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SOIL SUSTAINABILITY OF FOREST BIOENERGY FEEDSTOCKS ACROSS THE AMERICAS

By

Michelle E. Cisz

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In Forest Science

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This dissertation has been approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in Forest Science.

College of Forest Resources and Environmental Science

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Preface

The following chapters have been written by the author of this dissertation with the help and editing of advisors Dr. Rod Chimner and Dr. Sigrid Resh.

Acknowledgments

This dissertation was possible with the financial and professional help of several people over the years. The financial support of this research was through NSF-PIRE OISE under the management of Kathy Halvorson who entertained this vision and made it a reality. It was interesting to see the undertaking of such a large project as pieces came together at different times like a colorful knit hat. I would like to thank my advisors for their help at various steps of this research and for helping me make this dissertation possible. Rod guided me to think about concepts from a broader perspective while Sigrid inspired me to think deeper and pay attention to detail. They always gave me the room to improvise and learn while lending guidance and support at critical points over the years. Sigrid was especially key in helping me keep the ball rolling through the unexpected. I'd also like to thank my committee. Andy Burton always provided efficient, speedy help whether it was a question about shipping soil, details on an experiment, or random questions on statistics. His lab was generous in letting me occupy lab space and Jennifer Eikenberry provided professional help processing samples and keeping the lab in order to maintain productivity. Committee member, David Shonnard, has provided his external perspective on the research which has helped me in communicating science and in thinking about it from a different perspective.

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List of abbreviations

BD – bulk density (g/ cm^3)

%C – 1 gram of carbon per 100 grams of bulk soil

C – carbon

$cmol_c$ /kg – centimoles charge per kilogram of soil

COLPOS – College of post-graduate studies

ejidos – land that is owned by the state but used communally

EPA – Environmental Protection Agency

ha – hectare = 10,000 m^2

HF – a heavy fraction of carbon often associated with organic minerals (> 1.65 g/cm^3)

INTA –Instituto Nacional de Tecnología Agropecuaria

IPCC – Intergovernmental Panel on Climate Change

LF – light fraction including both free and occluded carbon (< 1.65 g/cm^3)

Mg – megagram = $1x10^6$, used in context Mg/ha

n.s. – not significant statistically

Pt – Petagram

µg – micrograms

yr – year

Abstract

Many sources of woody biofuels provide alternative options to fossil fuels that can help mitigate greenhouse gases. One key component of feedstock sustainability from an ecosystem service perspective is soil sustainability. We examined eucalyptus (*Eucalyptus grandis*) plantations in the warm-temperate entic and mollic soils of Northeastern Argentina, oil palm plantations (*Elaeis guineensis* Jacq.) in the tropical alfic soils of Southeastern Mexico, and aspen (*Populus tremuloides)* in the cool-temperate spodic soils of Northeastern United States. The following elements were measured in soil increments of 15 cm, down to a total of 60 cm: carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and aluminum (Al). In each country, we used a chronosequence design, measuring forest-stands of different age treatments (post-harvest or years since planted) to substitute for time. Each age treatment was comprised of at least three replicates.

In Argentina, the total soil C trended downward over the 50 years of Eucalyptus land-use ($p = 0.12$), however, these trends were driven by reductions in mineral-associated C fractions (heavy fractions) that normally represent persistent C and soil quality. The heavy fraction declines ($p = 0.06$) suggest long-term land degradation.

In Mexico, young oil palm stands, adult oil palm stands, pastures, and secondary forests were all similar in soil C, and most nutrients (0-60 cm). Adult oil palm stands had lower levels of N, while secondary forests had higher levels of iron (Fe) than other land-use treatment groups. Young oil palm stands had higher levels of C, N, and P in their weeded circles than adult oil palm stands, however the total weighted area of the weeded circles is not enough to make significant differences at the scale of 1-hectare.

Last, in the United States, Wisconsin coarse-textured aspen stands increased 42% in soil C from a chronosequence of stands aged from 10 to 56 years post-clear-cut in depths down to 45 cm ($p = 0.02$). Nutrients were relatively stable with increasing age of stands except for P, which increased exponentially with age and depth. While current management is sustainable, caution is warranted for sandy soils to be used as sources of biomass for biofuels, because they are more vulnerable to C and nutrient losses, especially in cases of intensive residue removal.

Each country's potential feedstock poses unique circumstances in which between countries, soils were impacted differently by climates, soil orders, and management. Biofuel management can offer promising sequestration of C in the short-term, and in the long-term, impacts will likely be influenced by proper residue management, preventing soil compaction, and not shortening rotation lengths in order to facilitate soil C and nutrient recuperation.

1 An Overview

"The nation that destroys its soil, destroys itself"- *Franklin Delano Roosevelt*

The context of this research lies in-part of a larger project under the auspices of the National Science Foundation grant PIRE-OISE that has allowed scientists and professionals from different disciplines to not only learn how to work together across regions and cultures, but also reflect upon and identify gaps in considering woody bioenergy feedstocks, research them, and offer interpretations for policymakers and future questions to be investigated. The ecosystem services team was just one of several teams within this project and focused on pollinator species, hydrology, and soil. The sustainability of soil is a critical ecosystem service and this chapter is meant to set the tone for why this research was conducted. Soil sustainability can be defined as the management of land facilitating essential physical, chemical, and biological attributes of soil that help plants grow. Sustainability is somewhat relative to time. The recuperation of any lost soil function is dependent not only on short-term factors but also on slower longer-term processes that influence soil.

Bioenergy and biofuels can help to mitigate greenhouse gases as an alternative energy source to fossil fuels. Not only this, but they are also recoverable on a much shorter time-scale of ten to a hundred years compared to fossil fuels that take millions if not hundreds of millions of years to form. Bioenergy can be a better alternative when the sum of its steps from source to emission upon ignition is reasonably lower than the fossil fuels already in place. Bioenergy feedstocks are the crops from which the bioenergy source comes through photosynthesis powered by solar radiation. This can take the form of both sugar/starch-based biomass such as corn and sugar-cane, or be extracted oils from seeds, beans, and fruits such as rapeseed, sunflower, jatropha, castor, soybeans, and palm fruits. Additionally, the lignocellulosic biomass from agricultural residues such as corn stover, wheat straw, and cane bagasse as well as fast-growing woody products such as willow, pine, eucalyptus, and poplar have also been under consideration as energy sources.

Biomass can be considered an important part of ecosystem services. Ecosystem services are a means to value functions that an ecosystem provides, or rather consider the opportunity cost-what gains are lost by choosing alternative scenarios. For example, wetlands can filter many pollutants from the water. If we consider draining them, we not only lose that service of filtration, but also face alternative scenarios to pay for the chemical neutralization of pollutants, or perhaps do nothing and eventually pay more in healthcare. Costs could also add up in the long-term through the release of greenhouse gases, less predictable weather, sea-level rise and real estate damage. Natural forest ecosystems can provide habitats for wildlife, reduce flooding, and at the land-scape scale provide corridors for wildlife, and maintaining watersheds. Plantations are notably less diverse in plants and animals, and in some cases negatively affect watersheds, however, they can still be considered sustainable in the context of offering a lower C footprint compared to alternative fossil fuel sources. Sequestering carbon dioxide (CO_2) from the atmosphere through photosynthesis, and converting this C to live biomass is one-way trees and plants provide this ecosystem service. Life cycle assessments include the stand initiation, maintaining the stand, harvesting the stand, as well as transporting the biomass to the processing plant to generate fuel or energy. If the life cycle assessments confirm a lower C footprint, then this makes the alternative energy source worthwhile.

Though above-ground biomass is an important consideration and the one we think of most because it is visually apparent, below-ground sustainability is just as critical and needed in supporting C sequestration, soil health, as well as C storage. Soil organic matter provides the service of retaining soil moisture and needed nutrients for productivity, but also facilitates the storage of C that might otherwise be released to the atmosphere contributing to greenhouse gases. An example is crops such as oil palm, which are fast-growing plants that sequester C relatively fast compared to most tree species. When oil palm stands are established on suitable land, this can create a very low C footprint, but when established on drained wetlands, can lead to the release of enough CO_2 and methane that it would take hundreds of years for that crop to sequester compensating CO_2 equivalents (Gibbs et al. 2008) as defined by the IPCC.

The storage of soil C is influenced by many factors including the climate, topography, parent material, and management history of the land (Brady et al. 2008), as well as the biological control of organisms. Trees species, as well as the macrofauna and microfauna, play roles in recycling and storing soil C. The microbial flora is complex and one of the essential movers and converters of soil organic matter. They can help provide aggregate stability and structure, which also facilitates soil C content and stability. They are responsible for decomposition, much of the soil respiration (the release of CO_2 from the soil), and their efficiencies and preferred substrates can vary as a community of diverse members each carries out different roles. Through decomposition they are also essential in converting nutrients to available forms for plants.

Soil nutrients are important consideration for biofuel management because they can be exported with harvested biomass. Fertilizers are often applied to plantations opposed to natural forests, and if done excessively, can lead to eutrophication in nearby water surfaces. With intensive management and frequent harvests, nutrient can become limited. Depending on the nutrient, nutrient limitations can lead to slowed growth and make feedstocks more susceptibility to disease.

To this extent, potential bioenergy feedstock forests and the forest soil can respond differently depending on all these factors. Soil sustainability of bioenergy feedstocks is not only an indicator to assure the productivity of biomass but is also important for reducing CO_2 emissions. This can be done through C sequestration via photosynthesis, and through increasing soil C storage, or at least reducing losses. One strategy is through managing land-use changes that are directed in creating greater C storage (Don et al. 2011; Laganiere and Angers 2019). This can be for both above and belowground C stocks, but soil C and nutrients are ultimately critical in supporting above-ground productivity and C stocks. Other strategies to reduce the loss of soil C to the atmosphere include reducing disturbances 1.) while clearing land for the establishment of bioenergy feedstocks, 2.) during thinning throughout the stands life, and 3.) during harvesting while retaining residues on site. Long-term management is key for assuring prospects of the land that can continue to meet the needs of local communities and broader societies.

To examine soil sustainability within various types of forest feedstocks that could be used as bioenergy, we measured soil C, macro-nutrients, and other relevant soil characteristics. We used a chronosequence design for each study, a technique of substituting “space for time” that does carry limitations and heeds some level of caution for interpretation. Management changes over time such as use of fire, improvements in fertilization, and harvesting equipment, as well as environmental changes related to disturbance events such as floods, frosts, fires, and overlooked fine-scale attributes such as microbial community composition, soil moisture, pH, and soil weathering impacts on biogeochemistry can all be factors to consider that might not fully be accounted for when designing a chronosequence. For this reason, the sites in each of these three chapters were chosen from soils with similar soil characteristics in texture, color, and slope. Large differences in soil development were avoided when possible.

The overarching objectives of this research were to quantify soil C and nutrients within various types of bioenergy feedstocks in different countries of the Americas. We set out to 1.) provide perspective as to whether current “business as usual” management was the best scenario compared to other alternative land uses in that area; 2.) quantify soil C to understand to what extent soil C is being gained, lost, or having no net change over time; and 3.) quantify nutrients to see if depletions were occurring relative to the duration of the feedstock land use.

2 The Effects of Multiple Rotations of *Eucalyptus grandis* on Soil Carbon

2.1 Abstract

In addition to storing vast amounts of atmospheric carbon (C), soil C in forest plantations also improves soil health by retaining moisture and nutrients, buffering soil pH, supporting microbiota, and improving soil structure and aggregate stability. Eucalyptus plantations are used throughout the tropical/subtropical world for wood production, timber, and pulp. Eucalyptus' utility and adaptability have led to expansions throughout South America.

While there is a large amount of literature that focuses on Eucalyptus and soil C in the first 1 or 2 rotations, the long-term impact of plantations on soil C quantity and quality. Here we assess the quantity and quality of soil C down to 60 cm in both the short-term and the long-term chronosequences of *Eucalyptus grandis*. The short-term chronosequence is represented by second rotation stands in their early, mid, and late-stages of a ~10-12 year harvest cycle. The long-term chronosequence is represented by late-stage stands of rotations 1 - 4 with similar rotation length.

We found no significant differences in soil C and nutrients across the second rotation, although the longer-term chronosequence shows a strong downward trend in soil C by the 4th rotation ($p = 0.12$). Total nitrogen declined across the chronosequence ($p = 0.02$). Other nutrients, P, K, Ca, and Mg, varied by soil depth and among rotations, showing no significant changes except at a few select depths. To assess C quality, C was separated by density into two fractions: 1) heavy fractions (HF) and 2) free light and occluded fractions (FLF+OC) at 0-15 cm and 45-60 cm. The HF reveals that recalcitrant C is declining in both surface mineral soil (0-15cm) ($p \leq 0.10$) and in deeper soils (45-60 cm) ($p = 0.06$). This suggests that not just the quantity, but also the quality of soil C has declined in *E. grandis* stands over four rotations. This suggests a degradation in soil and less sustainability as biofuel.

Keywords: (soil carbon, long-term, multiple rotations, *E. grandis,* carbon stabilization)

2.2 Introduction

Nearly half of the world's terrestrial carbon (C) is stored in forests, which contain roughly 787 Pg of C (Dixon et al. 1994; Johnson and Curtis 2001). Forest C is distributed both above and below ground, with nearly two-thirds of forest ecosystem C located in the soil. Soil C improves soil health by retaining moisture, providing nutrients, buffering soil pH, supporting microbiota, and improving soil structure and aggregate stability. In a future with increased forest use and intensive management, long-term forest sustainability is reliant on the maintenance of soil C and its implicit functionality.

Of the world's forests, ~ 7 million ha are planted with non-native trees (Del Lungo 2006; Pan et al. 2013). In South America many governments provided subsidies to plant non-native tree plantations in the 1980s and in many places those plantations still exist (Palo and Mery 2012). *Eucalyptus grandis*, one of the more common species used for plantations in South America, are primarily used for pulp and timber because of their rapid growth. Eucalyptus plantations are managed intensively with a typical rotation length in the wet tropics of 5-15 years and 10-30 years in the dry tropics (Del Lungo 2006). Eucalyptus plantations are often managed on multiple harvest rotations. Silvicultural managers can employ various methods for regrowth after a harvest including single stem selection, coppice regrowth, or planting new saplings between old stumps (Sandoval López et al. 2018).

While many studies of Eucalyptus plantations show limited changes in soil C over one to two rotations (Lepsch 1980; Bashkin and Binkley 1998; Binkley and Resh 1999; Guo and Gifford 2002; Neufeldt et al. 2002; Zinn et al. 2002; Mendham et al. 2003; Binkley et al. 2004; Hernández et al. 2016; Soares et al. 2017), few studies have examined the effects beyond two rotations. The studies that have looked at long-term changes over multiple rotations have generally found that total soil C decreases or remains relatively constant. For instance, a study by Cook and others (2016) showed changes in soil C in ~300 Eucalyptus stands across three regions of Brazil. The stands were managed on short rotations (6-8 years), and overall, soil C slightly declined over 26 years. The decline was driven by one region making up about 1/3rd of the study, and the decline occurred over what was likely the first and second rotation (17 years). The other two regions did not change significantly in soil C. Another long-term study found that a chronosequence of nine Eucalyptus stands had soil C at 0-10 cm that peaked by the end of the 3rd rotation (21 years) and then did not statistically change after (Lima et al. 2006). In a separate study, a chronosequence of 30 Eucalyptus stands managed on rotation lengths of 12-14 years in Entre Rios, Argentina displayed soil C peaks in the second rotation (~24-28 years) and then trended downward with subsequent rotations. Stands in their second rotation held similar C levels to adjacent pastures (Sandoval et al. 2012).

These studies looked at changes to the quantity of soil carbon, but changes to the quality of carbon are also important. Separating carbon into fractions based on their density, e.g. light vs heavy, is one method used to describe the quality of C (Golchin et al. 1994; Swanston et al. 2005). The lighter carbon characterizes labile carbon with fast turnover rates in years to decades that are usually derived from detritus inputs like leaf litter. The heavier carbon typically characterizes older C that is bound to clay minerals and can persist in the soils for centuries (Crow et al. 2007; Jiang et al. 2019). It is also referred to as mineral associated organic matter (MAOM), but we will be referring to it as heavy carbon. The persistence of heavy carbon is due to several different mechanisms including chemical and physical occlusion, which prevents access for microbial decomposition. In general, an accumulation of heavy carbon indicates that soil C is more protected and resistant to change and represents a longer-term pool, whereas, an accumulation of lighter C is typically more vulnerable to accelerated loss from climate or land-use change. It is not uncommon for soil C accrual to be mostly in labile fractions initially, with heavy C fractions increasing later (Jiang et al. 2019; Laganiere and Angers 2019).

The quality of soil can also reflect management practices with the light fraction being most vulnerable. Practices such as burning, plowing, tilling, thinning, long-term chemical fertilization, and harvesting can decrease labile carbon (Tan et al. 2007; Dou et al. 2016). Disturbance can affect both heavy and light fractions, especially if the perturbation disrupts soil aggregates that help protect the carbon (Six et al. 2002; Kumar et al. 2014). Management practices that lead to long-term degradation are usually reflected in the heavy fraction while labile fractions are usually able to recuperate faster.

Our primary objective was to quantify changes in soil C quantity and quality across a single rotation and over four rotations in *Eucalyptus grandis* stands, using a chronosequence approach. We hypothesized that soil carbon would not significantly change within a single rotation, but would decline after four rotations, especially in the surface soils (< 20 cm). We also hypothesized that most of the carbon loss would be from the light C fractions and the heavy C would not change. Our secondary objective was to assess changes in soil nutrients and metals within single and across multiple rotations of *E. grandis* stands.

2.3 Materials and Methods

2.3.1 Site descriptions

This study was conducted in the Argentina province of Entre Rios, near Concordia, which is part of the temperate biome within the subtropic dry forest zone (Holdridge, 1947, 1967). The study sites (31°15'18"S, 58°16'39"W to 31°58'49"S, 58°18'22"W) were located just west of the Uruguay River and received an average of 1376 mm precipitation per year ranging from 52 mm in the cooler month of July to 163 mm in April from 1981-2010 (Figure **2-1** and Figure **2-2**) (SMN).

Near the Uruguay River, soils were sandy to loamy and the region was historically used for livestock breeding. West of the sites were finer textures, often Vertisols, and currently used for citrus, pasture, or agriculture. Wetter patches of shrubs, or what is referred to locally as "espinal", could also be found scattered throughout the landscape and are generally not suitable for *E. grandis* due to the risk of poor drainage and frost damage. Soil maps in this region indicated the soil could be a variety of different types, including Mollisols and Entisols, Inceptisols, and Vertisols. All *E. grandis* stands in this study were growing in soil with a sandy loam texture in the top 30 cm. Soil below 30 cm depth was either sandy loam or sandy clay loam with clay ranging from 5 to 27% composition. The area was at risk for erosion and during this study, the erosion varied from light to moderate, indicated by rills, grooves less than 1 m wide that were caused by the overflow of rain and the movement of sediment. (Tasi 2009). Most stocking densities in this area were 1000 plants/ha.

