio observed that there was an increase in mineral soil carbon stock and the C:N ratio, but N stocks were reduced. Hume et al., also observed that over time, the soil carbon and nitrogen stocks declined and C:N ratio was reduced. Whereas, Johnson and Curtis (2001) noted that stem-only harvest of deciduous and mixed-deciduous forests had little effect on carbon and nitrogen stocks. Finally, Jerabkova et al. (2011) in a meta-analysis of clearcut and variable retention harvests on soil nitrogen in boreal and temperate forests found that clearcut harvest significantly increased soil and soil solution nitrogen concentrations. The increase in the various nitrogen forms in deciduous forest were found to have occurred immediately post-harvest and generally decreased to pre-harvest levels within 5 years.

4.4.2.2 Base cations

At the 5 year post-harvest interval, the only extractable base cation concentrations affected by treatment were Ca^{2+} and K^+ (Table 4.3; Fig. 4.5). However, there were no differences between extractable base cations in harvest treatments and control sites at the 22 year time interval. Variation in the changes of concentrations of exchangeable cations within the soil due to the effects of forest harvest have been reported within the literature. The concentrations of base cations have been shown to have increased post-harvest (Liechty et al., 2002; Knoepp et al., 2014), decreased post-harvest (Pennock and Kessel, 1997; Likens et al., 1998; Huntington et al., 2000) or have experienced no statistical change post-harvest (Johnson and Todd, 1998; Liechty et al., 2002).

Increased concentrations of exchangeable base cations within forest soils under clearcut treatments, such as that observed in our study 5 years after harvest, have been widely interpreted as the result of the decomposition and mineralization of slash material (Robertson et al., 2000; Piirainen et al., 2004). Liechty et al. (2002) studying shortleaf pine-hardwood stands in the Ouachita Mountains of Arkansas noted greater exchangeable Ca^{2+} concentrations within upper soil horizons post-harvest, but the concentrations of the other base cations remained relatively unchanged. Within pine-hardwood and oak-hickory forests of North Carolina, Knoepp et al., (2014) found that concentrations of exchangeable Ca^{2+} , Mg^{2+} and K^+ increased for 3 years post-harvest in stem-only treatments. After initial increases, Ca^{2+} and K^+ concentrations returned to pre-harvest levels; whereas, the concentration of Mg^{2+} remained elevated for 30 years. Calcium and K^+ have often been cited as the macronutrients most likely to become depleted following harvest due to net nutrient export off-site upon log removal and little return of these ions

to the soil via atmospheric deposition (Grigal, 2000). However, several longer term studies performed in temperate hardwood forests, show little to no Ca^{2+} and K^{+} depletion several decades after harvest (Knoepp and Swank, 1997; Johnson et al., 1997; Johnson and Todd, 1998).

Figure 4.5. Mean values of exchangeable (i) Ca^{2+} and (ii) K^{+} concentrations post-harvest for control and stem-only harvest at 5 years post-harvest, and control, stem-only and whole-tree harvest 22 years post-harvest at the 0 to 10 cm depth interval. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites. Means with ** are different at $p < 0.05$ and *** are different at $p < 0.01$.

4.4.2.3 Soil pH

Soil pH increased in stem-only treatments 5 years after forest harvest (Table 4.3; Fig. 4.6). There was no difference between harvest treatments and control sites at 22 years post-harvest. Clear-cut harvests have been documented to alternately increase

(Marschner and Noble, 2000; Jerabkova et al., 2011; Hynes and Germida, 2013) and decrease (Johnson et al., 1991b) soil pH. Increases in pH are primarily attributed to the addition of significant amounts of slash material. It is important to note that the phenomenon whereby slash additions affect pH is not widespread in the literature. More recent studies have begun to propose that forest litter addition may function to neutralize soil pH rather than simply creating acidic or basic conditions (Xu et al., 2006; Hong et al., 2018).

Figure 4.6. Mean values of soil pH for control and stem-only harvest treatments 5 years post-harvest and control, stem-only and whole-tree harvest treatments 22 years post-harvest. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites. Means with *** are different at $p < 0.01$.

4.4.2.4 Sulfur

A significant increase of total sulfur concentration was noted in stem-only harvests relative to control at 5 years post-harvest; however no differences were noted between treatments at 22 years post-harvest (Figure 4.7).

Figure 4.7. Mean values of total sulfur concentration post-harvest for control, stem-only harvest at 5 years post-harvest and control, stem-only and whole-tree harvest at 22 years post-harvest. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites. Means with *** are different at $p < 0.01$.