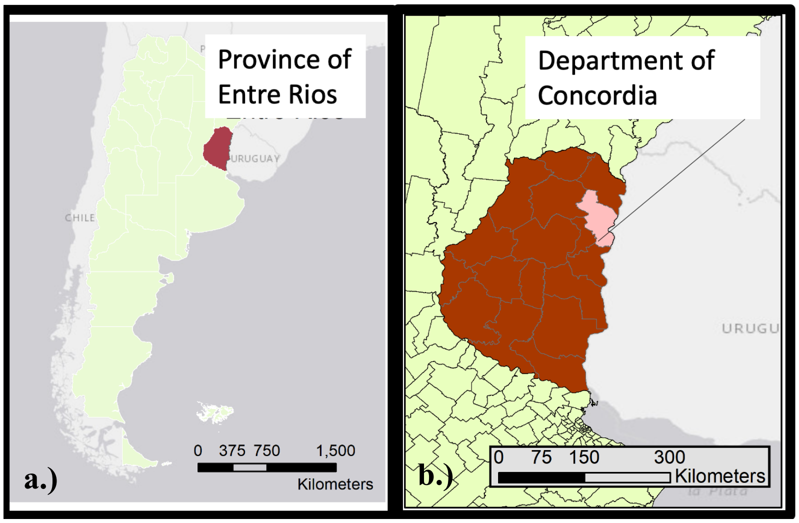
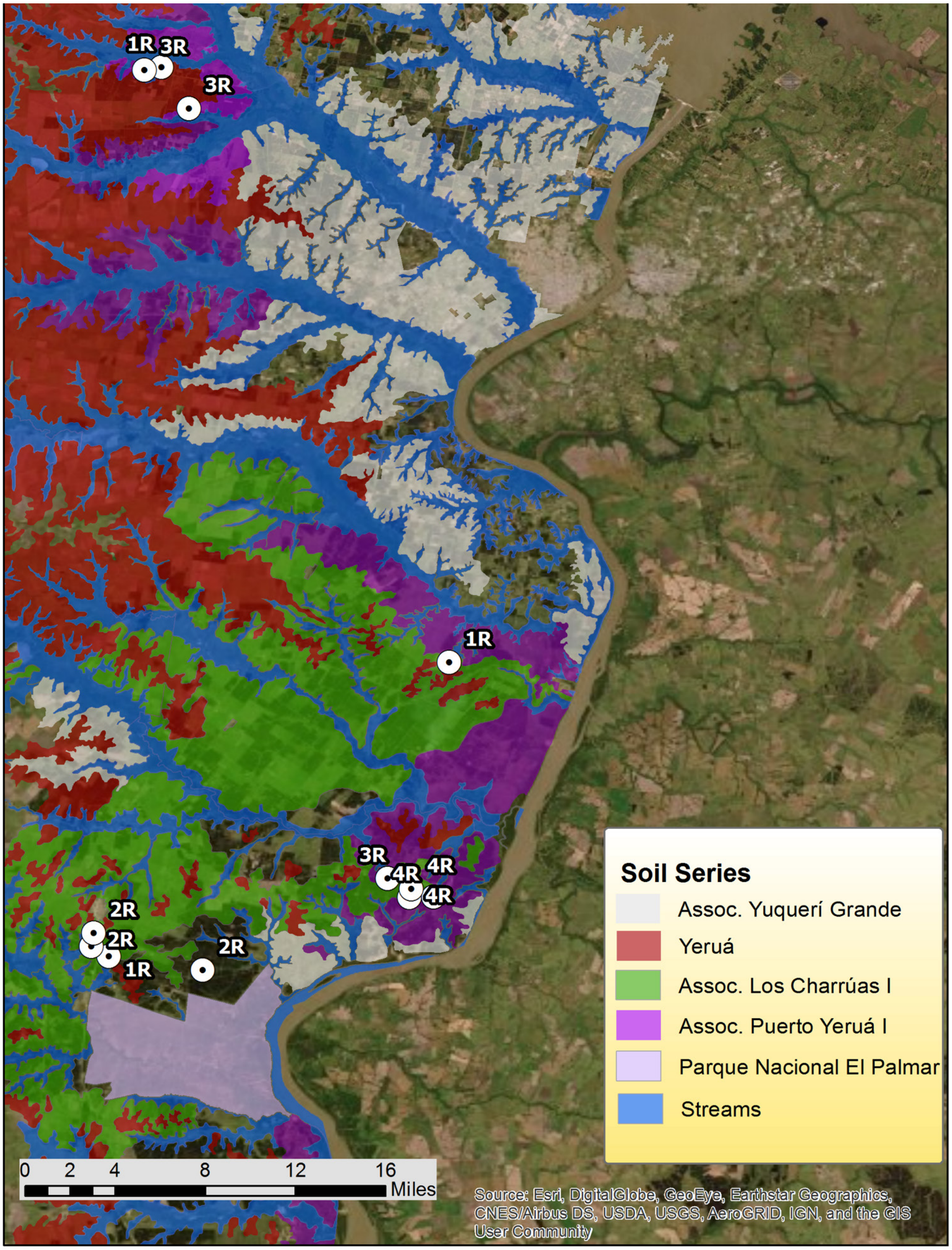


Figure 2-1. a.) Entre Ríos (red) in Argentina (yellow-lime) and b.) Concordia (pink) in Entre Ríos.



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 2-2. Concordia sites listed by rotation. Source: Google Earth Pro, see appendix for full citation.

2.3.2 Experimental design

Our study's design was limited to loamy soils of *E. grandis* and the results may not apply to other Eucalyptus stands growing in sandy soils. In late October through December 2014, we sampled soils of *E. grandis* stands across two chronosequence studies (a short time-frame second rotation chronosequence and a longer time-frame chronosequence across four rotations). Our chronosequence of stands in their second rotation is comprised of 8 sites, all of which were harvested one time after 10-13 years and then sampled during their second rotation, henceforth called the second rotation (2R). We sampled three age groups in the 2R study: 2yr, 5-6yr, and 12yr post-harvest. Each age treatment was replicated three times except for the 12-year group, which had one stand removed because it was predominantly sand (**Table 2-1**) and not similar to the other sites. The criteria for all our replicates in both chronosequence studies were that the stands must have formerly been pasture or grasslands (i.e., not agricultural cropland), they must have had <10% slope, they must have had separate harvest times, and replicates in the same group must not have been adjacent to one another.

In addition to the 2R study, we also sampled across a chronosequence of four rotations or roughly 40 years. Each rotation group (1R, 2R, 3R, 4R) is comprised of three replicate stands all representing the end of that particular rotation. The end of a rotation is 10-13 years after planting (1R) or harvest (**Table 2-3**). As previously mentioned, we were unable to obtain 2R's third replicate of age 10-13 yrs. Since the time scale of this chronosequence was much longer and there were no significant differences among the 2R stands, we used all 8 stands within the second rotation for the analysis.

Although we were unable to obtain a detailed history of every stand, we were able to obtain information about standard practices in this area, some of which were confirmed through observations. The stands of the first, second, and third rotations have a standard spacing of 2.5 m by 4 m between rows. Granulated fertilizer was spread by hand at the base of trees in the first couple of years after stand initiation. Usually, after 2 years, new sprouts were pruned, leaving the healthiest or straightest lead to grow and maximizing production later on. After ten to thirteen years of growth, the stands were clear-cut allowing a new stem to regenerate from the old stump once again. After the harvest of 3R the stand was burned to stunt new growth, and new saplings were planted between the old rows. The new saplings were locally referred to as "replantas". While the fourth rotation is not regrowth from the same stumps, they are referred to as 4R here to account for four rotations of trees grown to maturity.

2.3.3 Field sampling

At each site, we established three, 50 m transects, each 24 m apart. Along each transect, we sampled soil at least three different points, for a total of nine-ten points. Half of the samples were from both within the tree lines (intra-row) and the other half were within alleyways (between rows or interrow) to assure stand representation. The soil was collected using a dutch auger and a double ring barrel. The dutch auger was used at six location points, collecting soil in 15 cm increments down to 60 cm while the double ring barrel coring unit was used at all nine points to collect surface bulk density (BD) mineral soil at 0-15 cm. Bulk density was collected at two tree line locations and one to two points in the alleyway for a total of three to four points. We used the double-barrel core to collect BD down to 60 cm. We used the longer barrel of the two rings that had a diameter of 4.8 cm (from inside wall to inside wall of the ring) with a height of 5.5 cm. For the 60 cm profile, we cored within the middle of four, 15 cm depth increments (i.e., 0-15, 15-30, 30-45, and 45-60 cm). Additional soil was carefully extracted both above and below the bulk density sample itself to fully represent each depth for estimates of carbon and nutrient concentrations. We used BD measurements from all nine points to calculate surface (0-15 cm) C and nutrients while the remaining three to four BD measurements were used in calculating C and nutrients at depths 15-30, 30-45, and 45-60 cm. Soil samples from the field were put on ice in a cooler until air-dried later that same day. Although the leaf litter layer (> 2 mm) is a direct input of nutrients and C, it was not measured here. Under the Oi horizon of twigs and undecomposed leaves, neither the O_e nor the O_a horizons were apparent. If any light, partially decomposed debris < 2 mm on the surface was present, it was included as part of the 0-15 cm depth for nutrient analysis.

2.3.4 Lab analysis

Soil samples contained high levels of clay and silt, making aggregates difficult to break up and required a mallet to crush larger aggregates as they air-dried. Bulk density samples were dried only to 70º C due to facility constraints while the corresponding nutrient samples (by depth) were left to air dry for several days to weeks. Both BD and soil C and nutrient samples were sent to Michigan Technological University (Houghton, MI, USA) where they were ground by hand using mortar and pestle. After being ground, subsamples were dried again at 70º C, weighed, and then dried at 105º C for at least 48 hours. A moisture correction factor was applied to the dry weight of bulk density to assure accuracy and was used for all other analyses. All nine sample points per site were composited in the lab and each 15 cm depth was kept separated. Neither rocks nor roots contributed significantly to bulk density. All sites and depths were also checked for carbonates using 1 M HCl and visually assessed for effervescence, but none were found to have carbonates.

Soil C and N analysis were processed separately from other nutrients. To determine total C and N concentrations, soil samples were pulverized, oven-dried at 60º C, and combusted in the presence of oxygen using an elemental analyzer (ECS 4010, Costech

Analytical Technologies Inc., Valencia, CA, USA). To determine the mass of C and N on a per hectare basis (Mg/ha) we used the following formula:

$$C \text{ or } N \text{ } (Mg\ ha^{-1}) = \%C \text{ or } N \times BD \times Depth \times \text{unit conversion} \qquad (eqn.\ 2.1)$$

In this equation,

- % C is the mass of C (g) divided by 100 g oven-dried soil
- BD is mass of oven-dried soil (g) / volume (cm^3)
- Depth is the thickness of the layer measured in cm

Standards from the National Institute of Standards and Technology (NIST) were used to calibrate C and N concentrations and the standard was run every 12 samples to assure precision. Exchangeable nutrients Ca, Mg, K, and metals such as Fe and Al were extracted with NH_4Cl solution to mimic the capacity of extractable nutrients at native pH conditions. Twenty-five ml of molecular grade 1 M NH_4Cl solution was added to 2.5 g of soil in polypropylene cups and shaken for 30 minutes on an orbital shaker at 150 rpm. Supernatants were pushed through a syringe equipped with a 25mm Whatman grade 1 paper filter. Filtered extracts were refrigerated for 1-3 days until analyzed with inductively coupled plasma mass spectrometry (ICP-OES; Perkin-Elmer Optima 7000 DV, Waltham, MA, USA). Phosphorus was extracted from 5.0 g of soil using the Mehlich 1 Method (Kovar and Pierzynski 2009) and measured with colorimetry at 882 nm (Kuo 1996).

The pH was measured for each depth in 10 g of sieved, oven-dried (60º C degrees) soil after being diluted with 20 ml of deionized water (dH_2O). The slurry was stirred and soaked for 10 min to assure the solution was equilibrated. The pH meter was calibrated from standardized solutions with a pH of 4 and 7. Measurements were recorded to the nearest 0.01 **(Table 2-1)**. To estimate texture, samples were grouped by 0-30 cm and 30-60 cm for each of the 17 sites and processed using 50 g of oven-dried soil and following the Bouyoucos method (Staff 2014). Clay and silt were determined at 2-hour time points.

Density separations were conducted on the nine composited soil samples of the four rotation treatment groups at depths 0-15 cm and 45-60 cm. From each of the 12 sites, ~25 g/sample were sent to the USFS Northern Research Station (Houghton, MI, USA). Before the samples were separated by density, the gravimetric water content was determined. The soil was separated by a sodium polytungstate solution with a density of 1.65 g/cm^3 to determine the mass of each of the two fractions: free light plus occluded fractions (FLF+OC), and the heavy fraction (HF) (Strickland and Sollins 1987; Golchin et al. 1994; Swanston et al. 2005). We were unable to obtain the light-occluded fraction separately because native aggregates were broken up before analysis. Ninety-eight to 100% of the weight of the original sample was recovered after separation. After being separated the fractions were analyzed for %C using the elemental analyzer. The amount of each carbon fraction, CF, in Mg C ha^{-1} was calculated by 1.) correcting for soil moisture on the air-dried bulk soil before separation, and then 2.) calculating the mass of each carbon fraction per unit area. All masses are in the same units.

$$bulk_{wet} - (\theta_g * bulk_{wet}) = bulk_{dry} \qquad \text{(eqn.2.2)}$$

$$\%F * BD * Depth * \%C_f = CF \qquad \text{(eqn.2.3)}$$

In these equations,

- $bulk_{wet}$ is an air-dried mass of bulk soil
- $bulk_{dry}$ is the oven-dried mass of bulk soil (before separated)
- θ_g is the mass of water per mass of oven-dried soil
- %F is the dried mass of the separated C fraction / bulk dry
- $\%C_f$ is the concentration of fraction (i.e.) FLF+OC or HF, the value determined by the elemental analyzer (i.e.) HF(g) /100g of bulk soil
- BD = Bulk density is the dry mass of bulk soil (g) per unit volume (cm^3)
- Depth is the difference between the upper and lower range measured in cm

2.3.5 Statistical analysis

The relationship of soil characteristics with time and depth were tested with linear regression. Total soil carbon, carbon fractions, nutrients, aluminum, $[H^+]$ derived from pH values, and clay were the continuous dependent (response) variables while age, rotation, and depth served as the continuous independent variables. Soil characteristics were analyzed for each 15 cm layer increment separately.

Statistics were performed in the software package JMP Pro version 14 with an alpha level of 0.05. Transformations were not necessary for the 2R data set, the 1R-4R data set, nor the carbon fraction data set except for the square root taken of aluminum in the 1R-4R set at15-30 cm. Any statistically significant finding was assessed using post hoc comparisons with the Least Square Mean Differences Tukey HSD with an alpha level of 0.05. In any tests that did not satisfy the assumption of homoscedasticity, data were checked for outliers and in a few appropriate cases fit to a different non-linear model.

2.4 Results

2.4.1 All sites: soil properties

The most abundant soil texture of the sites was sandy loam (**Table 2-1** and **Table 2-2**). Most 0-30 cm soils along with half of the 30-60 cm depths were sandy loam. The other half of 30-60 cm samples had a little more clay and were classified as sandy clay loam. For the 0-30 cm depths, there was no significant difference in clay content across the four rotations. At lower depths 30-60 cm, clay was also similar among rotations. However, clay content significantly increased with depth for all sites from ~10% clay in the top 30 cm to ~20% clay in the lower 30-60 cm. Combining clay and silt together constituted between 25-50% of these hydric soils.

Soil pH was acidic and averages ranged from 4.5 to 5.6 (**Table 2-1** and **Table 2-2**). For the ages within 2R, pH changes were not significant for 0-30 cm or for 30-60 cm depths. Across all 4 rotations, soil pH for the 30-60 cm depths changed near significance with the greatest differences being between 1R and 4R. Depth had a stronger effect on pH than the progression of rotations. The pH values were more acidic at the top 30 cm compared with the 30-60 cm depth for the ages within 2R and the 1R-4R chronosequence.

Bulk density (BD) was similar for stands within 2R, (**Table 2-3**). The most BD change across age groups was within 15-30 cm. In rotations 1 through 4, the second rotation has the highest mean BD at all depths down to 60 cm (**Table 2-4**). In rotations 1 through 4, soil BD at the surface varied the most. This top layer held the greatest mean differences values with progressing rotations in a zig-zag pattern with each rotation being significantly different from the next sequential rotation and this was best fit to a sine curve. Bulk density below 15 cm was less variable among rotations. Soil bulk density significantly increased with depth across the age groups for the 2R chronosequence and across the four rotations (**Table 2-3** and **Table 2-4**).

Table 2-1. Soil characteristics for each age group within the 2nd rotation. Standard error is in ( ). Deeper soil (30-60 cm) contains more clay ($p = 0.01$), less sand ($p = 0.01$), and is less acidic ($p = 0.04$) than soil from 0-30 cm.

Years post-harvest	Soil Order	Depth (cm)	pH	%Sand	%Clay
Early	Mollisol/Entisol	0-30	4.8 (0.2)	71.5 (0.0)	11.6 (0.0)
2yr		30-60	5.5 (0.1)	61.3 (0.0)	22.4 (0.0)
Mid	Mollisol/Entisol	0-30	4.6 (0.1)	71.0 (0.1)	11.8 (0.0)
6-8 yr		30-60	5.1 (0.3)	64.5 (0.1)	18.8 (0.0)
*Late	Mollisol/Entisol	0-30	4.5 (0.2)	72.5 (0.1)	11.9 (0.1)
11-12 yr		30-60	4.8 (0.4)	66.0 (0.1)	18.2 (0.0)

*n=2

Table 2-2. Soil characteristics for 1R through 4R. Standard error is in parenthesis. The soil is more acid at the surface (0-15 cm, $p < 0.01$). The soil is becomes more acidic with progressing rotations at 30-60 cm ($p = 0.11$).

Rotation (n=3)	Soil Order	Depth cm	pH	%Sand	%Clay
1R	Mollisol/Inceptisol	0-30	4.8 (0.1)	68.7 (0.1)	9.3 (0.1)
		30-60	5.6 (0.3)	65.0 (0.0)	21.6 (0.0)
***2R**	Mollisol/Entisol	0-30	4.7 (0.1)	72.5 (0.0)	12.1 (0.0)
		30-60	5.1 (0.4)	66.0 (0.0)	19.2 (0.0)
3R	Inceptisol/Mollisol	0-30	4.5 (0.1)	76.4 (0.0)	08.7 (0.0)
		30-60	4.9 (0.1)	66.1 (0.0)	19.7 (0.0)
4R	Mollisol	0-30	4.7 (0.2)	82.6 (0.0)	07.0 (0.0)
		30-60	4.7 (0.1)	71.7 (0.0)	17.7 (0.0)

*In 2R, the third replicate is an 8-year stand instead of an 11-12 year stand

Table 2-3. Second rotation soil bulk density and nutrients by age treatments. Each age treatment is comprised of 4 different, 15 cm depths. Standard error is in parenthesis. The lower portion: *p ≤ 0.05

2R (Age)	**Depth (cm)**	**BD (g/cm^3)**	**C (Mg/ha)**	**N (Mg/ha)**	**P (Mg/ha)**	**K (Mg/ha)**	**Ca (Mg/ha)**	**Mg (Mg/ha)**	**Al (Mg/ha)**
2yr	0-15	1.5 (0.1)	31.6 (2.2)	2.5 (0.2)	0.039 (0.035)	0.17 (0.02)	2.3 (0.6)	0.3 (0.1)	0.01 (0.01)
	15-30	1.6 (0.0)	27.1 (2.5)	2.1 (0.2)	0.004 (0.000)	0.14 (0.00)	3.2 (1.2)	0.4 (0.1)	0.06 (0.05)
	30-45	1.6 (0.0)	19.2 (1.4)	1.5 (0.0)	0.003 (0.000)	0.19 (0.02)	4.7 (1.1)	0.5 (0.1)	0.02 (0.01)
	45-60	1.6 (0.0)	13.9 (1.6)	1.2 (0.1)	0.001 (0.000)	0.34 (0.02)	7.5 (0.9)	0.9 (0.1)	0.01 (0.01)
	0-60	---	91.8 (7.7)	7.4 (0.7)	0.048 (0.035)	0.84 (0.01)	17.7 (3.7)	2.2 (0.4)	0.11 (0.08)
6-8yr	0-15	1.4 (0.1)	37.5 (1.6)	3.0 (0.3)	0.013 (0.008)	0.14 (0.01)	2.4 (0.5)	0.4 (0.1)	0.06 (0.01)
	15-30	1.5 (0.1)	27.3 (4.7)	2.0 (0.3)	0.005 (0.001)	0.13 (0.01)	3.3 (1.2)	0.4 (0.1)	0.08 (0.06)
	30-45	1.6 (0.0)	20.9 (3.1)	1.6 (0.2)	0.004 (0.000)	0.28 (0.09)	6.6 (2.6)	0.8 (0.3)	0.03 (0.02)
	45-60	1.7 (0.0)	17.2 (1.3)	1.3 (0.1)	0.003 (0.000)	0.26 (0.07)	7.5 (2.1)	0.8 (0.2)	0.02 (0.02)
	0-60	---	102.9 (6.8)	8.0 (0.2)	0.026 (0.009)	0.81 (0.15)	19.8 (6.4)	2.3 (0.6)	0.18 (0.11)
11-12yr	0-15	1.6 (0.0)	33.2 (4.3)	2.2 (0.1)	0.005 (0.000)	0.16(0.02)	3.0 (1.3)	0.4 (0.2)	0.06 (0.03)
	15-30	1.7 (0.0)	26.6 (7.7)	2.0 (0.4)	0.004 (0.000)	0.15 (0.05)	4.2 (3.1)	0.6 (0.2)	0.14 (0.12)
	30-45	1.7 (0.0)	19.1 (1.3)	1.6 (0.0)	0.003 (0.001)	0.17 (0.06)	5.6 (3.1)	0.6 (0.2)	0.06 (0.04)
	45-60	1.7 (0.1)	16.7 (0.0)	1.3 (0.1)	0.002 (0.001)	0.22 (0.04)	7.0 (2.8)	0.7 (0.2)	0.04 (0.03)
	0-60	---	93.6 (12.8)	7.0 (0.6)	0.014 (0.002)	0.70 (0.13)	19.8 (10.3)	2.3 (0.7)	0.30 (0.16)

- *continued next page*

-Table 2-3 (continued from previous page)

Linear regression p-value results among age groups (not transformed)

2R	**Depth**	**BD**	**C**	**N**	**P**	**K**	**Ca**	**Mg**	**Al**
	0-15	0.75	0.88	0.63	0.19	0.39	0.55	0.61	0.13
	15-30	0.12	0.99	0.74	0.89	0.71	0.71	0.55	0.37
	30-45	0.20	0.82	0.93	0.61	0.98	0.71	0.74	0.33
	45-60	0.38	0.25	0.20	0.94	0.12	0.88	0.50	0.59
	0-60	---	0.78	0.75	0.48	0.43	0.80	0.88	0.23

Table 2-4. Soil bulk density (BD) nutrients by rotation treatment for 4 different depths. Standard error is in parenthesis. The second rotation includes all 8 sites in the calculations.