Significant variation in total S concentration within soil post-clearcut harvest sites has been reported (Mitchell et al., 1989; Spratt Jr, 1997; Likens et al., 2002; Piirainen et al., 2004). In organic (O) horizons, Mitchell, et al. (1989) reported little change in sulfur concentration in plots post-harvest at the Hubbard Brook Experimental Forest (New

Hampshire, USA). However, soils in the in E and B horizons exhibited greater S concentrations after a whole tree harvest. Mass balance calculations have shown that 88% of the organic forms of sulfur added to the O horizon can be accounted for in the E and B horizons (Mitchell et al., 1989). In contrast, other sites have shown losses in S after harvesting activities (Merino et al., 1998; Piirainen et al., 2004). Prior studies at MOFEP (Spratt, 2002) and the nearby Deer Run State forest (Spratt Jr, 1997) indicated post-harvest losses of total and organic S, although a large amount of yearly variation in S concentrations was observed. Our reported increase in total sulfur concentration and Aryl activity at 5 years post-harvest within stem-only harvest runs counter to the decreased response reported by Spratt (1997, 2002). Increased sulfur concentration in soil at Hubbard Brook post-harvest (Mitchell et al., 1989) was attributed to decreased soil pH causing increased sulfate adsorption within the E and B horizons. Mitchell et al. and Likens et al. (Mitchell et al., 1989; Likens et al., 2002) documented increased organic matter decomposition as contributing to the pool of available sulfur. Although the increases in soil total sulfur observed in our study do not agree with previously published results at the MOFEP site, they are consistent with results seen in other experimental sites. Furthermore, they represent a positive development in sulfur cycling, reflecting the potential minimal losses of sulfur from forest harvest via leaching.

4.4.2.5 Phosphorus

Singh et al. (2015) conducted extensive analysis into soil P within the Ozark Highlands. Total and available (Mehlich-3 and Bray-1) mean concentrations of phosphorus were found to be low (116.2, 7.87 and 5.81 mg kg⁻¹, respectively) and concluded that the Missouri Ozarks may be P-limited. Our reported finding of a

significant decrease in Bray-1 P in stem-only harvests relative to control treatments at the 5 year post-harvest interval represents a concerning development; however, there were no significant differences between treatments and controls at the 22 year interval (Figure 4.7). It should be noted that the differences in treatments at 22 years post-harvest although not significant, were highly variable (Table 4.3; Figure 4.8). Ponder and Eivazi (2008) examined Bray-1 P eight years post clear-cut harvest at the LTSP site, and similarly, found decreases in Bray-1 phosphorous concentrations between control and harvested treatments.

Hume, et al. (Hume et al., 2018) in a meta-analysis of 49 forest harvest studies found that forest floor (i.e., O horizon) P generally increased in concentration as a result of forest harvest; however mineral horizons experienced reduced P concentrations as a result of whole-tree and stem-only harvests. Our data seems to suggest initial decreases in Bray-1 P at 5 years post-harvest due to stem-only harvest are largely mitigated at 22 years post-harvest; however, we recommend caution in this interpretation of the results, due to the high variability of the 22 year post-harvest results.

Figure 4.8. Mean values of Bray-1 extractable phosphorus concentrations in soil post-harvest for control, stem-only harvest at 5 years post-harvest and control, stem-only and whole-tree harvest at 22 years post-harvest. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites.

4.4.3 Soil Biological Properties

4.4.3.1 Soil Enzyme Activity

Although no differences were seen in treatments within sites for β -glu and NAG enzyme activities, activities of the soil enzymes arylsulfatase and acid phosphomonoesterase were significantly different ($p < 0.05$) between the stem-only harvest and control at 5 years post-harvest; arylsulfatase activity increased and acid phosphomonoesterase activity decreased in stem-only harvest relative to the control

(Table 4.3; Figure 4.9). At 22 years post-harvest, acid phosphomonoesterase activity in the stem-only harvest was statistically similar to the control; however a marginally significant difference ($p < 0.10$) in acid phosphomonoesterase activity was noted between the whole-tree harvest and control treatments 22 years after harvest. The observed differences in enzyme activities mirrored changes in their associated elemental concentrations observed at the treatment sites. At five years post-harvest, both Aryl and total sulfur were found to have increased significantly; whereas the AcdP and Bray-1 P were both observed to have significantly decreased.

Figure 4.9 Mean values of (i) acid phosphomonoesterase and (ii) arylsulfatase activities post-harvest for control and stem-only harvest at 5 years post-harvest, and control, stem-only and whole-tree harvest 22 years post-harvest at the 0 to 10 cm depth interval. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites. Means with ** are different at $p < 0.05$ and *** are different at $p < 0.01$.

Other studies have documented changes in soil enzyme activities post-harvest; however, the magnitude and direction of change varies by site. Danielson et al.