Rotation	Depth (cm)	BD (g/cm^3)	C (Mg/ha)	N (Mg/ha)	P (Mg/ha)	K (Mg/ha)	Ca (Mg/ha)	Mg (Mg/ha)	Al (Mg/ha)
1R	0-15	1.3 (0.0)	38.6 (7.2)	3.0 (0.4)	0.004 (0.000)	0.19 (0.04)	3.2 (0.8)	0.5 (0.1)	0.03 (0.01)
	15-30	1.6 (0.0)	30.0 (3.8)	2.2 (0.1)	0.004 (0.000)	0.16 (0.02)	4.2 (1.5)	0.5 (0.1)	0.02 (0.01)
	30-45	1.6 (0.0)	23.8 (3.6)	1.7 (0.1)	0.005 (0.002)	0.24 (0.01)	6.6 (1.4)	0.7 (0.1)	0.01 (0.01)
	45-60	1.5 (0.0)	16.3 (1.2)	1.2 (0.1)	0.002 (0.000)	0.29 (0.03)	7.8 (1.6)	0.8 (0.1)	0.01 (0.01)
Ave Tot.	0-60	---	108.7 (13.0)	8.0 (0.6)	0.014 (0.001)	0.88 (0.03)	21.8 (3.0)	2.6 (0.2)	0.07 (0.00)
2R	0-15	1.5 (0.1)	33.7 (1.9)	2.6 (1.9)	0.018 (0.010)	0.16 (0.01)	2.6 (0.4)	0.4 (0.1)	0.05 (0.01)
	15-30	1.6 (0.0)	26.3 (2.5)	2.0 (0.2)	0.005 (0.001)	0.14 (0.01)	3.6 (0.9)	0.5 (0.1)	0.09 (0.04)
	30-45	1.6 (0.0)	19.5 (1.3)	1.5 (0.1)	0.003 (0.000)	0.22 (0.04)	5.7 (1.3)	0.6 (0.1)	0.04 (0.01)
	45-60	1.7 (0.0)	15.7 (1.0)	1.2 (0.1)	0.003 (0.000)	0.27 (0.03)	7.5 (1.1)	0.8 (0.1)	0.02 (0.01)
Ave Tot.	0-60	---	96.4 (4.9)	7.4 (0.3)	0.029 (0.004)	0.79 (0.03)	19.0 (1.1)	2.2 (0.10)	0.19 (0.01)
3R	0-15	1.2 (0.1)	29.6(3.0)	2.1 (0.2)	0.003 (0.000)	0.13 (0.01)	2.8 (0.5)	0.4 (0.0)	0.09 (0.08)
	15-30	1.5 (0.0)	27.8 (3.6)	2.0 (0.2)	0.004 (0.000)	0.14 (0.02	2.9 (0.9)	0.5 (0.1)	0.23 (0.09)
	30-45	1.6 (0.0)	22.6 (1.4)	1.7 (0.2)	0.002 (0.000)	0.20 (0.03)	5.7 (1.3)	0.7 (0.1)	0.09 (0.03)
	45-60	1.6 (0.0)	17.3 (0.5)	1.3 (0.0)	0.001 (0.000)	0.30 (0.02)	8.5 (0.7)	0.9 (0.1)	0.03 (0.01)
Ave Tot.	0-60	---	97.3 (7.2)	7.1 (0.4)	0.011 (0.000)	0.77 (0.04)	19.9 (1.4)	2.4 (0.1)	0.44 (0.04)
4R	0-15	1.5 (0.0)	34.1 (4.5)	2.3 (0.3)	0.004 (0.000)	0.13 (0.01)	3.2 (0.4)	0.3 (0.1)	0.01 (0.00)
	15-30	1.4 (0.0)	19.9 (4.5)	1.6 (0.3)	0.005 (0.002)	0.17 (0.02)	3.7 (1.9)	0.7 (0.3)	0.12 (0.06)
	30-45	1.5 (0.1)	17.0 (2.6)	1.4 (0.2)	0.002 (0.001)	0.13 (0.03)	2.3 (0.9)	0.4 (0.1)	0.19 (0.09)
	45-60	1.6 (0.1)	14.0 (1.4)	1.0 (0.0)	0.002 (0.001)	0.23 (0.02)	6.7 (1.7)	1.0 (0.3)	0.05 (0.03)
Ave Tot.	0-60	---	83.9 (12.4)	6.3 (0.8)	0.014 (0.001)	0.66 (0.02)	15.8 (1.0)	2.4 (0.2)	0.35 (0.04)

-Continued

Table 2-4 (continued from previous page)

		Linear regression p-value results for soil elements and rotations							
Rotations	**Depth (cm)**	**BD (g/cm3)**	**C (Mg/ha)**	**N (Mg/ha)**	**P (Mg/ha)**	**K (Mg/ha)**	**Ca (Mg/ha)**	Mg **(Mg/ha)**	Al **(Mg/ha)**
1,2,3,4	0-15	< **0.01** †	0.37	0.11	0.91	**0.03**	0.84	0.19	0.16
	15-30	**0.05**	**0.09**	**0.08**	**0.04**	0.68	0.78	0.36	**0.05**
	30-45	**0.02**	0.15	0.12	0.15	**0.10**	**0.07**	0.12	< **0.01**
	45-60	**0.02**†	0.40	**0.05**	0.61	0.07	0.75	0.31	**0.05**
	0-60	**--**	0.12	**0.02**	0.61	**0.06**	0.40	0.98	**0.01**

†Not a linear fit, but a polynomial to sixth degree fit for BD 0-15 cm and a parabolic fit for BD 45-60 cm.

2.4.2 Soil carbon

The stands within 2R had total soil carbon (0-60 cm) that ranged between 91.8 and 102.9 Mg ha^{-1} (**Table 2-3**; **Figure 2-3**), but these differences did not indicate a significant relationship with age since harvest. There were also no significant relationships in soil C across 2R at any single depth increment (**Table 2-3**; **Figure 2-3**).

Across the four rotations, average total soil C (0 to 60 cm) exhibited a downward trend from the first to the fourth rotation ($p = 0.12$) (**Table 2-4**; **Figure 2-4**). Total soil C (0-60 cm) in the first rotation averaged 108.7 (±13.0) Mg ha^{-1}, while the fourth rotation was 23% lower, 83.9 Mg ha^{-1} (±12.2) (**Figure 2-4**). This is an estimated rate of 8.3 Mg ha^{-1} being lost per rotation.

The declining trends in total soil C with rotations are gradual and the most changes occurred at the 15-30 cm layer ($p = 0.09$), and the least at 45-60 cm ($p = 0.40$). As would be expected, soil carbon significantly declined ($p < 0.01$) with depth for all rotations.

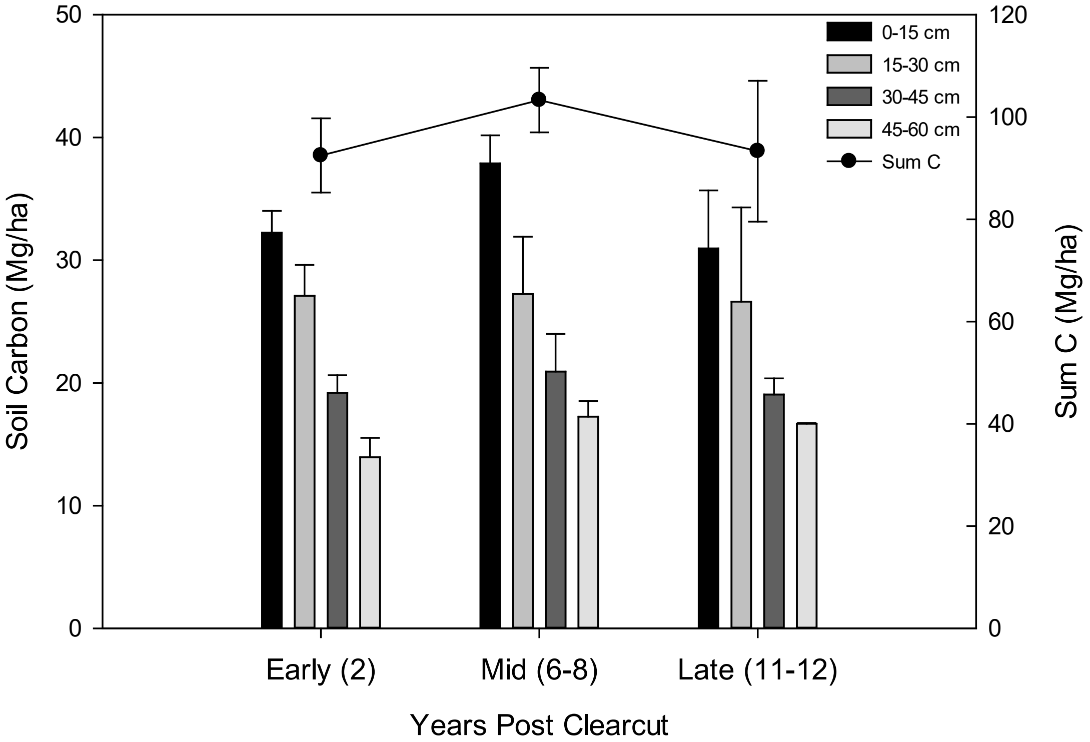


Figure 2-3. Soil carbon from Eucalyptus stands in their 2nd rotation with standard error bars showing no significant changes of soil C within the 2R. The two late replicates (45-60 cm) held equal values and therefore have a standard error of zero.

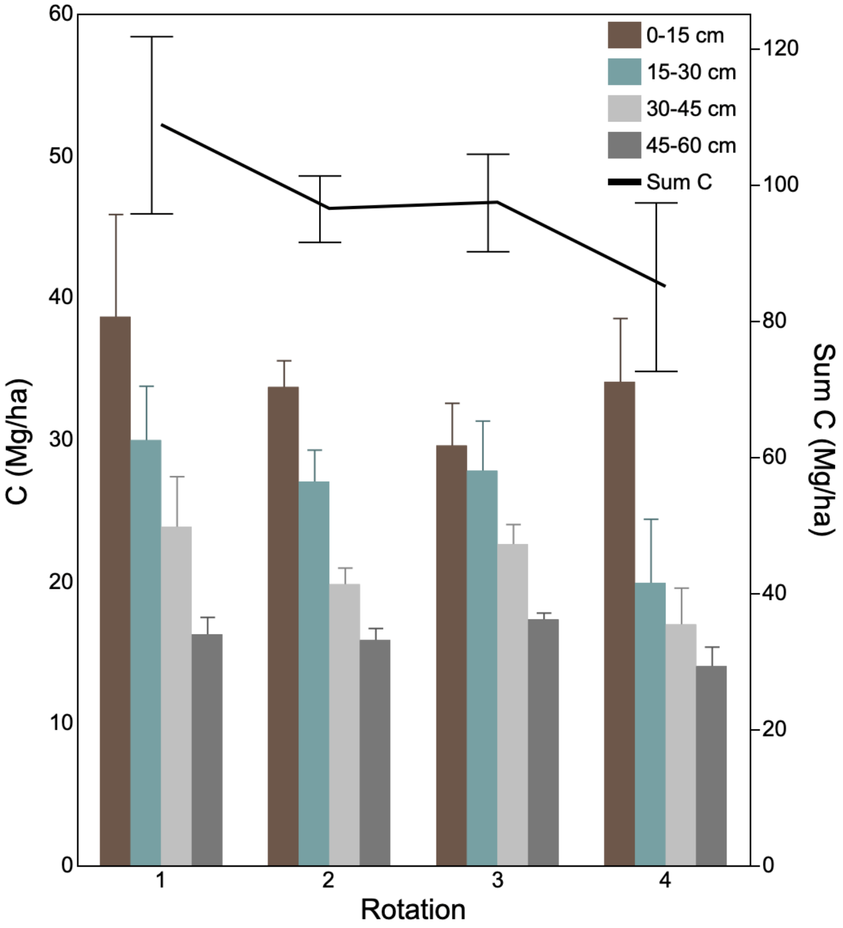


Figure 2-4. The soil C from 4 rotation treatments with standard error. The line depicts the 0-60 cm average of the 3 replicates summed by depth except 2R. The 2R is comprised of 8 replicates with varying post-harvest ages seen in Figure 2-3.

2.4.3 Soil carbon density separations

For the surface soils (0-15 cm), the heavy fraction (HF) of soil carbon decreased from 1R with 26.5 Mg ha^{-1} (±SE 4.9) to 4R with 14.4 Mg ha^{-1} (± 1.1) ($p = 0.10$) (**Figure 2-5**). The %C of HF ranged from 0.40 to 1.81%. The free light fraction plus occluded carbon (FLF+OC) exhibited a parabolic pattern across the four rotations ($p = 0.02$) (**Figure 2-5**), with the % C ranging from 20.80% to 95.68%.

In the deeper soils (45-60 cm), the HF decreased across rotations ($p = 0.06$). The FLF+OC on the other hand did not change across the rotations ($p = 0.53$). Both the HF and FLF+OC showed limited changes from the first rotation to the second rotation (**Figure 2-5**). The HF changes with rotation do not become evident until the fourth rotation.

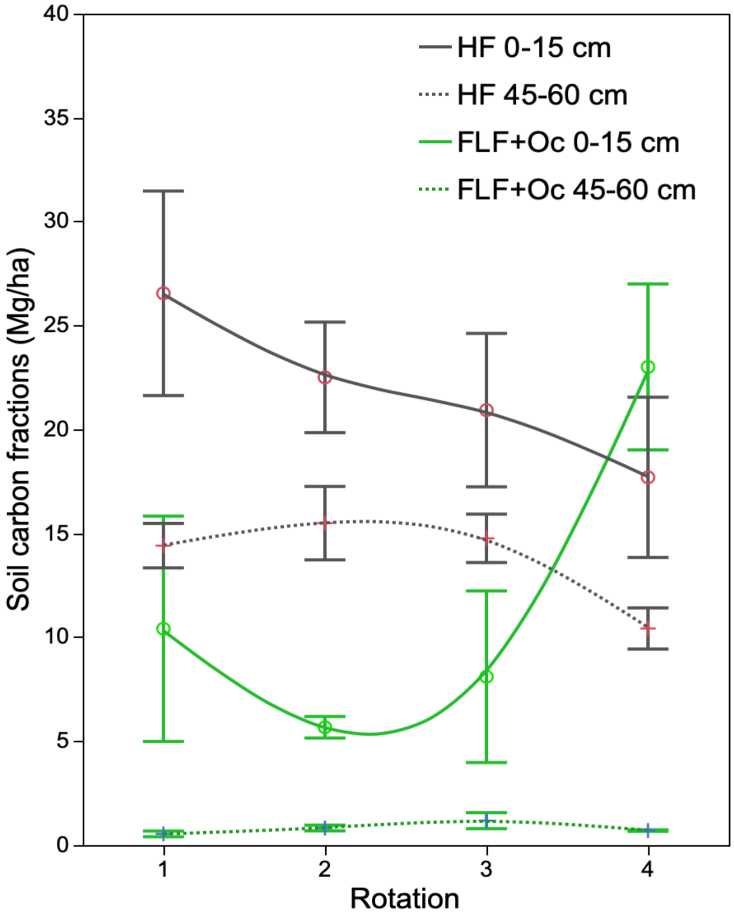


Figure 2-5. Density separated carbon fractions: the heavy fraction (HF) and the free light and occluded fraction (FLF+OC) at the two depths 0-15 cm and 45-60 cm. Error bars are standard error.

2.4.4 Soil nutrients and metals

Most nutrients exhibited a downward trend across the four rotations (**Table 2-3**, **Table 2-4**). All trends of nutrients pertain to both chronosequences. Any significant values were derived from the 1R to 4R chronosequence.

Total nitrogen (N; 0-60 cm) declined significantly across rotations ($p = 0.02$), as did N at 0-15 cm and 45-60 cm (**Table 2-4**). N tended to be more abundant at the surface of the soil profile (**Table 2-3**, **Table 2-4**). Soil C:N ratios had a narrow range from 12.3 to 14.8 across all rotations and depths (**Table 2-4**). The C:N ratio increased over the four rotations at 0-15 cm ($p = 0.03$), however there were no significant differences for the deeper soil. Across rotations, calcium exhibited a gradual downward trend but changes are not statistically significant. Calcium was less abundant at the surface (i.e., 0-15 cm) and increased with increasing depth ($p < 0.01$). Phosphorus (P) concentrations were variable from one rotation to another and often had various outliers at different depths. Phosphorus showed no change ($p = 0.70$) at 0-15 cm, but P did show a downward trend across rotations below 0-15 cm. Phosphorus changed at 15-30 cm and best fit a downward opening parabola ($p < 0.01$), but also could be interpreted as having a linear relationship decreasing with successive rotations if two higher outliers were removed (**Table 2-4**). With regards to vertical patterns in the profiles, P declines with increasing depth ($p = 0.02$). Potassium (K) and Magnesium (Mg) also exhibited a downward trend across rotations, but changes were not significant. Similar to Ca, both K and Mg increase with increasing depth ($p < 0.01$ for both).

Iron and aluminum were originally screened on a subset of stands from each rotation and depth. The concentrations for iron were negligible and for aluminum (Al) ranged from 0.00 to 0.38 Mg ha^{-1}. At all depths, Al showed an upward trend with progressive rotations (**Table 2-3**, **Table 2-4**). At 30-45 cm, Al increased exponentially. With regards to depth, Al is most abundant in the top 30 cm for 1R to 3R. In the 4th rotation, an E horizon was observed in two out of the three replicates at ~30 cm. Higher levels of Al can be seen at the 30-45 cm ($p = 0.01$) and at 45-60 cm ($p = 0.06$) (**Table 2-4**). To a lesser extent, the E horizon may also be responsible for lower levels of K, Ca, and Mg in these two sites at 30-45 cm.

2.5 Discussion

Many studies have examined soil carbon dynamics within the first and second rotations of Eucalyptus, and these have helped us understand detailed responses of soil carbon to land-use change, management, and soil attributes (e.g. soil texture, microbial diversity, pH, and fertility). Fewer investigations, however, have measured the long-term effects of multiple rotations on soil C (Lima et al. 2006; Maquère et al. 2008; Eclesia et al. 2012; Sandoval et al. 2012; Cook et al. 2016).

In our 2R chronosequence, we found no change in soil C across age classes for the 2R to 60 cm. Other studies of soil with moderate fertility or loamy texture also found limited changes in soil C within the second rotation (Mendham et al. 2003; Soares et al. 2017). In comparison to sandier sites with a higher potential to gain carbon, the soils with higher clay and silt likely have more protected and stable C, or possibly are closer to becoming more C saturated (Six et al. 2002). These studies, however, are not discerning faster-cycling carbon from persistent carbon. Soares et al. (2017) does discuss a correlation of molecules associated with chemically stabilizing organic matter (humic acid, fluvic acid, and humin), but this does not account for the physical parameters (aggregate occlusion, pore space inhibiting the activity of microbes) nor the biological controls of microbial communities. With an apparent lack of change in total soil carbon, persistent soil C could be lost and replaced with new vulnerable inputs of labile C. Alternatively, if the persistent C was not being disturbed, there could have been a new dynamic equilibrium. We do not know how these varied responses may affect productivity, and we also do not know if and how these short-term responses may impact long-term responses. Likely, the 2R time-frame in our study was not long enough to capture the declines we see in the longer-time frames. While soil C appears to be resistant to change even after the first harvest and through the second rotation, multiple rotations appear to influence soil differently.

We found that total soil C declined across rotations for the soil profile (0-60 cm). This long-term trend was observed by other work done in the same region at depths 0-30 cm (Sandoval et al. 2012). Soil C losses have been observed after planting eucalyptus from pasture at this precipitation gradient (>1200 mm), but decreases are generally counterbalanced with age at 0-20 cm (Berthrong et al. 2012; Eclesia et al. 2012). While the precipitation gradient may share an association with soil C loss, one difference with our study is that our sites are managed under multiple harvests. Clear-cut harvests generally disturb the soil more intensely than other types of harvests. Clear-cut harvesting can affect the forest floor as well as upper layers of mineral soil (Mayer et al. 2020). Although humid subtropic soils may be able to accumulate soil C faster, in temperate zones, long-term management of clear-cuts have been predicted in 50-year simulations to decrease net biome productivity by up to 58% (Peckham et al. 2013). Burns to reduce the thick forest floor between rotations can also lead to depletions in soil C (Mendham et al. 2003).

In addition to long-term forest management impacts on the quantity of soil C, we also found significant impacts on the quality of soil C. While it is not uncommon to see increased labile C with afforestation in the surface soil (Laganiere and Angers 2019), the decline we documented in heavy carbon in both surface and deeper soil suggests potential degradation. Losses of deep soil C have been seen in other studies (Turner and Lambert 2000; Nave et al. 2010; Santos et al. 2020). Due to the nature of heavy C being associated with persistent mineral organic matter, the loss of this fraction could suggest, lower future C storage capacity, higher emissions of CO_2 accompanied with the decomposition of the persistent fraction, and a loss of soil structure that helps reduce erosion rates and the formation of rills associated with land degradation (Lal 2012).

In addition to declines in soil C, nutrients were also found to trend downward, although variability across rotations was high. Our soil nutrient analysis is limited in that we do not have information on estimated inputs or nutrient mineralization. Nevertheless, significant declines in total N were observed at the surface and in the 45-60 cm layer. This and isolated declines in P at 15-30 cm could suggest amendments would be useful to sustain productivity (Corbeels et al. 2005).

Several management practices help prevent soil C and nutrients from declining and agree with best management practices that can be used at various stages of plantations undergoing multiple rotations. These stages include the initial time of site preparation, harvesting, and time between rotations that can all help restore soil C and nutrients. Initial site preparation and the type of land use that is being replaced accounts for a large portion of the initial soil C loss (Don et al. 2011). Choosing the best-suited species for the local site can prevent plantation damage and the need to uproot dying or partially damaged trees. Interplanting nitrogen-fixing ground cover or tree species can also be effective not only in preventing N-limitation but also in preventing erosion and nutrient leaching. N-fixers or vegetation can also increase N and P availability not to mention increasing plant diversity leading to a more sustainable system as a whole (Lehmann et al., 2020). In serving these functions, it can help reduce fertilizer and mechanical treatments(Ulloa and Villacura 2005; Schoeneberger et al. 2012).

Intensive harvesting such as clear-cuts is associated with soil C and cation losses through leaching and erosion, especially if residues are not left on site (Blanco-Canqui and Lal 2009b). Alternatively, staggering harvests can increase forest structure and biodiversity. Since plantation soils are somewhat a cross between agriculture and forests, maintaining harvest residues or ground vegetation early in site development or even after a harvest may help lessen the damaging effects of freeze-thaw and frost (Layton et al. 1993; Williams et al. 2009; Miner et al. 2013). Processing and leaving biomass residues can help sustain nutrients, while the removal of whole trees or stems with bark leads to a direct decrease in carbon and nutrient inputs. Nutrient declines can lead to increases in acidity. If post-harvest soils are subjected to burning for the next rotation this can also lead to soil C losses (Nave et al. 2010).

Over time, multiple harvests can weaken aggregate stability and size distribution by decreasing the amount of soil organic matter inputs after a clearcut, especially if a residue burn is set before the new growth, leading to erosion and reduced soil organic matter (SOM) (Hammerbeck et al. 2012; Muhammad et al. 2012). Lower levels of SOM can lead to lower water-holding capacity, surface crusting, increased soil strength, and decreased infiltration. Lower SOM can also lead to shifts in microbial communities and soil fauna, which can change decomposition rates, nutrient immobilization (Karlen et al. 1994; Bailey et al. 2002; Blanco-Canqui and Lal 2009a).

Although there are practices to help amend soil C and nutrient losses incurred from multiple harvests, the damage is usually not caused by a single factor. Site quality affected by soil characteristics, climate, and management all weigh into the sustainability of the soil. The plantations' multiple rotations can represent many factors endured through time, some making soil C more vulnerable or ultimately altering the development of the soil. The gradual changes seen in this study are not alarming, however do elicit concern as to whether the soil is heading in the best direction for not only the plantations but future land use.

2.6 Conclusion

In this study, we found that the quantity and quality of soil carbon declined over multiple rotations of *E. grandis* land use. Macronutrients N, P, K, Ca, and Mg also trended downward with increasing rotation number with a few select depths being statistically significant (**Table 2-3**). These findings are important for long-term planning whether it be bioenergy policy or consideration of suitable land use in the future. Soil is generally more sustainable when rotations are lengthened and fire management is kept to a minimum, however more work is needed to understand the processes for which gradual degradation is occurring. With the intent of timber, wood, pulp, or carbon sequestration, *E. grandis* provides an effective and economically valuable option. Bioenergy policy and regulations should consider that the benefits of Eucalyptus in this region can decline after roughly 20-25 years of land use despite sustained above-ground productivity with soil amendments (Sandoval López et al. 2018).

3 Impacts of Palm Plantations on Soil Carbon and Soil Nutrients in Tabasco, Mexico

3.1 Abstract

The expansion of oil palm (*Elaeis guineensis* Jacq.) in Mexico as well as the adoption of industrial agricultural techniques has led to questions about its ecological sustainability. Heavy tractors can lead to soil compaction and herbicide application can lead to less diverse ground vegetation. We studied the soil carbon and nutrients of palm stands ~10 hectares in size in Tabasco, Mexico. We compared young palm stands (3-4 years old) and old palm stands (17-19 years old) with alternative land-use scenarios of secondary forests and pastures. Young and adult palm stands were systematically sampled in weeded circles, harvest pathways, and under palm fronds for adult stands. Among the following groups, young palm stands, adult palm stands, pasture, and secondary forests, each with three replicates, we found no differences in soil carbon 0-60 cm ($p \leq 0.05$). Other nutrients such as Ca, Mg, P, and K were similar among groups with no specific trend. When comparing within-stand locations of palm stands, we found adult palms had lower nitrogen at 45-60 cm in harvest pathways than young palm stands. Also weeded circles in young palm sites held higher levels of C ($p = 0.02$), N ($p = 0.02$), and P ($p = 0.04$) at 0-15 cm than older palm sites. The weeded circles make up only a small percent of the total area of the plantation (~ 4%), however, thereby not influencing the plots as a whole when these areas are weighted.

Keywords: (soil carbon, *palm*, *Elaeis guineensis* Jacq., bioenergy, soil nutrients,)

3.2 Introduction

In Mexico, the area occupied by oil palm (*Elaeis guineensis* Jacq.) plantations has tripled between 2000 and 2010 (Aguilar-Gallegos et al. 2015). For regions like southeastern Tabasco, oil palm production has the potential to continue expanding. To prevent environmental degradation, landowners, users of communal lands (ejidos), and the state of Tabasco need to understand the soil sustainability of this crop.

Currently, most data for oil palm plantations in Mexico such as soil carbon (C), land-use changes, and other parameters are based on IPCC frameworks and/or parameters taken from literature of other countries (Germer and Sauerborn 2008; Hassan et al. 2011; Flynn et al. 2012). While research of other countries' adoptions of oil palm production has improved our understanding of the impacts of both direct and indirect land-use change, soil erosion, and acidification (Nelson et al. 2011; Guillaume et al. 2015; Benami et al. 2018), few published studies have assessed the soil impacts of land change use from secondary forests or pasture to oil palm plantations in southern Mexico.

Land-use changes that result in shifts in soil C are often dependent on the land-use history as well as the current vegetation inputs. Pastures, most agricultural lands, and marginal lands that do not compromise food security are better options for accommodating the expansion of oil palm plantations than forested land or drained wetlands (Germer and Sauerborn 2008). Sustainable oil palm practices, starting with the conversion of pastures or grasslands, can recuperate carbon losses incurred from initial stand disturbance as early on as ~10 years compared to other biofuel crop options (Gibbs et al. 2008). While above-ground biomass growth is important for carbon accounting and bioenergy development, soil C is also an important component of C storage. Soil C can be a net sink or source of C for the atmosphere, depending on site management. Soil C plays a pivotal role in sustaining productivity not just for current biomass production, but also for subsequent land uses in addition to climate change mitigation. Climate, soil type, and management can all influence soil carbon and nutrients making it difficult to draw inferences from one tropical region to another.

With the expansion and oil palm in southern Tabasco, our goal was to assess the impacts of oil palm on the soil to provide information on how the oil palm stands are impacting the soil sustainability. Our first objective was to measure carbon and soil nutrients in smallholder plantations (~10 to 20 ha). Our second objective was to compare these soil attributes of oil palm stands to alternative land uses such as pastures and secondary forests.

3.3 Materials and Methods

3.3.1 Site description

The study sites are in the lowland humid tropics of Tabasco, Mexico (17°46'N, 92°45'W) where most of the primary forests and have been converted to pastures and agriculture. The mean average temperature in this region was 26.5 °C from 1961 to 2004. The mean annual precipitation is 2,688 mm year^{-1} also based on data from 1961 to 2004 with the drier months between March and July averaging 185 mm/month and the wetter months between August and December averaging 392 mm/month (SMN accessed 02/05/2015).

Most of the soils we encountered within oil palm plantations were Luvisols, however, Gleysols are another prominent soil type in the municipalities of Jalapa, Tacotalpa, and Teapa. Tabasco's earlier assessments of the best-suited soils for oil palm were determined as chromic and haplic Luvisols as well as Chromic and Eutric Cambisols according to the World Resource Base Soil Classification System (Aceves Navarro et al., 2008). These two groups are equivalent to USDA's classification for Alfisols with high clay activity and newer soils of Inceptisols, respectively (Environmental Fate Technology Team 2011). Luvisols in this region tend to have a cation exchange capacity ≥ 24 cmol_c /kg with 50% base saturation (Palma-López et al. 2017).

The sites extend approximately 30 km along the river La Sierra belonging to the Grijalva watershed (**Figure 3-1**). To the south of the study sites, sharp outcrops expose karst while the volcano El Chichón lies to the west. El Chichón erupted in 1982 depositing fine-grain ash comprised of sharp glassy shards of silica with potential potassium, sodium, and sulfur beyond the range of the study sites (Varekamp et al. 1984; Rose and Durant 2008). Most of the geology of this area is quaternary alluvial sediment, giving rise to gleyic and fluvic soil with scatterings of Alisols and Luvisols.

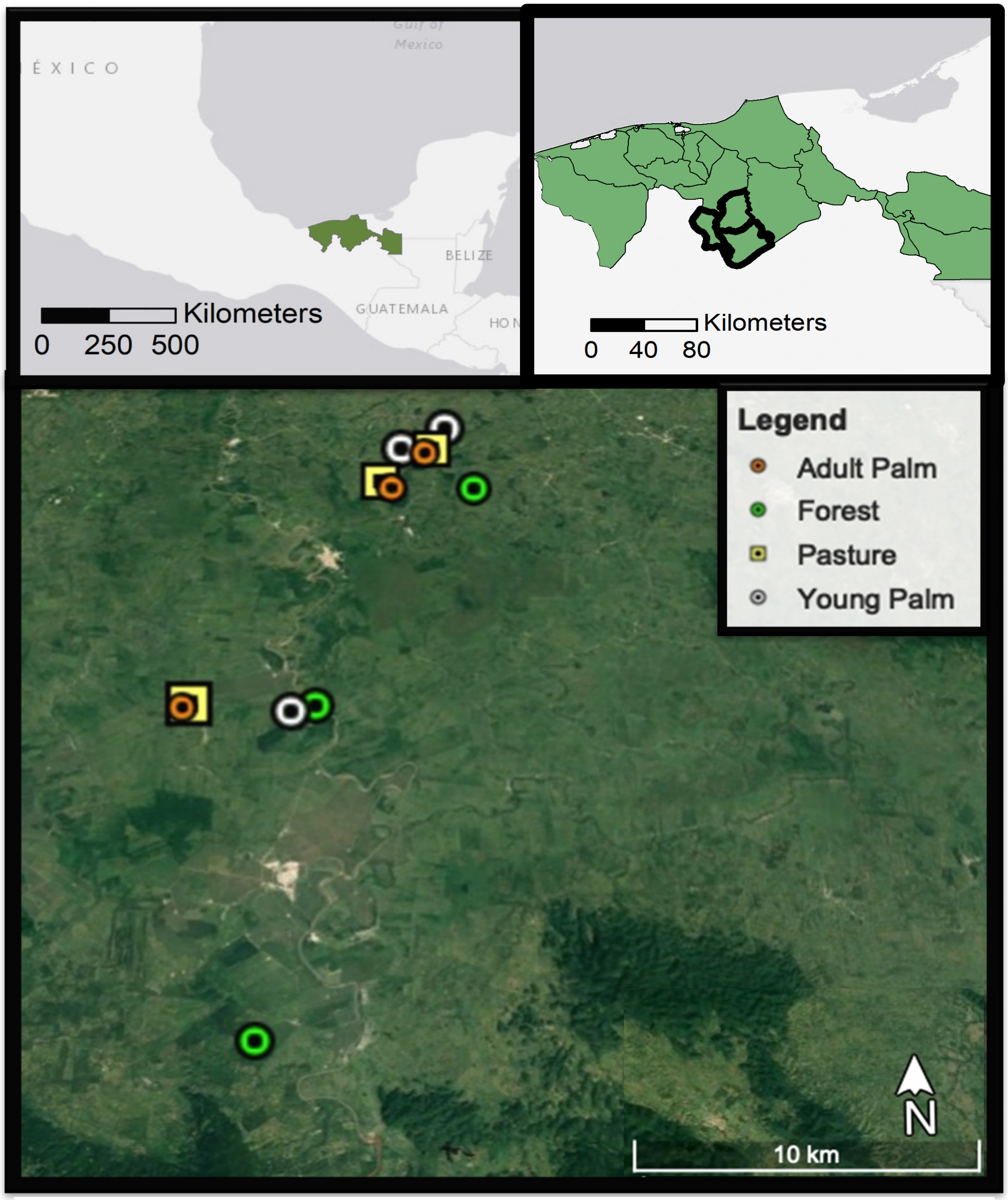


Figure 3-1. The state of Tabasco (green, top left), the outlined municipalities (top right), and the landscape view of sites alongside the river (bottom).

3.3.2 Experimental design

Our study's design was limited to oil palm grown on loamy soils, and the results from this study may not apply to nearby regions of Tabasco due to the topographic and hydrologic heterogeneity of the landscape. Between February and late-August 2015, we sampled two age groups of oil palm—young (3-4 years old) and adult palm (17-19 years old)—along with two alternative land uses of pasture and secondary forests. Each of these four groups served as a different land use treatment for this study. Each land use treatment is comprised of three replicate sites.

All oil palm stands were converted from pastures. The oil palms were planted in a staggered spacing creating an equilateral triangle 9m x 9m x 9m with a stocking of ~143 palms/ha. One exception was an adult plantation that had palms spaced at 7m x 7 m. The plantation stands ranged between two and twenty hectares in size and we sampled an area of approximately 0.5 ha for each stand/site. Most sites were flat with two exceptions. One young palm stand had a gradual slope of ~5% and one secondary forest site had a slope of ~25%. From both sites, we sampled at the top, mid, and bottom positions of the slope.

Although we were unable to obtain a detailed history of every site, we were able to obtain general information as to how sites in this area are managed. The oil palm stands were established with minimal disturbance by transplanting potted seedlings with a shovel (personal communication). Chemical fertilizers such as granulated triple 17 (17% N,17% P_2O_5, and 17% K_2O) were generally applied to a weeded circle around the palm stem just after palm fruits start to develop (~2-4 years old). The weeded circle was an area under the palm that tended to be devoid of weeds, partially created by the drip line of the crown, but also weeded to prevent competition with other vegetation. Pruning and residue distribution of fronds did not start until after the fruit bunches were harvested. With palm roots growing away from the stem with age, chemical fertilizers were applied freely through the stand, not just within the original weeded circles. Residue management was similar among stands. Frond piles were arranged beside the palm stem away from the harvesting pathway that equipment used. Otherwise, the piles were left in every other row. Fruit bunch residues were not returned to the field after being harvested although this practice can be found in the area.

3.3.3 Field sampling

Land-use treatment groups were young palm, adult palm, pasture, and secondary forest. Each group consisted of three replicated sites ranging in size from 10-20 hectares. At each site, soil was extracted from nine points. While the oil palm sampling was systematic with points at defined locations, pastures and secondary forests were randomly sampled. The oil palm stands had the following defined locations to assure the representation of areas that may have higher amounts of soil nutrients or carbon: 1. Within weeded circles, which were 0.9 m away from the base of the palm stem); 2. Within harvest pathways, which were at 3.3 m and 4.5 m from a palm where the tractors would pass; 3. Under palm frond piles that were only found and therefore sampled at adult palm sites. The intra-row areas are assumed to be similar to the harvest pathways and are included in its weighted area (**Figure 3-2**). We navigated to randomly generated points in pastures and forests, chosen from a grid with points spaced 25 m apart. Extra points were generated for pastures to deal with situations where points coincided with tractor ruts, nearby tree roots, or cow manure. In forests, if a point coincided with animal burrows or insect mounds, it was omitted and a next random point was used.

At each site, plots (~0.5 ha) were established in oil palm stands with soil sampling along three-transects, each 108 m long and 27 m (**Figure 3-2**). Along each transect, three points were evenly spaced out, totaling 9 points. The soil was extracted down to 60 cm at three of the nine dispersed points in the plot using a 5.08 cm diameter gouge auger. Extracted soil was divided into four, 15 cm increments. These cores were used for BD measurements, C, and N analysis. Alongside the original three points as well as the remaining 6 points, soil was collected using a 3.3-5.0 cm diameter dutch auger with a 15 cm length that would later be used for nutrients, pH, and texture analysis (**Figure 3-2**). These nine points also marked the resin bag installation locations for all sites. Specific to only the palm stand sites, the three 60 cm cores were extracted from the harvest pathway while three additional bulk density (BD) cores were extracted per defined location down to 30 cm. Soil samples were put on ice in a cooler until air-dried later that same day.