(Danielson et al., 2017) observed increases in β -glu activity and variable site specific responses in NAG and phosphatase activities at one year post-harvest. Previous work at MOFEP found that β -glu and NAG enzyme activities initially decreased in the clearcut treatments relative to the control; however, at the two year post-harvest interval values within clearcuts were increased over control sites (Hatch, 2014). Ponder and Eivazi (2008) examined AcdP, Aryl, β -glu, and NAG soil enzyme activities at a whole tree harvest site relative to control eight years post-harvest at the LTSP site. Contrary to our observed results, they found significant differences only in β -glu activity. Ponder and Eivazi also examined the following properties: soil carbon, phosphorous sulfur and soil pH. With the exception of soil P, they found no significant differences between treatments. Soil P was found to be reduced in the harvested plots relative to control. The lack of change in the soil chemical properties between treatments may explain the relative lack of response of soil enzyme activities to treatment in the data of Ponder and Eivazi. A recent meta-analysis of global soil enzymes activities concluded that soil enzymes were more sensitive to nutrient addition than to atmospheric or climate change (Xiao et al., 2018). Although the changes in concentrations and enzyme activities between Ponder and Eivazi and our research are not similar, the relative relationship between changes in nutrient status and enzyme activity was consistent, indicating potential for enzyme activity to act as an indicator of change in the soil chemical environment.

4.4.3.2 Fungal:Bacterial ratio

A significant decrease in the fungal:bacterial ratio was observed in stem-only harvest relative to control at 5 years post-harvest; whereas, at 22 years post-harvest no

differences were observed between the harvest treatments and control (Table 4.3; Figure 4.10).

Figure 4.10. Mean values of the Fungal:Bacterial ratio in soil post-harvest for control, stem-only harvest at 5 years post-harvest and control, stem-only and whole-tree harvest at 22 years post-harvest. Means with different letters are significantly different within a site at $p < 0.1$. Lowercase letters are used for the MOFEP, and uppercase letters for the LTSP sites.

Alterations of soil microbial community structure as a result of forest harvest are well documented (Marshall, 2000; Frostegård et al., 2011; Hartmann et al., 2012; Holden and Treseder, 2013; Fichtner et al., 2014). The effects of harvest activity on fungal communities appear to be greater than on bacterial populations (Siira-Pietikäinen et al., 2001; Hartmann et al., 2012; Holden and Treseder, 2013), thus leading to the proposition that the fungal:bacterial ratio may be a suitable indicator of soil disturbance (Frostegård and Bååth, 1996). However, variability of microbial community structure observed in

other studies indicate that an element of site-specificity is associated with microbial change (Hartmann et al., 2012a; Lewandowski et al., 2015). The causal phenomena for shifts in microbial community structure has been attributed to microclimate factors (e.g., air and soil temperature and moisture), availability and quality of soil organic matter (Malik et al., 2016), and soil chemical properties (e.g., soil solution nutrient concentrations and pH) (Högberg et al., 2013a).

In particular, the fungal:bacterial ratio has been shown to be sensitive to changes in carbon cycling (Malik et al., 2016), carbon to nitrogen ratio (Högberg et al., 2006, 2013b) and changes in soil pH (Bååth and Anderson, 2003; Högberg et al., 2006). Soil fungi are less sensitive to changes in soil C:N ratio due to the ability of certain fungal species to decompose more recalcitrant carbon forms (e.g., lignin). Conversely, bacteria are advantaged by lower C:N ratios (Högberg et al., 2013b). Increases in soil pH generally favor bacterial over fungal populations (Högberg et al., 2006). Our research suggests that the observed increase in soil pH paired with a decrease in soil C:N ratio 5 years post-harvest, particularly within the upper 5 cm where microbial activity is the greatest (Lladó et al., 2017), created a soil environment favoring bacteria rather than fungi. Other factors, such as changes in microclimate created within a clearcut can affect microbial populations. Within the clearcut, direct solar irradiation on the forest floor increases daytime soil temperature during the summer months (Dan Moore et al., 2005). Previous studies at the MOFEP and LTSP sites have shown greater variability in air and soil temperatures and humidity in the even-aged management (clearcut) sites as compared to control and selection harvests (Zheng et al., 2000; Ponder, 2005). A number of authors

have shown a correlation between increasing soil temperature and increased microbial activity (Davidson et al., 2000; von Lützow and Kögel-Knabner, 2009).

4.4.4 Comparison to other forest soil health assessments

Valle and Carrasco (Valle and Carrasco, 2018) assessed the applicability of 27 potential soil health indicators in Chilean Andisols under various cover, including native forest. They found the following indicators were sensitive to treatment: bulk density; plant available water, wide coarse pores; air conductivity; soil organic carbon; extractable Al; pH; extractable P (Olsen method) and; sum of bases. The authors note that their results are largely in alignment with indicators offered by other researchers, with the exception of extractable Al, which is strongly related to the inherent properties of volcanic soils. Leite Chaves et al. (Leite Chaves et al., 2017) applied the following soil health indicators to Brazilian Oxisols under various landcovers, including native forest: soil organic matter content; cation exchange capacity; base saturation; pH; phosphorous content (method not specified); soil infiltration; penetration resistance; soil cover; and number of dung beetles per unit area. They found all indicators were sensitive to changes in the six different landcovers assessed (native forest, savannah, pasture, maize agriculture, 1 year post-reforestation and 10 years post reforestation) with the exception of pH and soil organic matter. Organic matter varied only if the landcover was forested or cleared; whereas, pH did not change in any of the landcover types. Moscatelli, et al. (2017) examining soil indicators in mountain forests across Europe found that soil organic matter, pH and microbial biomass size, activity and functional diversity are reliable and sensitive indicators for monitoring changes in soil systems.