We measured net N-mineralization by installing ion exchange resin bags. We loaded approximately 7.5 g (~12 ml) of mixed ion exchange resin beads (IONAC NM-60 H+/OH- form, Type I Beads, 16-50 Mesh, JT Baker 4631-01) into small square nylon bags (Binkley et al. 1992). The bags were constructed from No Nonsense ® nylon stockings (color nude) and approximately 5 x 5 cm, sealed with non-soluble glue using a hot glue gun. The glue was tested for ionic leaching before deploying the bags into the field to assure no interference.

The bags were installed for six relatively dry months from March of 2015 to late August 2015, and four months with the onset of the wet season, late August 2015 - December 2015. Resin bags were inserted into a narrow, 45-degree angle cut made with a tree planting shovel in efforts to lessen the effects of preferential water flow coming from directly above the bags. The resin bags were ~10 cm deep and a distance of ~ 0.5 m away from the soil sampling locations to assure no interference. A fishing string was tied to the corner of each resin bag. The other end of the fishing string was tied to non-adhesive flagging tape that remained above ground so we could retrieve the bags at a later date. When the dry season bags were removed, the wet season bags were installed less than 0.5 m from the first bag. After resin bags were removed from the field, they were placed in individual Ziploc bags and shipped to the Michigan Technological University lab where they were temporarily stored in a refrigerator until ions were extracted.

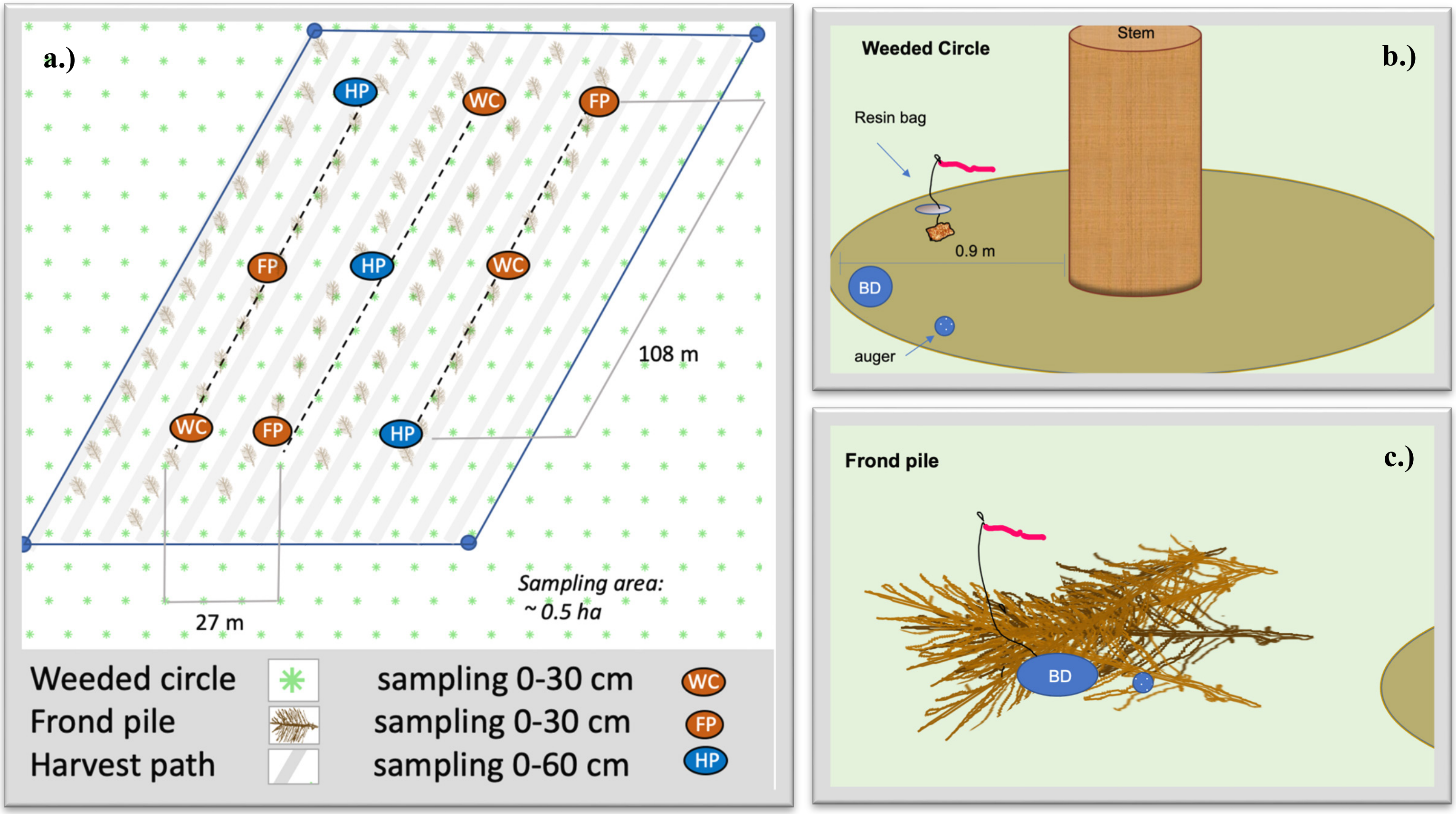


Figure 3-2. a.) Adult palm sampling design with three transects and three location types and their soil sampling depths. b.) Sampling at the weeded circles. c.) Sampling under frond pile (not in weeded circle)

3.3.4 Lab analysis

At the COLPOS University campus in Cárdenas, bulk density samples were oven-dried to 100 °C until weights stabilized. Samples analyzed for nutrients were air-dried and shipped to the Michigan Technological University (Houghton, MI, USA). All soil samples were ground by hand using mortar and pestle. Three points from each defined location type of palm stands were composited by depth. For pastures and forest sites, the nine points were composited for each of the four 15 cm depths.

Total C and total N were analyzed by dry combustion while P, K, Ca, Mg, Fe, an NH_4/NO_x were processed by various extraction methods. Total C and N concentrations were analyzed from the subsamples of the bulk density. Standard protocols for C, N were followed using the ECS 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA) that was calibrated with standards from the National Institute of Standards and Technology and included blanks to ensure accuracy. All sites and depths were also checked for carbonates using HCl and visually assessed for effervescence. One site tested positive, and was dropped out of analysis (not in study). The C and N concentrations were used to calculate total soil C and N in Mg ha^{-1} with:

$$C \text{ or } N\ (Mg\ ha^{-1}) = \%C \text{ or } N \ x\ BD\ x\ Depth\ x\ unit\ conversion \qquad (eqn.3.1)$$

where %C is g C/100 g oven-dried soil, BD is grams of dry soil per unit volume (g/cm^3), and depth is the layer's thickness in cm.

For the nutrient extractions of Ca, Mg, K, and the metal Fe, 2.5 g of soil from each defined location (for palm sites) and depth was added to a polypropylene cup containing 0.025L of 1M NH_4Cl. We used NH_4Cl to mimic the extractability of nutrients in native conditions. The cups were shaken for 30 minutes at 150 rpm. Any sediment in the cups was allowed to settle ~15 minutes after being removed from the shaker. The clear supernatant was suctioned up with a syringe and then a filter unit containing a 25 mm Whatman grade-1 paper was fitted to the tip of the syringe. Filtered extracts were refrigerated at 4°C for one to three days until they were run on the inductively coupled plasma mass spectrometry (ICP-OES) (Perkin-Elmer Optima 7000 DV, Waltham, MA). Phosphorus was extracted from 5.0 grams of soil using the Mehlich 1 Method (Kovar and Pierzynski 2009) and measured with colorimetry at 882 nm (Kuo 1996). The total soil nutrients in Mg ha^{-1} were calculated using:

$$Soil\ nutrient\ (Mg\ ha^{-1}) = 0.025L/2.5g\ \ x\ \ [Y]\ \ x\ \ BD\ \ x\ \ Depth\ x\ 100 \qquad (eqn.3.2)$$

where 0.025 L is the amount of NH_4Cl used to extract the nutrients, 2.5 g is the initial dry mass of soil used, [Y] is nutrient concentration results from the ICP given in ppm or mg/L, and seen here as [Y], BD is grams of dry soil per unit volume (g/cm^3), and depth is the layer's thickness in cm. This yields an answer in mg/cm^2 which needs to be multiplied by 100 to convert the units to Mg/ha.

At each palm stand location (weeded circles, harvest pathways, frond piles), carbon and nutrients were weighted. To do this, the nutrient amount ($Mg\ ha^{-1}$) was multiplied by the percent area their sampling location represented in a stand. The results for each location type in the palm stand were then added together yielding a weighted value for a specific replicate and depth. In young oil palm stands, weeded circles were estimated as 4% of the area and harvest pathway measurements were estimated as the remaining 96% of the area which includes the intra-row areas that are not frond piles. In adult oil palm stands, we estimated that weeded circles still made up 4% of the area, frond piles made up 11.6% of the area, and the remaining 84.4% of the area was harvest pathway.

For each depth, the pH was measured in 10 g of sieved, oven-dried soil after being diluted with 20 ml of deionized water (dH_2O). The pH meter was calibrated from standardized solutions with a pH of 4 and 7. Measurements were recorded to the nearest 0.01 (**Table 3-1**). To estimate soil texture, samples were grouped by 0-30 cm and 30-60 cm for each of the sites and processed using 50 g of oven-dried soil following the Bouyoucos method (Staff 2014). Clay and silt were determined at 2 hour time points.

The dry resin beads were removed from the nylon bags and weighed. To extract the ions from resin bags, we used 20 ml of 2M KCl adapting our protocol from the S. Castle Aridland Ecology Lab. Both ammonia (NH_4) and nitrate species (NO_x) were measured using Perstop continuous flow autoanalyzer. The NH_4^+ and NO_3^- extract concentrations (ppm) were divided by the final mass of beads that were collected from the field and also divided by the number of weeks they remained in the ground.

3.3.5 Statistical Analysis

To check for differences in nutrients and other soil characteristics between the two location-types within a young palm stands and between young and adult palms, the Student t-test was used (one-tailed). To assure the criteria of the t-tests were met, the Shapiro-Wilks test was used to assess normality along with qq-plots. The Bartlett test was used to detect whether variances were equal. If criteria were violated, the non-parametric test, Wilcoxon Mann-Whitney U rank test was used. The ANOVA tests were used to test the differences between the three location-types within adult palm as well as the four land use treatment groups with an alpha level of 0.05. Any differences that were found (along with near significant differences > 0.10), were further analyzed by comparing each pair of means with the Student t-test also referred to as the multiple comparison procedure. Statistics were run in the SAS based, software package JMP Pro version 13. To analyze differences in soil acidity, the pH measurements were converted to hydronium ion concentrations.

3.4 Results

3.4.1 Soil C and nutrients within oil palm stands

In young oil palm stands, soil C was higher in the top 15 cm of the weeded circles (45.4 Mg ha^{-1}) than in harvest pathways (35.8 Mg ha^{-1}; p = 0.02; **Figure 3-3**), but no differences were found below 15 cm. Soil N is also higher in the weeded circles at 0-15 cm than in the harvest pathways (p = 0.10). For all other nutrients (P, K, Ca, Mg) and metals (Al, Fe) levels were similar between weeded circles and harvest pathways.

Within adult palm stands, soil C and N were each statistically not different among the three sampling locations (weeded circles, harvest pathways, and frond piles). (**Figure 3-3**). While C and N were similar at all depths, extractable potassium (K) to 15 cm was higher in weeded circles than in the frond piles and harvest pathways (p = 0.07). Extractable phosphorus (P) to 15 cm was lower in the frond piles than that in the weeded circles (p = 0.07).

3.4.2 Young palm versus adult palm

Most differences among C and nutrients were at the surface (0-15 cm) when comparing soils of young and adult stands. Young palm-weeded circles were higher than adult weeded circles in the following: soil C (p = 0.04); soil N (p = 0.03), and soil phosphorus (P) (p = 0.06; **Figure 3-3**). However, iron was higher for the adult weeded circles (0-15cm, p = 0.03). Nitrogen was low in adult harvest pathways compared to young palm harvest pathways (p = 0.04). The pH was similar between the two palm ages. The pH was also similar in its vertical distribution from depths 0-30 cm to 30-60 cm (p = 0.53).

To put both young and adult stands in a better perspective, the landscape scale differences we found within the stands are no longer significant when weighted by area. For example, the weeded circles' nutrient hot-spots in the young palm only make up 4% of the total area in a hectare.

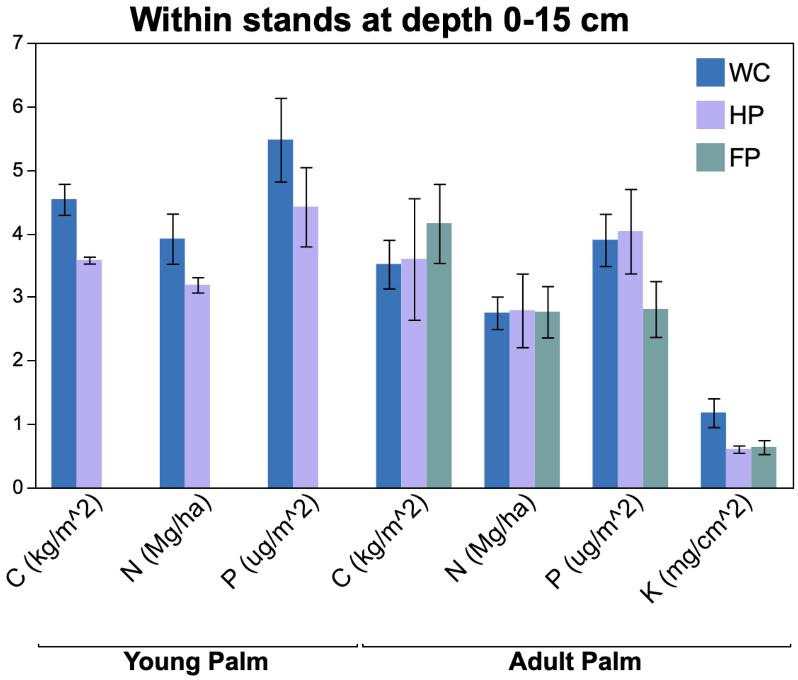


Figure 3-3. **Young Palm** (Left): Within young palm stands, there is higher carbon (C) in weeded circles (WC, blue) than in harvest pathways (HP, purple) (p = 0.02) and there is higher soil nitrogen (N) in weeded circles than in harvest pathways (p = 0.10). **Adult Palm** (Right side): Within adult palm stands there is higher extractable phosphorus (P) in weeded circles than found in frond piles (FP, green) (p = 0.07) and there are higher levels of potassium (K) in weeded circles than both harvest pathways and frond piles (p = 0.07). **Young vs Adult**: Young palm WCs were higher than adult WCs in soil C (p = 0.04.

3.4.3 Land use type

The four land-use groups were similar in soil C at each depth and by total depth (0-60cm). The average total C ranged from 95.1 to 116.3 Mg ha^{-1} (**Table 3-2**, Figure 3-4). Soil C was highest at the 0-15 cm layer and declined with depth ($p < 0.01$). Within land uses, adult palm stands were the only treatment group that did not show significant declines of soil C with depth ($p = 0.18$).

Adult oil palm stands had lower soil N than all other land-use types at the 30-45 cm layer ($p = 0.11$) and 45-60 cm ($p = 0.12$). Across the land-use treatments, the vertical profile of nitrogen was more abundant at the surface and decreased with increasing depth ($p = 0.09$). Most other nutrients were similar among land-use groups, making it difficult to see trends with a low sample size (n=3). Secondary forests held higher levels of iron than all other land use types ($p = 0.01$) and this was significant at 30-45 cm. Though not statistically significant at other depths, forests still maintained levels 1.5 times than the average of other sites.

The four land-use groups were also similar in bulk density. Each depth showed no significant difference between land uses. The land-uses were also similar in pH, at both 0-30 cm and 30-60 cm (**Table 3-1**). Secondary forests ranged in pH from 4.4 to 7.6 (30-60 cm). The land-use groups ranged in soil texture from sandy loam to clay loam. Clay was similar between land-use groups and the percent content of clay increased with increasing depth ($p = 0.05$).

3.4.4 Net N mineralization

Net ammonium production was more abundant than NO_3^- for all land-use groups during the dry season ($p < 0.01$). Ammonium decreased slightly in the wet season ($p = 0.06$) and during this time the two N-ions were not significantly different ($p = 0.32$, Figure 3-5). Net ammonification or the production of NH_4^+ was negligent from the dry season to the wet season (Figure 3-6). Increases in NO_3^- from the dry season to the wet season could infer net nitrification, the production of NO_3^-, however nitrification varied greatly from site to site within land-use treatments. The young palm group was the highest estimate of nitrification but likely by default of not having other replicates to average. In the secondary forest group, one stand increased in NO_3^- by 62.3 (µg/g resin) $month^{-1}$ while another stand had decreased in NO_3^- by 45 (µg/g resin) $month^{-1}$. The pasture group had the lowest average nitrification of 6.03 (µg/g resin) $month^{-1}$. Total N mineralization ($NH_4^+ + NO_3^-$) was similar between the dry season and the wet season and did not differ significantly between the four land-use treatments ($p = 0.19$).

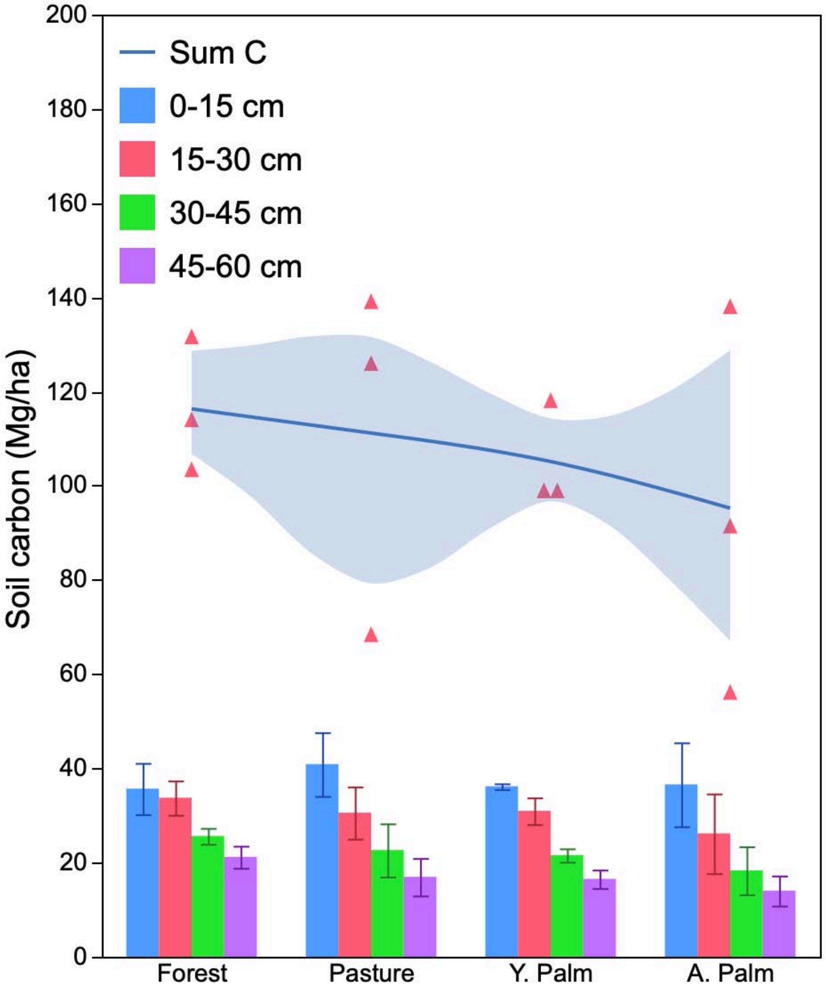


Figure 3-4. Smoothed line with light blue confidence fit is the summed depths of soil C from 0 to 60 cm that does not change significantly across land use types nor does soil C change at any of the 15 cm depth increments (bars with standard error). Red plus signs mark the summed C for each land use replicate.

Table 3-1. Soil characteristics by land use and ANOVA tests of the four land-use treatments.

Land use	**Depth (cm)**	**C:N**	**Clay%**	**Sand %**	**pH**
Y. Palm	0-30	11.3 (0.8)	19.9 (2.2)	48.3 (3.3)	5.0 (0.4)
	30-60	11.1 (1.4)	25.0 (1.2)	47.2 (3.0)	4.9 (0.7)
A. Palm	0-30	12.5 (0.9)	19.3 (2.6)	44.7 (3.2)	5.0 (0.1)
	30-60	11.8 (1.6)	24..9 (1.1)	44.4 (2.3)	4.9 (0.4)
Pasture	0-30	11.7 (1.4)	23.6 (1.0)	50.5 (4.3)	4.4 (0.2)
	30-60	11.7 (1.6)	25.4 (5.8)	51.2 (8.9)	4.4 (0.2)
Forest	0-30	11.1 (1.2)	18.7 (5.1)	42.3 (2.2)	4.4 (0.8)
	30-60	11.5 (0.9)	26.0 (9.5)	43.0 (9.6)	4.2 (1.0)
ANOVA	*0-30*	*0.82*	*0.64*	*0.23*	*0.93*
p-value	*30-60*	*0.99*	*0.90*	*0.80*	*0.83*

Table 3-2. Weighted nutrient and metal averages by treatment group with standard error (SE) in parenthesis

Land use	Starting Depth (cm)	C (Mg/ha)	N (Mg/ha)	P (Mg/ha)	K (Mg/ha)	Ca (Mg/ha)	Mg (Mg/ha)	Al (Mg/ha)	Fe (kg/ha)
Y. Palm	0	36.2 (0.6)	3.2 (0.1)	0.45 (0.06)	0.09 (0.02)	1.5 (0.9)	0.2 (0.1)	0.03 (0.02)	0.34 (0.21)
	15	31.0 (2.8)	2.8 (0.2)	0.45 (0.11)	0.07 (0.02)	1.8 (1.2)	0.2 (0.1)	0.06 (0.03)	0.99 (0.88)
	30	21.6 (1.4)	1.9 (0.1)	0.42 (0.17)	0.07 (0.02)	2.0 (1.2)	0.3 (0.1)	0.12 (0.07)	0.92 (0.81)
	45	16.5 (2.0)	1.6 (0.0)	0.73 (0.33)	0.08 (0.02)	2.1 (1.5)	0.3 (0.2)	0.21 (0.11)	0.28 (0.10)
A. Palm	0	36.6 (8.9)	2.8 (0.5)	0.39 (0.06)	0.07 (0.01)	1.5 (0.4)	0.2 (0.1)	0.03 (0.01)	1.32 (0.37)
	15	26.2 (8.5)	2.1 (0.5)	0.39 (0.11)	0.07 (0.02)	1.8 (0.9)	0.3 (0.2)	0.05 (0.02)	1.53 (0.76)
	30	18.3 (5.1)	1.4 (0.2)	0.38 (0.16)	0.07 (0.02)	2.5 (1.1)	0.4 (0.2)	0.06 (0.03)	1.14 (0.66)
	45	14.0 (3.2)	1.2 (0.2)	0.31 (0.18)	0.09 (0.02)	3.1 (1.3)	0.6 (0.2)	0.09 (0.08)	0.81 (0.66)
Pasture	0	40.9 (6.8)	3.5 (0.3)	0.37 (0.11)	0.15 (0.04)	1.8 (0.9)	0.3 (0.2)	0.05 (0.02)	1.10 (0.39)
	15	30.6 (5.5)	2.5 (0.1)	0.38 (0.14)	0.13 (0.04)	2.0 (0.9)	0.4 (0.2)	0.10 (0.05)	1.48 (0.59)
	30	22.7 (5.6)	1.8 (0.2)	0.27 (0.09)	0.13 (0.05)	2.8 (1.6)	0.6 (0.4)	0.17 (0.08)	2.05 (0.47)
	45	17.0 (4.0)	1.5 (0.2)	0.25 (0.14)	0.14 (0.06)	3.1 (1.9)	0.8 (0.5)	0.20 (0.10)	1.84 (0.24)
Forest	0	35.7(5.4)	3.2 (0.2)	0.32 (0.04)	0.12 (0.04)	3.0 (2.0)	0.4 (0.2)	0.05 (0.04)	1.60 (0.87)
	15	33.8 (3.6)	3.1 (0.1)	0.32 (0.10)	0.12 (0.04)	3.8 (2.9)	0.4 (0.3)	0.13 (0.08)	4.24 (0.67)
	30	25.6 (1.7)	2.3 (0.2)	0.41 (0.18)	0.15 (0.07)	5.2 (4.3)	0.5 (0.4)	0.27 (0.14)	4.06 (0.37)
	45	21.2 (2.3)	1.8 (0.2)	0.21 (0.12)	0.17 (0.05)	5.4 (4.4)	0.5 (0.3)	0.40 (0.20)	1.56 (0.82)

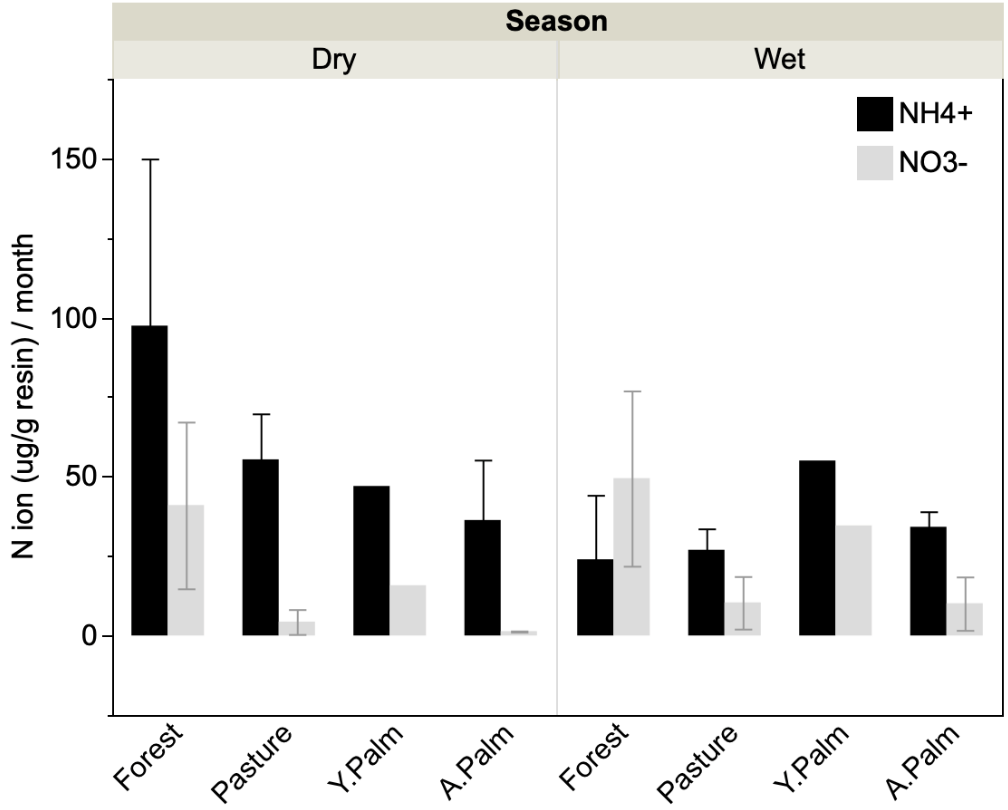


Figure 3-5. NH_4^+ levels are greater than NO_3^- (p = 0.01) and NH_4^+ levels decline from the dry season to the wet season (p = 0.06).

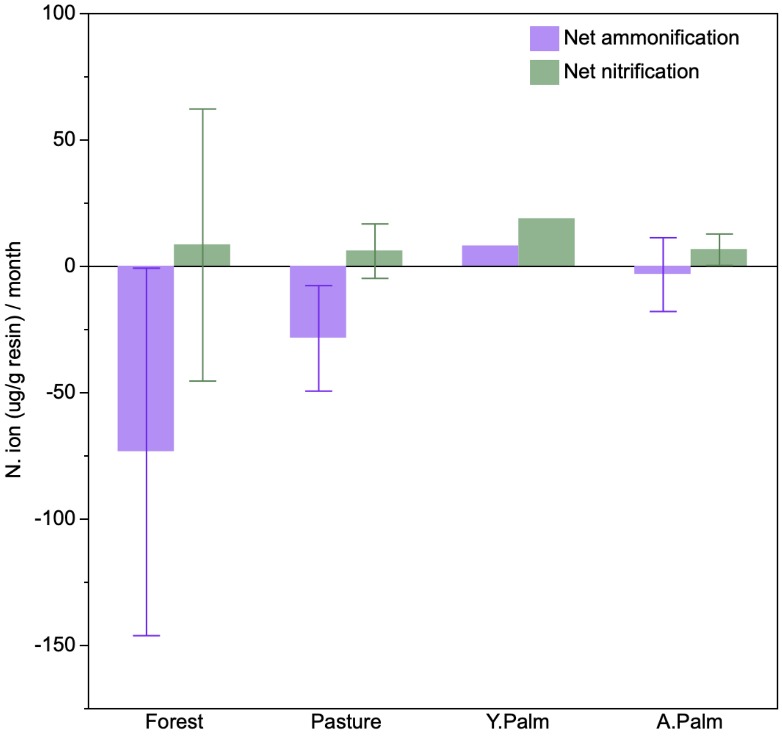


Figure 3-6. Estimates of ammonification (Wet-Dry) is negligent and nitrification (NO_3 wet - NO_3 dry) is low. Among the young stand replicates, resin bags were recovered for both wet and dry seasons in one site.

3.5 Discussion

This study sought to determine how palm plantations influenced soil C and nutrients and to compare palm plantations against alternatives land uses like pasture and secondary forest. While some carbon and nutrients were elevated in localized areas within palm stands, the effects were not apparent at the stand scale of one hectare. Within the group of young palm stands, it appears that nutrients in the weeded circle or ‘hot-spots’ were likely due to fertilization and elevated soil carbon possibly due to new root inputs, but we lack the management information required to be certain of our suspicion. In a carbon isotopic study, the fine roots of oil palm were identified as the main driver of soil C stabilization and the patterns observed in that study support some of our within-plot findings but do not provide larger-scale perspectives (Rüegg et al. 2019).

Contrary to our expectations, soil C did not increase from the 4-yr old stands to the 20-year-old palm despite minimal land disturbance upon planting the seedlings and the incorporation of C from decaying palm residues and fine root inputs over time. This could suggest all the ecosystems are near maximum C storage given the local climate. With the introduction of new organic matter inputs from oil palm, perhaps this stimulated the mineralization of pasture-derived C, allowing a net stabilization of total soil C. The steady inputs of oil palm in addition to shade-tolerant ground vegetation could continue on the same trajectory until pasture-C is depleted, leading to net increases in total soil C. An example of where increases occurred in a later time-frame was in a Columbian plantation where it took 36 years before losses of C derived from pasture were counteracted by new oil palm C inputs (Quezada et al. 2019). However, most palm plantations operate on shorter time frames because fruit productivity of oil palms often declines after plants reach 25 years old at which point oil palms are cut down and replanted (Yusoff 2006). Other studies have also seen no significant changes to soil C with increasing age (Smith et al. 2012; Khasanah et al. 2015). If oil palm does not impact soil C significantly, it is also possible that ground vegetation productivity could eventually be reduced from canopy closure, thereby leading to a decrease in soil C. The similarities we see between land-uses in this study could also be due to a low number of replicates (n=3) per land-use type given the site-to-site variability seen in groups like the adult palm (**Figure 3-3**).

Oil palm stands and pasture soil C levels were similar to secondary forests. Some studies have found older palms and pastures to have greater quantities of soil C than secondary forests (Frazão et al. 2013; Goodrick et al. 2015). One study showed that converting primary forest to pasture or secondary forest resulted in similar soil C loss (Chiti et al. 2014), supporting the feasibility of these land uses to be equal – assuming they might have started with similar C levels (Neumann-Cosel et al. 2011). Hughes et. al (1999) reported stable mineral soil C of secondary forests in Southeast Mexico in a 50-year chronosequence (1.5 m depth). These secondary forests showed no differences in soil C from primary forests, cornfields, and pastures. This is contrary to what Don et al. (2011) found in the tropics where grasslands converted to secondary forest typically gained 17.5 ±8 Mg ha^{-1}. It is also possible that the storage capacity of soil C is limited as it has been found in other tropical soils(Sayer et al. 2019). Though secondary forests have not proven to be higher in soil carbon in this study, other ecosystem services such as above-ground biomass and biodiversity of plants and animals in forests outweigh those of the other land uses.

While nutrient patterns were similar between most land-use types, nitrogen mineralization and nitrification varied from site to site. Resin bags are a mere estimation of N-ions on these plots and many bags were destroyed from roots growing directly into the bags leaving holes for the resin to fall out. It is interesting though that NH_4^+ levels were elevated for most groups in the dry season compared to NO_3^-. The dry phases of soils undergoing dry and rewetting cycles can elevate levels of NH_4^+ and decrease NO_3- (Gao et al. 2020). We expected to see some higher levels of total N-ions in the wet season thinking there could be faster decomposition rates and increases in mineralization, but there were no significant differences in total N-ions between wet and dry seasons.

Within the scope of this study in Southeastern Mexico, soil carbon does not appear to change significantly from 4-yr to 20-yr old oil palm stands and is not different from other land uses. Land-owners of these small-scale plantations (< 20 ha) could benefit from some practices that are already taking place within the municipalities. While many farmers already take precautions to avoid soil compaction, other practices such as intercropping with nitrogen-fixing species or returning empty fruit-bunches from the processing mills to the stand to retain soil organic matter could be useful.

3.6 Conclusion

Based on our sampling regime and palm stands spanning roughly 13-16 years, our data show that palm stands are not significantly different in soil C from pastures and secondary forests (0-60 cm). Forests had higher levels of iron ($p = 0.01$) at 30-45 cm compared to all other land uses. Most nutrients were similar among land-use groups. The only differences found were that adult palm had lower nitrogen levels than young palm at depths 45-60 cm in harvest pathways, and young palm stands had higher levels of C, N, P within the soil surface of localized weeded circles compared to adult stands' weeded circles. We hope that this data can be used for future modeling for Luvisols in this region. Future studies would benefit from obtaining a greater number of replicates and also including other types of soil in this region such as Gleysols or Acrisols on which future oil palm is also likely to expand. Data produced by this study offers important real-world constraints on potential changes in the soil for future biogeochemical models.

4 Soil Carbon Responses in a Forty-Year Chronosequence of Naturally Regenerated *Populus tremuloides*

4.1 Abstract

Aspen *(Populus tremuloides* Michx.*)* are fast-growing trees that are managed on 40-55 year rotations throughout the Great Lakes Region. Currently, aspen forests are used primarily for pulp and particle wood with harvests typical being stem-only and occasionally whole-tree harvest. In northern Wisconsin, aspen stands are being considered as a bioenergy feedstock, however, many aspen stands occur on sandy-textured soils potentially making them more susceptible to soil carbon and nutrient losses. Our objectives were to test if soil carbon and soil macronutrients (N, K, Ca, Mg, P, as well as Al and Fe) declined across a chronosequence of harvested aspen sites in sandy Wisconsin soils. We sampled soil down to 60 cm in fifteen separate stands of aspen with a similar sandy texture that spanned from 10 to 56 years post-harvest. Ages were grouped into 10-, 20-, 30-, and 45-yr post-harvest groups for analysis. We found that soil carbon increased from 10 to 45 years post-harvest by 42% (58.5 Mg ha^{-1} to 83.1 Mg ha^{-1} ; 0-60 cm: $p = 0.02$), but most of the change was in the top 30 cm ($p = 0.02$). Nutrients N, K, Ca, and Mg were similar among all age groups, however, P increased exponentially with age and depth. Our results over one rotation indicate that soil carbon increased over time since harvest, but the removal of additional residues if done for biofuels could lead to depletion of soil carbon and nutrients in the long-term.

Keywords: aspen, coarse-textured soils, biofuels, soil carbon

4.2 Introduction

P. tremuloides forests are being considered as a bioenergy feedstock in the Great Lakes Region because of their fast growth and high volume. In the state of Wisconsin, Oneida County is the third-largest holder of aspen forests with an amount of biomass equal to 3,428,000 dry short tons (WDNR 2018). However, many aspen stands proposed for bioenergy use occur on sandy textured soils, making them susceptible to losses of nutrients such as N, P, K, Ca, and Mg (Ruark and Bockheim 1988; Curzon et al. 2020). While guidelines suggest a higher percentage of harvest residues should be left behind on nutrient-poor sites, further investigation of the carbon and nutrient status of these forests is required before they should be considered for bioenergy.

Aspen studies in the Great Lakes region over the past 40 years have informed us about variations of the aspen forest communities, their successional pathways, how they are impacted by forest management (Roberts and Richardson 1985; Nave et al. 2010; Klockow et al. 2013; Curzon et al. 2020), and how soil carbon (C) and nutrients change through time (Ruark and Bockheim 1988; Alban and Perala 1992; Tang et al. 2009). Aspen are adaptable to a wide range of soils and can be found on both fine and coarse-textured soils. Coarser textured soils are prone to low nutrient retention and relatively lower soil moisture. As a pioneer species, aspen in the Great Lakes Region are usually replaced by maples and yellow birch in mesic areas, while in drier areas they can succeed to oak and conifer (Roberts and Richardson 1985; Pastor and Post 1986). Many coarse-textured soils in Northern Wisconsin are Spodosols which are occupied by both aspen and conifers. Although harvesting on sandy soils is recommended with caution, a meta-analysis study on temperate forests showed Spodosols were not significantly affected by harvest despite some soil C losses in deeper soil (>30 cm) counteracted by some small gains at the surface (Nave et al. 2010). These larger-scale patterns however are more applicable to regular harvest management and based on more than just aspen stands. One of the aspen studies in the meta-analysis was examined by Alban and Perala (1992). In their study, total carbon in the forest initially decreased after harvest, and showed a net increase by 10-years, similar to other studies (Ruark and Bockheim 1988; Tang et al. 2009), but showed relatively constant soil C as aspen stands were replaced with northern hardwood species. In contrast to this, Tang et al. (2009) observed increases in soil carbon as stands aged from ~10-years to 50 years old. Harvesting forests for bioenergy is more intensive with more biomass typically removed from the stand, leaving longer-term effects in question. Klockow et al. (2013) observed that within a year of whole-tree harvest that both above-ground biomass and soil mineral C declined compared to stem-only harvest. Moreover, the amount of slash residue taken off-site corresponded to the decreases in soil C. A 20% slash retention was also included in this study and the impacts were between that of the whole-tree harvest and stem-only harvest treatments. Soil C in a Long Term Site Productivity (LTSP) study was monitored 25 years after a whole-tree aspen harvest with the removal of shrubs (Curzon et al. 2020). Sites included sandy, loamy, and clayey-textured soils for comparison. On sandy sites, soil C decreased and biomass was reduced which could lead to nutrient depletion and soil moisture losses. Whole tree harvests did not negatively affect loamy and clayey soils after 25 years, but the fine-textured soils were more prone to compaction. In a 500-year simulation of sugar maple in this region, all harvest treatment scenarios showed losses of nutrients and soil carbon, including 50-year and 100-year rotation lengths, and varying residues retention up to 35% compared to no harvest (Peckham et al. 2013). While sandy, loamy, and clayey soils can be affected differently in the short-term, the increased biomass removed with a whole-tree harvest is likely to affect all soil types regardless of texture, leading to a loss in nutrients, lower pH, and lower productivity (Wall 2012). A meta-analysis study showed soil carbon decreases 13.3% after whole tree harvests compared to stem-only harvest (James and Harrison 2016).