Our research highlights the variability in appropriate indicators for assessing changes in soil health as a result of changes to forested ecosystems. Several indicators were found to be sensitive to change in our study were also held in common with the aforementioned studies: soil pH; available phosphorous and base saturation. However, we uncovered additional soil health indicators that appeared to be more specific to the Missouri Ozarks.

4.5 Conclusion

Of the 25 potential indicators evaluated, 15 (60%) were observed to have changed five years post-harvest in stem-only clearcut harvest relative to the control. Most of the observed changes could be viewed as either neutral (i.e., F:B ratio, CF%) or as positive (i.e., BS, exchangeable Ca^{2+} K^+ , C:N, ECEC, pH, TN, TOC:AC, TS and Aryl) developments – indicating increases in nutrient concentrations or availability (i.e., C:N, TOC:AC, pH) at the treatment sites. The exceptions were declines in Bray-1 P and acid phosphomonoesterase activity and the increase in soil fine bulk density. At 22 years post-harvest, few potential indicators identified differences between stem-only and whole-tree harvest treatments compared to control sites. The exceptions were C:N ratio (which remained low in whole-tree harvest treatments) and reductions in acid phosphomonoesterase activity.

Several indicators studied provided information regarding similar soil processes, and this was an intentional means to evaluate indicator sensitivity. Practitioners do not require such duplicity, thus we recommend eliminating fungal:bacterial ratio and activities of the soil microbial enzymes Aryl and AcdP from inclusion in a minimum dataset for soil health evaluation. Changes in the fungal:bacterial ratio at the MOFEP and

LTSP sites have a direct connection to improvements in pH and C:N ratio.

Furthermore, the soil enzyme activities Aryl and AcdP likely could also be eliminated, as their changes are directly mirrored by corresponding elemental concentrations (sulfur and extractable phosphorus, respectively). Additionally, all three of these assays are relatively expensive relative to the other suggested indicators and they require more care during sample collection, preparation and analysis compared to the other properties. As such, these indicators not being consistent with the rules for good indicators suggested by Doran and Parkin (1996) – specifically that they are not easy for non-soil scientists to utilize.

Our recommendation for a comprehensive list of soil health indicators for the Missouri Ozarks would include the aforementioned soil properties which were sensitive to forest harvest, while still meeting the test of relatively inexpensive and easy to interpret: BS; CF%; exchangeable Ca^{2+} and K^{+} ; C:N; ECEC; pH; TN; TOC:AC; T; and Bray-1 P. Although not as sensitive to treatment we would also recommend retaining soil fine fraction bulk density as an indicator. Although the bulk densities measured in our studies largely did not exceed the point of limiting root growth (i.e., 1.40 g cm^{-3}), it is our assessment that the reduced range of values was related to the judicious use of the best management practices set forward by the Missouri Department of Conservation, and not due to compaction associated with harvesting equipment .

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Supplemental Materials 4.A – Comparison of selected soil health indicators at MOFEP and LTSP to other long-term forest management research sites.

Table A.1 Mean soil physical properties of the top mineral horizons (~0-10cm) for the Missouri Ozark Highlands and other similar long-term forest harvest experimental sites.

<u>Site</u>	<u>Db (total)</u> <u>(g cm⁻³)</u>	<u>Db (fine)</u> <u>(g cm⁻³)</u>	<u>Coarse Fragment</u> <u>(%)</u>
MOFEP (this study)	1.31	0.78	49
LTSP (this study)	1.37	0.9	53
LTSP (Ponder, 1997)	1.26	-	-
LTSP (Page-Dumroese et al., 2006)	1.48	-	44
Clarksville series (Sauer and Logsdon, 2002)	1.41	0.98	47
Coweeta (Knoepp and Swank, 1994)	-	0.87	-
Coweeta (Price et al., 2010)	1.22	-	-
Hubbard-Brook (Johnson et al., 1991a)	-	0.64	-

Table. A.2 Mean concentrations of control treatments of total organic carbon and total nitrogen stocks of the top mineral horizons (~0-10cm) for the Missouri Ozark Highlands and other similar long-term forest harvest experimental sites.

<u>Site</u>	<u>TOC</u> <u>Mg/ha</u>	<u>TN</u> <u>Mg/ha</u>
MOFEP (this study)	12.8	0.73
LTSP (this study)	19.6	1.15
Coweeta (Knoepp et al., 2014)	32.1	1.4
Hubbard-Brook (Federer, 1984; Fahey et al., 2005)	31.7	1.9

Table A.3 Mean concentrations of exchangeable Ca^{2+} , Mg^{2+} and K^{+} of the top mineral horizons (~0-10cm) for the Missouri Ozark Highlands and other similar long-term forest harvest experimental sites.

<u>Site</u>	<u>Ca^{2+}</u> <u>($\text{cmol}_\text{c} \text{ kg}^{-1}$)</u>	<u>Mg^{2+}</u> <u>($\text{cmol}_\text{c} \text{ kg}^{-1}$)</u>	<u>K^{+}</u> <u>($\text{cmol}_\text{c} \text{ kg}^{-1}$)</u>
MOFEP (this study)	1.13	0.42	0.117
LTSP (this study)	2.63	0.733	0.167
Coweeta (Huntington et al., 2000)	3.2	0.72	0.38
Hubbard-Brook (Whittaker et al., 1979)	0.97	0.16	0.11

Table A.4 Mean concentrations of total sulfur and extractable phosphorous (Bray-1 P, Olsen P) of the top mineral horizons (~0-10cm) for the Missouri Ozark Highlands and other similar long-term forest harvest experimental sites.

<u>Site</u>	<u>TS</u> <u>(g S kg^{-1})</u>	<u>Extractable P</u> <u>(mg P kg^{-1})</u>
MOFEP (this study)	0.1	7.87
LTSP (this study)	0.18	5.23
Coweeta (Autry et al., 1990; Walbridge et al., 1991)	0.226	1.9
Hubbard-Brook (Yanai, 1991; Zhang et al., 1999)	0.18	1.9

CHAPTER 5: OBSERVATIONS ON CLEARCUT HARVEST AT MOFEP

Based upon the observed results of the analysis of soil chemical and biological properties from soil solution and solid phase samples collected at MOFEP five years post-harvest, it is apparent that clearcut bole-only harvest altered soil nutrient cycling. Within two to 14 months after the clearcut harvest, slash material left behind after the harvest had decomposed sufficiently, to trigger the assart effect – a nutrient flush within soil solution. Concentrations within soil solution during the flush phase for important soil nutrients varied, but increases in concentration ranged from 3024%, 2475%, and 1826% for NH_4^+ , NO_3^- , and TN, respectively, to 195%, 162% and 130% for Mg^{2+} , K^+ and Ca^{2+} , respectively. Soil pH was found to have increased within the clearcut treatments from 4.3 to 5.47, also as a direct result of the decomposing slash material.

The changes in these plant-essential nutrients within soil, coupled with changes in the microclimate of the clearings created by the clear-cut harvest (increased soil temperature and reduced soil moisture), and changes in substrate quality, as measured by total organic carbon to total nitrogen ratio and total organic carbon to active carbon ratio, served to alter the soil microbial populations resident within the soil. Soil saprophytic fungal populations were reduced relative to soil bacterial populations within the clearcuts; whereas actinobacterial and arbuscular mycorrhizal fungal populations were found to have increased within clearcut harvest treatments relative to the total microbial population.

The aforementioned changes are largely indicative of a healthy recovering forest, post-clearcut. Increased nutrient concentrations, improved availability and quality of

organic matter, and shifts in microbial populations are all positive developments – effectively priming the clearcut for the appearance of successional ground flora, and eventually the regrowth of the native oak, hickory and pine extant at the site prior to the clearcut. At five years post-harvest, visual observation of the clearcut sites revealed dense growth of vegetation, making travel through the sites difficult – potentially good indicator of healthy succession. Results from the soil geochemical analysis at the adjacent LTSP site at 22 years post-harvest suggest that the changes observed at 5 years post-harvest did not translate into long-term effects.

The exception to this generally favorable outcome are the results of this research specifically concerning phosphorous. Available phosphorus and acid phosphomonoesterase activity – an important soil enzyme indicating phosphorous turnover – were significantly reduced in clearcuts relative to control sites. The Missouri Ozarks are well-documented to be phosphorus limited and phosphorus levels are low, relative to other parts of Missouri. Our research has shown that the phosphorus being released from decomposing slash material is present in soil solution at the upper zero tension sampler interval (15cm depth), but is not present at the lower depth intervals. This suggests that the available phosphorus is being lost by a mechanism other than leaching or overland flow. We suggest that the phosphorus is likely being lost via strong inner-sphere complexation to iron and manganese oxides and are being rendered unavailable to plant growth. The results at the LTSP site (22 years post-harvest) displayed high variability in available phosphorus; however, soil acid phosphomonoesterase activity was reduced in whole tree harvest relative to control sites, potentially indicating long-term impact to phosphorus cycling due to even-aged management.