Long-term research on woody biofuel management in the United States is limited, therefore whole-tree harvesting is the best example we have for understanding the potential effects. **Therefore, our goal was to look at soil C and nutrients patterns under traditional aspen management to help determine if aspen has the potential to be used for biofuels in sandy soils.** We hypothesized that there will either be a slight decline after 40 years or overall no net change in soil C levels across a ~60 year chronosequence.

4.3 Materials and Methods

4.3.1 Site description

The study sites were located in Northern Wisconsin (45° 43’N, 89° 32’W) near Rhinelander (**Figure 4-1**) on flat, glacial moraines and outwash plains. The mean annual rainfall in this region is 800 mm. Snowfall occurs 6.5 months out of the year averaging 1016-1270 mm/yr. Mean annual temperatures range between 14-26 °C in the warm season and -15 to -5.5 °C in the cold season (Climate-Data.org ; NOAA).

Aspen-mixed hardwood stands in this study were naturally regenerated and a few stands were regenerated with young red oak and conifer saplings left uncut as part of the prescription. Natural aspen stands are generally managed in this area on 40-55 year rotations at which more point shade-tolerant secondary succession spp. are replacing them (WDNR 2019). Stands with poorer soils generally have extended rotation periods. The DNR has established guidelines for biomass residue retention based on the percent of total biomass that has been harvested. Higher percentages of slash and residues are retained on poorer sites and these tend to be sites on sandy textured soil. Aspen stands are often removed as a stem only harvest and to a lesser extent whole-tree harvest. For stem-only harvests, residues can be carried off-site and chipped, left on-site and chipped, or left on-site as not chipped. Most of the sites were likely stem-only harvests. In younger stands, round wood residues remained within the stand’s extraction paths to help provide traction for machinery and prevent soil compaction. Residue management was not part of our site criteria. Stands representing each decadal age class had an average diameter breast height of 3.72 cm in 10-yr, 6.49 cm in 20-yr, 10.76 cm in 30-yr, and 21 cm in 40-yr (56-yr old not included) (Phifer 2017).

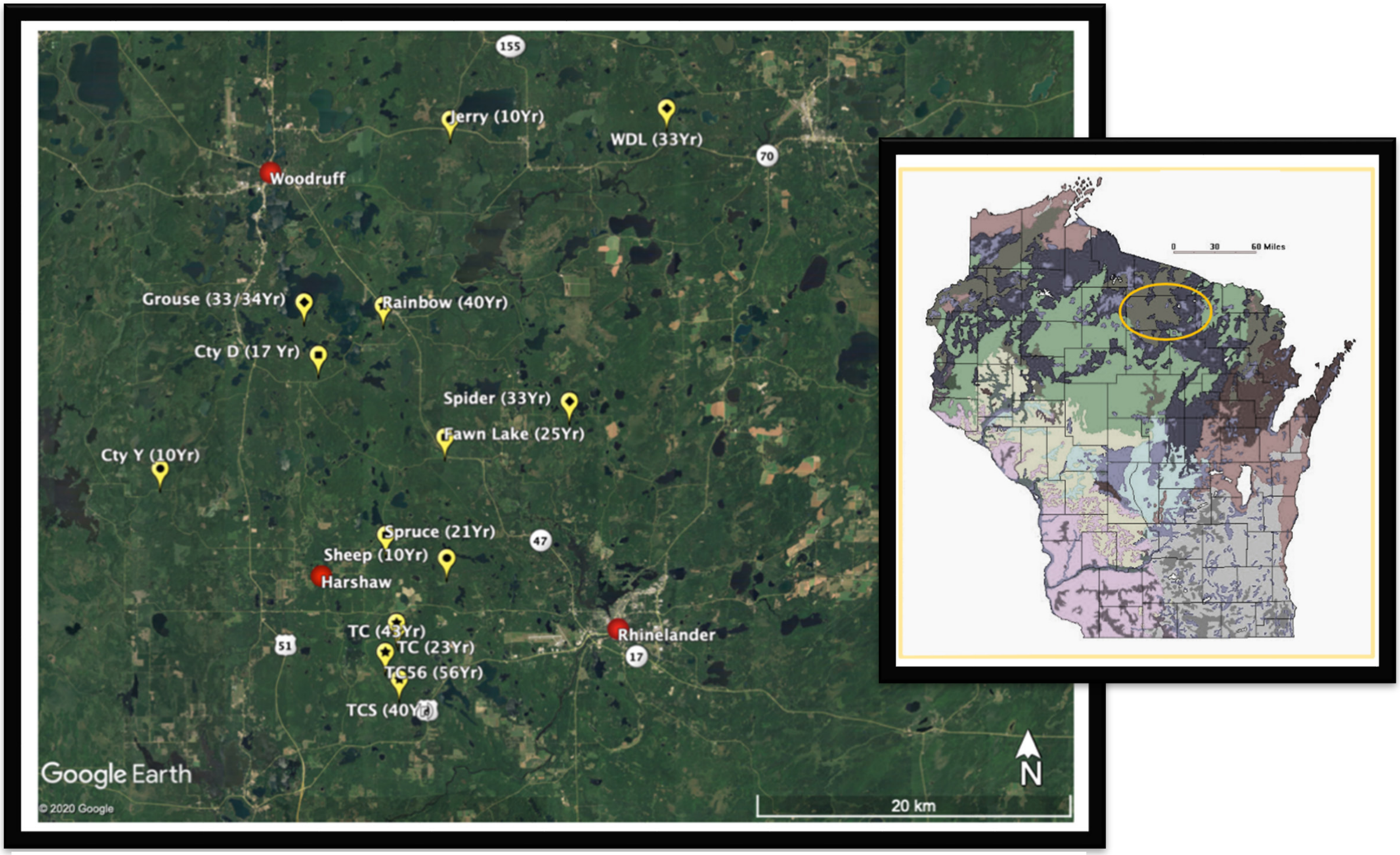


Figure 4-1. Study sites in Oneida County and along the southern border Vilas County. The inset map displays the counties in the state of Wisconsin.

4.3.2 Experimental design

Each site was chosen from stands greater than ten hectares with slopes less than 15%. Contiguous stands were selected over stands interrupted by wetlands or roads. To assure independent replicates, within a stand, we avoided any two blocks that were clear-cut at the same time. In efforts to reduce edge effects within a plot, the soil was not extracted within 10 m of roads and principal harvest routes used by semi-trucks. The soil survey maps indicated that the soils cored were Spodosols, mostly Haplorthods within the series of Padus, Pence, Goodman, and Sawyer although E-horizons were not always distinct. The fifteen sites were grouped into a chronosequence design, based on age or years post-harvest (10, 17-25, 32-34, 40-56). Each age group is comprised of four replicate stands except for the 10-year that was comprised of three replicates. Late succession stands ≥ 40 years are used as an alternative land use scenario to the bioenergy feedstock. For each age group, we selected sites we thought included both nutrient-poor and moderately fertile soil based on soil series according to Wisconsin's Guidelines of biomass removal (Bronson et al. 2014).

4.3.3 Field sampling and lab analysis

Six soil cores were collected per site in a random sampling scheme with at least a 25 m spacing between points. Next to the cored sample, we collected forest floor (Horizon O_i) between June and August 2014 using 25 cm x 25 cm quadrats and the litter layer was oven-dried litter at 65 ºC to constant weight. We extracted the remaining O_a and O_e horizons as well as the mineral soil with a soil corer. The corer was 6 cm in diameter and was pounded vertically into the ground to a depth of 60 cm using a 9-kg slide hammer weight. The corer was lined with a plastic sleeve of 60 cm height which was carried offsite and immediately placed on ice.

In the lab, cores were stored frozen and when thawed were cut into four equal 15 cm depths. Upon thawing, soft aggregates were manually crushed using a rolling pin, and samples were sieved through a 2mm screen. Dry weights were obtained at 65 ºC for bulk density. A set of subsamples were dried out to 105 ºC to assure that any further moisture loss was negligent. Roots, decaying wood, and rocks were removed. If any of these contributed significantly to the total volume or mass, they were weighed separately and adjusted in the dry weight and/or subtracted from the bulk density volume.

Soil C and N were analyzed apart from nutrients and metals Ca, Mg, K, P, Al, and Fe. Carbon and N samples were determined using the ECS 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA). Standards from the National Institute of Standards and Technology (NIST) were used to calibrate C and N concentrations. The C and N concentrations were used to calculate total soil C and N in Mg ha^{-1} with:

$$\%C \times BD \times Depth \times unit\ conversion \qquad \text{(eqn 4-1)}$$

where %C is the mass of C in grams per 100 g of bulk soil, BD is the bulk density which is the mass of bulk soil per unit volume (g/cm^3), and depth is the layer of soil's thickness.

Exchangeable nutrients, Ca, Mg, K, and metals such as Fe and Al, were extracted with NH_4Cl solution to mimic the capacity of extractable nutrients at native pH conditions. Nutrients were extracted by adding 25 ml of molecular grade NH_4Cl solution (1M) to 2.5 g of soil in polypropylene cups, shaken for 30 minutes on an orbital shaker at 150 rpm. Supernatants were pushed through a syringe equipped with a 25 mm Whatman grade 1 paper filter. Filtered extracts were refrigerated for 1-3 days until analyzed with inductively coupled plasma mass spectrometry (ICP-OES) (Perkin-Elmer Optima 7000 DV, Waltham, MA). Aluminum and iron were negligible on preliminary analysis from different sites, therefore, were not analyzed further. Phosphorus was extracted from 5.0 grams of soil using the Mehlich 1 Method (Kovar and Pierzynski 2009) and measured with colorimetry at 882 nm (Kuo 1996).

For pH and texture, the cores from each site were composited by 0-30 cm and 30-60 cm depths. The pH was measured by adding 20 ml of deionized water (dH_2O) to10 g of sieved, oven-dried soil. The pH meter was calibrated from standardized solutions with a pH of 4 and 7. Measurements were recorded to the nearest 0.01. The soil texture was measured for each 15 cm depth for a site and was processed using 100 g of oven-dried soil and following the Bouyoucos method (Staff 2014). Clay and silt were determined at two-hour time points. All sites and depths were also checked for carbonates using HCl and visually assessed for effervescence.

4.3.4 Statistical analysis

ANOVA tests were used to examine any differences in soil characteristics among rotation treatments including total soil carbon, carbon fractions, nutrients, [H^+] derived from pH, and clay. Soil characteristics were analyzed for each 15 cm increment separately. Phosphorus was the only dependent variable that was log-transformed. Aside from age, depth was also used as an independent variable to look at differences in soil characteristics. The carbon from the forest floor was estimated as 50% of the mass.

Statistics were performed in the software package JMP Pro version 14 with an alpha level of 0.05. Unequal variances were tested with the Bartlett test. For any significant differences, post hoc comparisons were performed with the Least Square Mean Differences Tukey HSD with an alpha level of 0.05. Transformations were made to phosphorus to correct for heteroscedasticity.

4.4 Results

Total soil C (0-60 cm) increased with age across the chronosequence (**Table 4-1**, **Figure 4-2**; $p = 0.02$). Most of the change in soil C was found in the 15-30 cm soil depths where the 45-yr group was significantly higher in soil C than the 10-yr and 30-yr groups (p <

0.01). At the depth 30-45 cm, soil C increased ($p = 0.01$, **Table 4-1**). In this statistic, we had an unequal variance that violated the normal 1-way ANOVA so we used Welches ANOVA that weights the variance. Nitrogen did not statistically change across the age groups as soil carbon did (**Table 4-1**, **Figure 4-3**). The C:N increased with age until the 30-yr stands followed by a trend downward (0-15 cm). The 10-yr old stands had low N that drove the C:N ratio up. Both levels of C and N decline with depth ($p < 0.01$).

Phosphorus, (P), data were log-transformed at all depths due to exponential increases causing uneven variance. Phosphorus increased with age (all depths, **Table 4-1**) and this was the only nutrient that increased with depth ($p = 0.02$). Extractable potassium (K), magnesium (Mg), and calcium (Ca) did not change with increasing age. Each of these nutrients did decrease with depth: K, $p = 0.03$; Mg, $p = 0.02$, Ca, $p < 0.01$.

Soil acidity decreased slightly with age from 4.3 to 4.8 (0-30 cm, $p = 0.11$, **Table 4-2**). At lower depths, the pH was similar across age groups. Soil textures were all sandy with percent sand ranging from 74 % to 89%. Percent sand trended slightly down with age and was more abundant with depth, but neither trends were significant. With regards to structure, most soils held little structure below the dark A-horizon (< 30 cm) except for an occasional sandy aggregate (~2.5 cm wide).

While the mass of the forest floor did not change with increasing stand age, the bulk density of mineral soil (including O_e and O_a horizon) increased with increasing depth. When bulk density was examined across age treatments it did not change significantly at any single layer tested, but did trend downward with increasing age for depths below 15 cm. Downward trends are seen more prominently after 20 years.

Table 4-1. Mean soil bulk density (BD) and nutrients by age treatment age treatment for 4 different depths. Standard error is in parenthesis. The ANOVA p-values test for changes across age groups (bottom of table). * denotes $p \leq 0.05$, ** $p < 0.01$

Age Group	**Depth (cm)**	**BD (g/cm^3)**	**C (Mg/ha)**	**N (Mg/ha)**	**P (Mg/ha)**	**K (Mg/ha)**	**Ca (Mg/ha)**	**Mg (Mg/ha)**
10 YR	0-15	0.7 (0.1)	35.7 (4.1)	1.8 (0.4)	1.15 (0.10)	0.06 (0.01)	0.3 (0.1)	0.04 (0.01)
n=3	15-30	1.2 (0.0)	11.4 (0.1)	1.0 (0.2)	2.3 (0.40)	0.05 (0.01)	0.1 (0.0)	0.02 (0.00)
	30-45	1.3 (0.0)	6.4 (0.4)	0.5 (0.1)	3.98 (0.53)	0.07 (0.03)	0.1 (0.1)	0.03 (0.01)
	45-60	1.4 (0.0)	4.9 (1.4)	0.4 (0.1)	4.46 (0.85)	0.04 (0.01)	0.3 (0.2)	0.04 (0.02)
	0-60	---	58.5 (2.4)	3.7 (0.8)	11.88 (1.81)	0.21 (0.02)	0.8 (0.3)	0.13 (0.04)
20 YR	0-15	0.8 (0.1)	39.1 (2.0)	2.0 (0.3)	0.54 (0.12)	0.06 (0.01)	0.5 (0.1)	0.05 (0.01)
n=4	15-30	1.1 (0.0)	20.4 (5.3)	1.4 (0.3)	0.98 (0.16)	0.05 (0.01)	0.2 (0.0)	0.03 (0.00)
	30-45	1.2 (0.0)	10.6 (3.2)	0.6 (0.1)	1.84 (0.03)	0.04 (0.00)	0.2 (0.1)	0.03 (0.01)
	45-60	1.4 (0.1)	5.4 (0.7)	0.4 (0.0)	2.19 (0.06)	0.05 (0.01)	0.3 (0.1)	0.05 (0.02)
	0-60	---	75.5 (6.0)	4.3 (0.3)	5.55 (0.24)	0.2 (0.01)	1.2 (0.3)	0.16 (0.03)
30 YR	0-15	0.7 (0.0)	48.3 (6.0)	2.1 (0.2)	4.13 (2.57)	0.06 (0.01)	0.3 (0.0)	0.05 (0.01)
n=4	15-30	1.0 (0.0)	17.0 (2.3)	0.8 (0.1)	3.98 (2.46)	0.04 (0.01)	0.2 (0.0)	0.03 (0.00)
	30-45	1.3 (0.0)	9.9 (1.0)	0.4 (0.0)	12.43 (7.05)	0.04 (0.01)	0.2 (0.0)	0.02 (0.00)
	45-60	1.4 (0.1)	5.4 (1.0)	0.3 (0.0)	10.96 (5.08)	0.04 (0.01)	0.2 (0.0)	0.03 (0.01)
	0-60	---	80.7 (8.4)	3.6 (0.4)	31.5 (16.55)	0.17 (0.02)	0.9 (0.2)	0.13 (0.02)
45 YR	0-15	0.7 (0.1)	36.1 (3.6)	2.0 (0.2)	6.87 (2.07)	0.06 (0.01)	0.6 (0.1)	0.06 (0.01)
n=4	15-30	0.9 (0.0)	27.7 (3.5)	1.5 (0.1)	6.93 (1.41)	0.04 (0.00)	0.3 (0.1)	0.03 (0.01)
	30-45	1.2 (0.0)	13.4 (1.0)	0.6 (0.1)	14.04 (1.61)	0.04 (0.00)	0.2 (0.0)	0.03 (0.01)
	45-60	1.4 (0.0)	5.9 (1.0)	0.3 (0.1)	39.44 (23.94)	0.04 (0.00)	0.2 (0.0)	0.04 (0.01)
	0-60	---	83.1 (1.3)	4.1 (0.1)	67.29 (24.58)	0.18 (0.01)	1.3 (0.1)	0.16 (0.01)

-Continued on next page

Table 4-1 continued from previous page

One-way ANOVA p-value of macro-nutrients among age groups

Age group	Depth (cm)	BD (g/cm^3)	C (Mg/ha)	N (Mg/ha)	log(P) (Mg/ha)	K (Mg/ha)	Ca (Mg/ha)	Mg (Mg/ha)
10,20,30,45	0-15	0.98	0.13	0.82	*0.03	0.87	0.27	0.30
	15-30	*0.01	*0.03	0.17	*0.05	0.16	0.48	0.80
	30-45	0.29	*0.01	0.27	*0.03	0.98	0.74	0.90
	45-60	0.76	0.92	0.71	*0.02	0.72	0.43	0.80
	0-60	-	*0.02	0.36	-	0.46	0.39	0.73

Table 4-2. The pH and texture do not significantly differ across the four age treatments.