Overall, this research suggests that the MOFEP clearcut bole-only harvest is displaying evidence of a health recovery. The impact of clearcut upon phosphorus cycling is an area of concern and may warrant monitoring and perhaps some mitigating remediation during later stages of regrowth, to include limited phosphorus fertilization of the clearcut sites, if regrowth appears to be impacted. Further study of phosphorus cycling at MOFEP and the LTSP sites is strongly suggested by the results of this research and is recommended by the authors.

APPENDIX 1. SOLVITA – A POTENTIAL FOREST SOIL HEALTH INDICATOR?

A.1 Introduction

Doran and Parkin (1996) recognized five features that good quantitative soil quality (health) indicators should possess:

- They should correlate well with ecosystem processes.
- They should integrate physical, chemical and biological properties and processes, and/or serve as basic input needed for estimation of soil properties or processes which are more difficult to measure.
- They should be easy to use in the field and assessable by both soil specialists and by agricultural producers.
- They should be sensitive to variation in management and climate.
- They should be components of existing soil databases, whenever possible.

Although progress has been made in identifying and quantifying many potential soil health indicators, the majority violate the third tenet; specifically, that they are easy to use and accessible by agriculture producers. In particular, suggested biological and biochemical indicators such as soil enzyme activities, soil microbial community structure and soil respiration tests are difficult for producers to perform, require sophisticated sampling, processing and analytical techniques. They can also be difficult for non-scientists to interpret.

The Solvita® test was developed specifically to bridge this gap with respect to ease of use and relatively low cost (Haney et al., 2008). The Solvita® test consists of a pH-

sensitive reactive gel embedded within a plastic probe. The probe is placed within a sealed jar with a soil sample for a specified period of time. After the incubation period the color of the gel is compared to a color chart to determine the amount of CO₂ produced during the incubation period. The Solvita® test is marketed as a nearly error-free determination of microbiological activity which can be performed by anyone (Haney et al., 2008).

The objective of this study was to determine the applicability of the Solvita® CO₂-Burst test to measuring harvest impacts on biological activity of forest soils.

A.2 Methodology

A.2.1 Soil Sampling

To focus on the zone of greatest potential biological change (Lladó et al., 2017), the top 5 cm of the mineral soil was sampled for analysis. Three replicates for each treatment-nutrient status pair were collected in May of 2017. The samples were air-dried and sieved to ≤ 2 mm, then stored for subsequent analysis.

A.2.2 Solvita® Methodology

The published Solvita® methodology is as follows (Solvita®, 2016). Forty grams of air-dry soil is placed into a 50 cm³ graduated beaker. The soil is settled by tapping the beaker 3 to 4 times, and the volume of soil is estimated allowing an estimate of the soils bulk density to be calculated. A plastic screen is placed on the surface of the soil to minimize soil disturbance and to promote dispersion of the water. Enough water is slowly added to fill 50% of the available pore space within the soil. A Solvita® test probe is placed with the filled graduated beaker into an air-tight 250 cm³ glass jar. The jar is then placed in a

controlled temperature room at 20 to 24°C. After 24 hours the probe is then removed from the jar and read on the supplied Digital Color Reader to determine the change in color of the probe, which is reported in both color value and converted to $\text{mg kg}^{-1} \text{CO}_2\text{-C}$.

A.2.3 Other Methods

The Solvita® response results were compared to a number of selected soil health indicators, specifically: Soil microbial functional groups as measured by phospholipid fatty acid (PLFA) analysis; pH; total nitrogen; total organic carbon; carbon:nitrogen ratio; and four soil enzyme activities (acid phosphomonoesterase, arylsulfatase, β -glucosidase and N-acetyl- β -D-glucosaminidase). The methods for each of these assays can be found in chapter 3 of this document.

A.2.3 Statistics

A two-way analysis of variance (ANOVA) using a linear mixed model (Proc Mixed) in SAS 9.4 (SAS Institute; Cary, NC) was used to evaluate effects of treatment (clear-cut, single tree selection, and control) and nutrient status (low and medium) on soil microbial communities, enzyme activities, and geochemical properties. The data distribution was tested for normality using the Shapiro-Wilk test. Pearson's correlation coefficients were computed in SAS 9.4 to identify relationships between Solvita® Soil CO₂-burst test and selected soil properties ($p < 0.05$).

A.3 Results and Discussion

A.3.1 Methodology Adjustment

An initial test was performed on two samples found to have relatively high and low total organic carbon content. The high TOC sample was found to have a bulk density of 0.75 g cm^{-3} . This low bulk density overfilled the supplied 50 cm^3 graduated cylinder. Therefore, the mass of soil used was adjusted from 40 to 30 grams. The value of the reduced mass read on the Digital Color Reader was $222 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$. Adjusted to the proper ratio, the resultant value was $296 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$. The rest of the samples were run with the reduced soil mass of 30 grams. Results for the entire sample set ranged from 39.8 to $241.0 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$, with a mean of $147.8 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$, with adjusted results ranging from 53.1 to $321.3 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$, with a mean of $197.1 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$.

The Solvita® test was originally designed with an expected $\text{CO}_2\text{-C}$ concentration of 0.0 to 95 mg kg^{-1} produced in a 24 hour period (Solvita®, 2017). This range was consistent with early research (Pettersson et al., 1979) into probable ranges of expected microbial respiration rates. It is now recognized that soils higher in organic matter content, such as heavily manure-amended soils and forest soils may potentially exceed this respiration rate. The Solvita® probe response becomes fairly steep at $\text{CO}_2\text{-C}$ concentrations above 100 mg kg^{-1} (Figure 5.1). Concentration values in excess of 100 mg kg^{-1} may be inaccurate, although relative relationships should still be preserved (Solvita®, 2017). A recently published technical memo produced by Woods End Laboratories (the creators of Solvita®) recommends reducing the mass of soil used when testing soils high in organic matter content (Solvita®, 2017). The minimum amount of soil to be used should be no less than 20 grams. If the response at 20 grams of soil is still

above 100 mg kg⁻¹ CO₂-C produced in 24 hours, Woods End Laboratories recommends increasing the size of the incubation jar, rather than reducing the soil mass further. Soil masses under 20 grams may lead to difficulty in accurately predicting the volume of water required to reach 50% of available pore space filled with water. Based upon the range of Solvita® responses observed in our experiment, we estimate that a reduction of soil mass to 20 grams (50% of the nominal value) would likely still lead to an observed concentration range of 25 to 160 mg kg⁻¹ CO₂-C, with a mean near 100 mg kg⁻¹ – still well above the 0 - 100 mg kg⁻¹ optimal range. Taking into account the low bulk densities observed in the top 5 centimeters of the soils we sampled, we would recommend one of the two following methodologies:

1. A reduction in the mass of soil used to 30 grams and an increase in the volume of the incubation jar used to a quart sized canning jar. This would necessitate multiplying the observed response by 5.1 to correctly calculate the true CO₂-C concentration.

$$\left(\frac{40 \text{ grams (nominal soil mass)}}{30 \text{ grams (actual soil mass used)}} * 1.02 \text{ (recommended volume correction)} \right) * 3.75 \text{ (jar volume correction)} = 5.1$$

This would reduce the estimated response range from 10 to 60 mg kg⁻¹ (or probe color 2 to 4).

2. Alternatively, a larger graduated cylinder (e.g., 60 cm³) could be used to hold the soil within a quart sized canning jar. This would only require a simple

correction of 3.75, yielding an estimated observed response range of 15 to 85 mg kg⁻¹ (probe color 2 to lower end of probe color 5).

Figure A.1 Solvita® Optical Response versus concentration of CO₂-C in Soil, illustrating the steepness of the response curve above 100 mg kg⁻¹ (Solvita®, 2017).

A.3.2 Solvita® Response to Treatment

Significant differences in the Solvita®-derived concentrations of CO₂-C were observed between clearcut harvest and control treatments ($p < 0.01$) and between low and medium nutrient status soils ($p < 0.05$) (Figure 5.2). There were no observed interactions between treatment and nutrient status. The clearcut plots had significantly higher Solvita® response (42.3 mg kg⁻¹ CO₂-C) than both the control (183.6 mg kg⁻¹ CO₂-C) and single-tree selection (165.3 mg kg⁻¹ CO₂-C) harvest treatments. The single-tree selection treatment was statistically inseparable from the control treatment. The medium

nutrient status soil Solvita® response ($214.8 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$) was significantly higher than the low nutrient status soil response ($179.4 \text{ mg kg}^{-1} \text{ CO}_2\text{-C}$).

Figure A.2. Boxplots of the response of the Solvita® Soil CO₂-burst test for A) control (CON), single-tree selection (SEL) and clearcut (CC) harvest treatments and B) low and medium nutrient status soils. Means with different lower-case letters are significantly different at $p < 0.10$.

A table of the soil chemical and biological properties analyzed simultaneously with the Solvita® test can be seen in table 5.3. The microbial functional groups of arbuscular mycorrhizal fungi, was found to have significantly increased within clearcut treatments; whereas the saprophytic fungi was found to have decreased. The fungal:bacterial ratio was also found to have decreased within clearcut treatments. Gecochemical properties found to have increased in value within clearcuts were soil pH and total nitrogen. C:N ratio was observed to have decreased. The soil enzyme arylsulfatase was observed to have increased in activity within clearcuts; whereas, acid phosphomonoesterase activity was found to have decreased.

When the Solvita® responses were compared to the selected soil properties via a Pearson's correlation, the responses was found to be correlated with many of the soil chemical and biological properties assayed, with the exceptions of fungal microbial functional group, the fungal:bacterial ratio and the acid phosphomonoesterase activity (Table 5.3).

Table A.3. Pearson's correlation coefficients between selected soil properties and Solvita® Soil CO₂-burst response. Values highlighted with * are significant at the p<0.05 level. Properties in bold were found to be sensitive to clearcut harvest treatment.

<u>Property</u>	<u>Pearson's r</u>
Total Organic Carbon	0.6100*
Total Nitrogen	0.8983*
C:N Ratio	-0.5116*
β-glu	0.7199*
NAG	0.6662*
AcdP	0.1281
Aryl	0.8541*
pH	0.7620*
Bact	0.8068*
Fung	0.2641
AMF	0.9003*
Actino	0.8487*
TotPLFA	0.8078*
Fung:Bact	-0.1239

Key to abbreviations: TotPLFA – total microbial biomass via PLFA; Bact – total bacteria; Act – actinobacteria; AMF – arbuscular mycorrhizal fungi; Fung – fungal biomass; Fung:Bact – fungal to bacterial ratio; TN – total nitrogen; TOC – total organic carbon; C:N – total organic carbon to total nitrogen ratio; AcdP – acid phosphomonoesterase activity; Aryl – arylsulfatase activity; B-glu – β-glucosidase activity; NAG – N-Acetyl-β-D-glucosaminidase activity.

Findings on the correlation of Solvita® and other soil health tests have been mixed. Chahal and Van Eerd (Chahal and Van Eerd, 2018) found that Solvita®, results were not consistent from year to year with yield differences, wet-extractable carbon and nitrogen concentrations, C-mineralization rates and cover crop carbon and biomass determinations. They also observed similar findings for the Haney Soil Health Test and the Solvita® Labile Amino Nitrogen (SLAN) tests. Nkongolo et al. (Nkongolo et al., 2016) used Solvita® to estimate soil respiration in reclaimed mining properties in forested ecosystems in Canada. They observed the Solvita® response to be uncorrelated with the other tested properties of total microbial biomass, total number of trees, tree species and ground cover species richness measures.

Conversely, Muñoz-Rojas, et al. (Muñoz-Rojas et al., 2016) applied the Solvita® test as a measure of microbial activity in degraded semi-arid soils undergoing restoration. They found good correlation between the Solvita® response with soil organic carbon and microbial abundance and diversity. They concluded that Solvita® was an inexpensive and effective method of assessing soil functionality in restored soils. Ward, et al. (Ward et al., 2017) examined the effects of nitrogen fertilization on soil respiration using the Solvita® test. The Solvita® response was found to be sensitive to increasing levels of fertilization via an inverse relationship with increasing levels of nitrogen fertilization in longer term studies (decade level); however there was no correlation found in shorter term studies. A positive relationship between Solvita® response and pH was also found. The authors suggest that changes in dominant enzymes and fungal: bacterial dominance may be the controlling mechanism behind the changes in microbial respiration.

Dick et al. (Dick et al., 2016) in their assessment of biological and biochemical tests to assess soil fertility conclude that soil CO₂ emissions, assumed to be the product of microbial respiration, are still not well understood.

A.4 Conclusion

A key conclusion is that the controls on microbial respiration are still not well understood. Results of the research tying soil microbial respiration to soil fertility and productivity are mixed and more research is required to consider Solvita® as a dependable tool of evaluation forest soil health. We found Solvita® to correlate well with other commonly used soil health indicators. We conclude that the Solvita® test is a useful tool to assess changes to assess changes in soil health due to forest harvest, particularly when used in conjunction with other soil health indicators.

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VITA

Alexander Taylor was born on New London Naval Base in Connecticut. After twelve years of living on various naval bases, his family moved to northwest Arkansas. After an abortive educational start at the University of Tulsa, he transferred to the University of Arkansas. In 1989, he graduated with a Bachelor of Science in Geology from the University of Arkansas – Fayetteville. He worked towards a dual Master of Science degree in Geology and Electrical Engineering until 1993, at which point he received a Board of Regents Fellowship to pursue a PhD at Louisiana State University. He received a PhD in Geology from LSU in 1998. In 1997, Alexander began work at Texaco Exploration and Production Department as a geoscientist, applying structural geology and remote sensing techniques to petroleum exploration, production and environmental impact assessments. After 9/11, Dr. Taylor was recruited by the U.S. Army to provide remote sensing and geological expertise to the Army Intelligence Community. He worked at the U.S. Army National Ground Intelligence Center, assuming roles of increasing responsibility as a contractor, civilian senior scientist for image science, Division Chief for the Imagery Applications Division, to Special Projects Officer within the Office of the Commander. In 2013, Dr. Taylor left the intelligence community to pursue a second PhD in Soil Science at the University of Missouri with an expected graduation date of December 2018. In the fall of 2018, Dr. Taylor accepted the position of Assistant Professor at Truman State University, where he will be teaching soil science and geology.