	Depth (cm)	**10 YR**	**20 YR**	**30 YR**	**45 YR**
pH	0-30	4.3 (0.0)	4.6 (0.1)	4.5 (0.1)	4.6 (0.1)
	30-60	4.9 (0.1)	5.0 (0.1)	5.0 (0.0)	4.9 (0.1)
% Sand	0-15	83.2 (3.28)	77.8 (4.73)	81.8 (2.60)	74.9 (5.65)
	15-30	85.4 (1.88)	78.4 (5.39)	81.6 (4.21)	77.9 (4.74)
	30-45	86.5 (2.24)	79.3 (4.28)	84.6 (3.26)	78.6 (6.54)
	45-60	88.7 (2.25)	83.4 (4.25)	87.1 (3.07)	79.2 (6.6)
% Clay	0-15	3.0 (0.33)	2.9 (0.06)	3.3 (0.27)	3.5 (0.56)
	15-30	3.1 (0.17)	3.7 (0.59)	2.8 (0.66)	4.8 (0.98)
	30-45	3.0 (0.33)	4.0 (0.56)	2.8 (0.57)	3.8 (1.02)
	45-60	3.0 (0.17)	3.2 (0.64)	2.8 (0.35)	4.2 (0.91)
		One-way ANOVA p-values among age groups (non-transformed)			
pH	0-30 cm	0.11			
	30-60 cm	0.71			
%Sand	0-30 cm	0.64			
	30-60 cm	0.75			
%Clay	0-30 cm	0.39			
	30-60 cm	0.53			

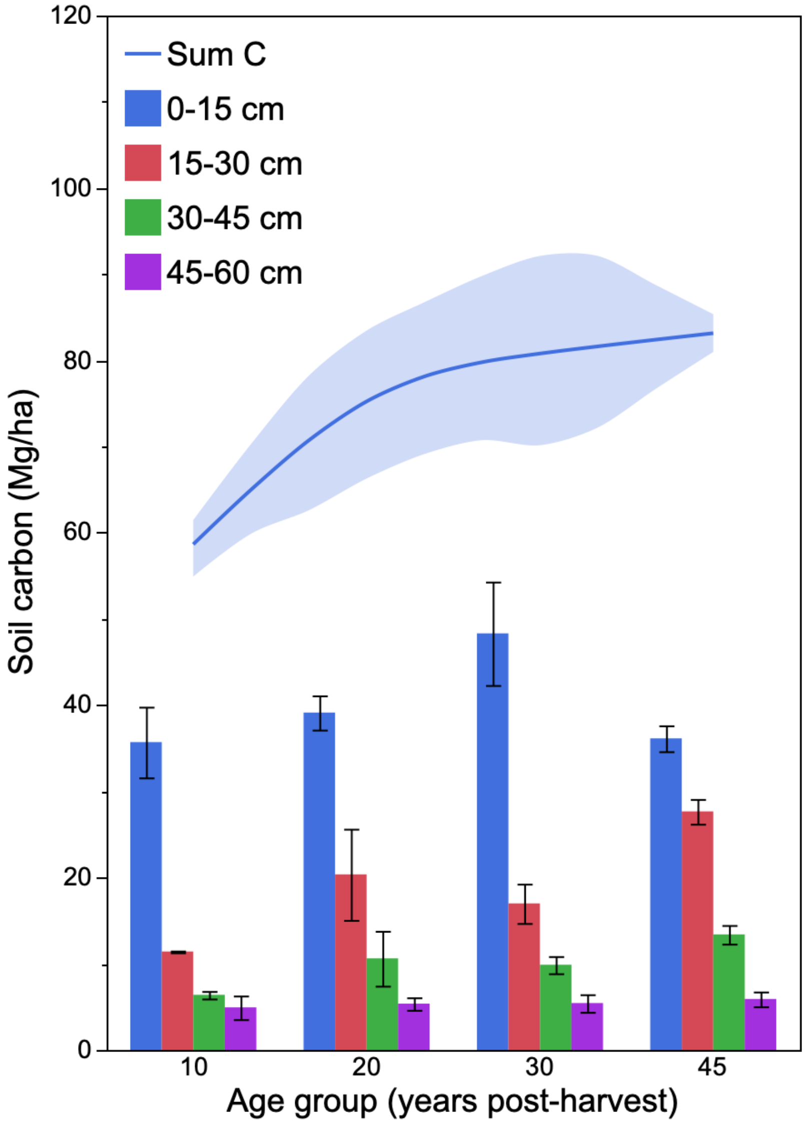


Figure 4-2. The summed depths of soil carbon (0 to 60 cm) is the smoothed line with a light blue confidence fit that increases across land-use types ($p = 0.02$). The soil C also increases at 15-30 cm and 30-45 cm ($p = 0.03$, $p = 0.01$ respectively). Bars represent the mean with standard error)

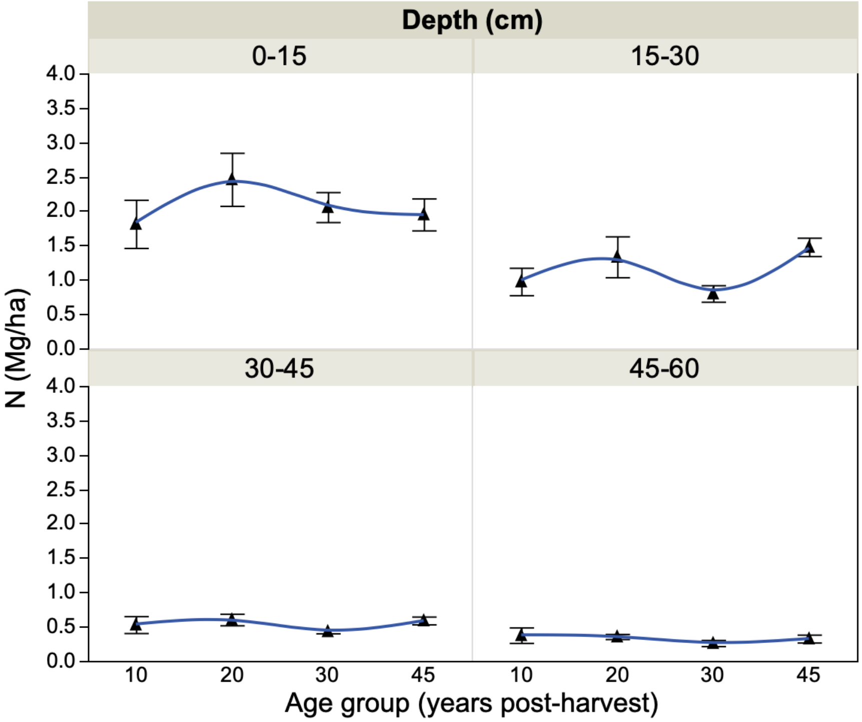


Figure 4-3. Average nitrogen with standard error bars for each depth.

4.5 Discussion

Changes in soil C from forest harvesting are influenced by several factors including land-use history, climate, topography, harvest intensity, and soil order (Nave et al. 2010; Don et al. 2011; Kolka et al. 2012; Wall 2012). Broad-scale analyses have observed most harvests as having no enduring effects on soil C in Spodosols and Alfisols, however these studies do not distinguish from old-growth conversions (first cut) or the number of previous harvests in plantations Nave et al. (2010); Wan et al. (2018). Many smaller-scale studies of aspen within the Great Lakes region or northern Midwest have also seen no significant change in soil C (Ruark and Bockheim 1988); Alban and Perala (1992); Bradford and Kastendick (2010); Premer et al. (2019). In contrast, our study found total soil C in aspen stands increased with age in the top 45 cm. Of the data collected in this study, most of this change comes from comparing the younger stands (10-yrs post-harvest) to the 20-yr stands. The increases we saw in the first twenty to twenty-five years may be part of the recuperation of soil C after harvest disturbances.

We found that most soil nutrients (N, Ca, K, Mg) did not change with stand age and that our sites' soil nutrient contents were relatively low compared to other aspen forests in the northern Midwest (Ruark and Bockheim 1988; Wang et al. 1995); Premer et al. (2019). Stabilized soil nutrient levels with aging aspen have also been observed by Wang et al. (1995) where they find nutrients continue to accumulate in tree stems and foliage with age. In our study, extractable phosphorus increased exponentially with stand age and also increased with depth. This is similar to Ruark and Bockheim (1988) which was also in sandy textured soils which found P to gradually increase eight years after harvest. Since phosphorus appears more abundant with depth, this could be from P leaching down the profile with time Jobbágy and Jackson (2001). Lower levels of P at the surface could also be due to plant uptake while higher P in older stands could be due to higher inputs of organic matter including wood debris, understory vegetation, micro-, mesofauna, and microflora. While the nutrient and carbon status of these stands appear to be improving or at least stable under the history of stem-only harvests and 40-50-yr rotations, it is not likely to stay this way if significant residues are removed for bioenergy.

Harvest disturbances can impact soils differently and for this reason guidelines caution against whole-tree harvest and heavy removal of slash on coarse textured soils or soils with low nutrient fertility. If we assume pre-harvest conditions in this study were similar to our 45-yr stands, then we could expect at least 42% of the soil C to be lost with harvest before it recuperated. Fine-textured soils that are generally fertile, such as Alfisols, have not shown the same extent of soil C losses (0-30 cm) after a harvest. However, these soils are still susceptible to decreases in nutrients and post-harvest productivity as seen with intensive whole-tree harvest Klockow et al. (2013). Aspen stands on fine-textured soils did not change in soil C ~ 10 years after harvest at 0-50 cm by Alban and Perala (1992) and at 10-30 cm by Tang et al. (2009). Aspen forests on fine-textured Luvisols and Brunisols in the boreal region were found to have the greatest nutrient stocks in mineral soil. Nutrients were increasing in overstory trees with age, suggesting a changing distribution of nutrients from the soil to the trees with age Wang et al. (1995).

In this light, harvesting slash from aspen sites in addition to merchantable boles could have more detrimental effects on soil C and nutrients. Aspen can be efficient in storing nutrients in perennial tissues. Before leaf senescence, nutrients are translocated from leaves back to perennial branches and twigs. This stresses the importance of slash retention on soils that have a lower nutrient stock in the soil (Pastor and Bockheim, 1984). While harvesting aspen from fine-textured soils in the winter can prevent compaction, coarse-textured soils may benefit from harvesting once leaves are fully leafed-out to maximize nutrient recycling and for leaves to be left on-site. Coarse-textured soils are generally less productive than fine-textured soils and therefore store less soil C (Vogt 1995). Removing slash for bioenergy may hasten drops in productivity over the long-term (Klockow et al. 2013) so stem-only harvests should be preferred over whole-tree harvest or 20% slash retention.

Despite soil C increasing in this study, these sandy soils retained most of their soil C in the upper 30 cm, making them more susceptible to accelerated losses if disturbance were to increase with harvest or if rotation lengths were shortened. Increases in biomass removal could lead to lower productivity, decreases in soil moisture, and over time affect soil structure and resilience to recuperate soil C and nutrients. In addition to soil C, we showed macro-nutrients were relatively balanced over the chronosequence, however, biomass removal of branches or biomass high in nutrients could lead to downward trends. For intensive biofuel management these coarse-textured soils may not be ideal, especially for long-term management (Curzon et al. 2020), however, if biofuel management extracts the same type and amount of biomass that is currently removed for pulp in addition to leaving stands on 40-yr cycles, then management would appear to be equally sustainable with current harvesting practices.

We originally hypothesized that we would see no significant changes in soil C based on literature values of both sandy and fine-textured soils, however, we found a significant increase in soil C, not supporting our hypothesis. This may be explained by the paucity of the data for soil C change on coarse-textured soil in the literature. Our sandy soils may be displaying larger changes than fine-textured soils because it has a higher potential to gain soil C. Although the increase in soil C may seem to assure sustainability, these sandy soils are more vulnerable to soil C loss after a harvest, and the changes we see are likely a recuperation of what was originally lost with initial land-use disturbance.

4.6 Conclusion

Naturally regenerated *Populus tremuloides* stands on 40-55 year rotations increased in soil carbon ($p = 0.02$) over time. On average, mineral soil C in the age group of 40-56year-old stands (0-60 cm) was 42% greater compared to 10-year old stands. Soil nutrients, pH, and soil texture were similar among age groups and showed no significant change except for increases in phosphorus. While the stands in this study appear to gain soil C with age, stands on sandy-textured soils are more sensitive to harvest residue removal. Caution is required if stands used for biofuel are located on course textured soils as the short-term effects we see may not necessarily translate to longer-term patterns.

5 Soil C sustainability relative to time.

The overarching goals of this research were to understand how potential biofuel feedstocks may impact soil C and nutrients and to understand if these impacts were sustainable. We fulfilled the objectives of measuring soil carbon and nutrients in each ecosystem, in three different countries. We also set out initially to provide perspective on whether current forest management was the best scenario for soil sustainability compared to other local land-uses, and this goal was partially fulfilled.

In Northeastern Wisconsin, we measured aspen stands at different ages since their last clear-cut. The soil of Oneida county is sandier than most other parts of Wisconsin (soils map of Wisc.), however could be relatively similar to other areas in the Great Lake Region. From 10-yr old aspen stands to 56-yr old aspen stands, soil C increased (0-60 cm; $p = 0.02$). The nutrients appeared to be stable with the increasing age of stands. While current management appears to be sustainable, these soils are likely more susceptible to losses of soil C and nutrients than fine-textured soils after a harvest on account of their texture (Curzon et al. 2020). In terms of forest management, these coarse-textured soils are less likely to be susceptible to compaction. If no management changes occur on-site for the potential biofuel feedstocks, then this could feasibly be sustainable for soil. Some consideration of the land's history should also be considered whether it be changes from different land-use or how many previous harvests of aspen have occurred.

In Tabasco, Mexico, oil palm was measured in a drier region mainly on one type of soil near a local river. Soil C was not found to be different than other land-use alternatives such as secondary forest and pasture. It is not clear if this soil was at maximum capacity for this local climate or if it was a coincidence that all four land-uses were not statistically different from one another. Although there were declining trends comparing secondary forests and pasture to oil palm stands, these declines were not statistically significant. If oil palm expands in the landscape, replacing pastures, it is unclear how different soil types such as Gleysols would be impacted by the potential feedstock. In this study, we only looked at small plantations ~ 4-20 hectares and there was a diversity of management practices outside of the measured study sites. It is unclear whether oil palm expansion would lead to the adoption of industrial practices such as digging large drainage ditches, applying heavier amounts of herbicides and fertilizers. In the same regard, more time and resource-consuming practices such as intercropping with N-fixing species, and returning fruit residues from the mill to the plantation to increase soil organic matter and nutrients could be phased out. Since fruit productivity decreases after ~20 years, most farmers have to decide whether to clear the land to replant new oil palm, leave fallow, or clear the land for different use.

Lastly, in Argentina, soil from multiple rotations of *E. grandis* was measured for soil C quantity and quality. While a single rotation showed no changes in soil C, multiple rotations exhibited slight declines ($p = 0.12$). The soil quality of density separated fractions showed both surface (0-15 cm) and deep (45-60 cm) decline of persistent carbon as opposed to fast-cycling soil C that increased after the second rotation. This suggested that soil C was not sustainable in this ecosystem. Nutrients trended down across rotations however declines were not significant.

Soil C changes over time in most ecosystems. Soil C is generally lost from changing native vegetation to secondary types of land-uses such as pastures or agriculture. In considering the climates of each of these three countries, the tropical and subtropical climates have faster decomposition rates and can recuperate soil C quicker than what we see in temperate dry regions in the Northern U.S. that have shorter growing seasons. While this seems like it would be an advantage in the tropics, these regions also tend to have more weathered soils, which may weaken their ability to hold onto soil C and nutrients. The changes we see in soil C are relative not only to the climate but also to the tree species. Tree residue inputs from eucalyptus created a thick forest floor of twigs, bark, and leaves, choking out most understory vegetation. While C stocks in the forest floor may be abundant, a N-limited environment with chemically recalcitrant residues can delay decomposition and carbon storage in the soil. In contrast, oil palm plantations retain a considerable amount of understory vegetation with a larger spacing density and the soil C stability is likely to come from fine roots rather than oil palm fronds and residues (Rüegg et al. 2019).

While disturbances from land-use changes, climate, and organic matter inputs can slow down soil C accrual, time can counterbalance this. Most forests and pastures accrue carbon with time or reach a dynamic equilibrium. Because soil C changes slower than many other parameters we measure, and because there are so many factors that influence soil C, it can be challenging to decipher what part of the ultimate pattern a single rotation is representing. In this project, we believe we measured stands from a single rotation chronosequence of both oil palm and aspen. In addition to the single rotation we measured in eucalyptus, we were able to measure multiple rotations which gave us insight into a different pattern. The sustainability of biofuel management will depend on whether rotation lengths are shortened and whether residues taken from the site are more than current management. Likewise, sustainability will also depend on the time-frame. The changes in soil C seen from a single rotation do not necessarily reflect the patterns we see in the long-term.

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A Supplementary Information

A.1 Chapter 2 Entre Rios, Argentina

Table A-1. Individual site soil C and Nutrients

Rotation-years post harvest-ID	**Depth (cm)**	**%C**	**%N**	**P (mg/kg)**	**K (mg/kg)**	**Ca (mg/kg)**	**Mg (mg/kg)**	**Al (mg/kg)**
1R-11-N	0-15	2.470	0.173	1.9405	12.840	139.90	28.96	0.614
	15-30	1.264	0.098	1.4383	5.458	123.20	17.51	0.465
	30-45	0.835	0.066	0.9532	9.252	215.30	28.64	0.121
	45-60	0.728	0.056	0.9787	11.580	277.90	34.02	0.106
1R-12-F	0-15	1.286	0.106	1.6341	5.649	95.39	16.32	2.257
	15-30	0.964	0.078	1.7532	7.854	97.45	18.44	1.928
	30-45	0.797	0.066	0.8255	8.963	206.80	24.71	1.182
	45-60	0.614	0.049	0.7915	11.500	278.70	32.38	0.936
1R-12-L	0-15	2.097	0.155	2.1109	8.661	242.80	31.86	0.737
	15-30	1.619	0.104	1.6122	7.302	317.80	27.04	0.242
	30-45	1.307	0.083	3.8282	11.120	391.80	36.36	0.184
	45-60	0.802	0.057	0.8061	15.350	469.90	44.73	0.153
2R-2-Lb	0-15	1.474	0.115	30.7479	8.606	84.80	20.50	0.260
	15-30	1.316	0.102	1.6428	5.956	103.70	25.60	0.314
	30-45	0.890	0.066	1.0816	7.331	203.00	31.29	0.344
	45-60	0.722	0.054	0.7347	11.890	278.30	45.67	0.246
2R-2-Lg	0-15	1.372	0.113	2.1675	6.130	143.40	11.49	1.076
	15-30	1.068	0.078	1.6607	5.488	230.10	14.54	4.464
	30-45	0.769	0.062	1.0625	9.669	272.80	21.73	1.500
	45-60	0.501	0.044	0.4196	13.940	368.20	30.78	0.820
2R-2-C	0-15	1.446	0.116	15.2325	7.895	73.56	10.89	3.165
	15-30	0.934	0.080	3.8203	5.376	58.80	11.14	0.486
	30-45	0.690	0.061	1.7970	6.665	108.90	12.78	0.287
	45-60	0.480	0.043	1.5026	15.180	257.70	29.39	0.304
2R-6-L	0-15	1.800	0.136	3.2414	6.533	149.90	25.40	1.325
	15-30	1.576	0.114	1.8071	7.395	214.00	24.33	0.672
	30-45	1.206	0.087	1.3503	18.480	434.60	50.30	0.184
	45-60	0.821	0.060	1.2894	9.626	367.90	32.26	0.193
2R-8-L	0-15	1.708	0.127	1.7564	7.249	133.10	21.00	3.423
	15-30	1.327	0.094	1.5939	5.644	192.80	19.47	8.420
	30-45	0.780	0.059	1.6345	13.010	347.10	36.71	3.119
	45-60	0.635	0.050	1.2487	14.630	397.20	41.92	2.074

2R-8-C	0-15	1.722	0.157	1.9017	5.915	53.82	6.73	4.099
	15-30	0.753	0.064	1.7500	4.707	39.77	9.24	1.067
	30-45	0.681	0.054	0.5089	4.295	59.01	10.29	0.583
	45-60	0.616	0.049	0.5803	6.120	130.60	16.10	0.393
2R-11-A	0-15	1.608	0.107	1.9797	6.429	194.90	25.85	1.024
	15-30	1.378	0.098	1.6955	7.999	291.90	30.54	10.480
	30-45	0.797	0.060	1.4924	8.947	342.30	30.32	3.872
	45-60	0.619	0.045	1.3503	9.616	361.50	32.23	2.803
2R-12-A	0-15	1.031	0.073	1.7617	6.788	69.34	9.82	13.100
	15-30	0.768	0.064	1.6851	4.100	42.25	13.41	2.520
	30-45	0.696	0.062	0.8170	4.589	98.05	16.01	3.450
	45-60	0.662	0.053	0.4255	7.030	166.70	21.96	1.537
3R-10-F	0-15	1.777	0.128	1.8680	6.865	117.40	19.24	1.191
	15-30	1.540	0.105	1.4518	7.368	197.70	26.68	16.000
	30-45	1.093	0.085	1.2386	10.830	336.40	32.82	5.672
	45-60	0.754	0.053	0.5990	13.990	393.70	37.21	2.027
3R-11-F	0-15	1.382	0.102	1.5635	6.328	125.90	18.00	0.242
	15-30	1.011	0.081	1.5533	4.867	58.00	12.90	11.970
	30-45	0.889	0.068	1.0355	5.599	131.10	18.28	1.820
	45-60	0.659	0.052	0.8122	10.660	293.50	31.03	0.551
3R-11-H	0-15	1.697	0.111	15.4425	7.040	195.70	23.76	0.219
	15-30	1.103	0.079	3.8203	6.593	131.00	21.44	2.359
	30-45	0.901	0.063	1.3807	9.086	261.50	33.42	4.077
	45-60	0.740	0.053	0.7411	12.530	362.20	44.76	3.995
4R-12-H	0-15	1.177	0.079	1.9017	6.267	159.90	11.55	0.133
	15-30	0.608	0.052	1.3214	7.194	53.29	9.25	10.250
	30-45	0.642	0.052	0.7321	6.637	69.57	12.44	3.713
	45-60	0.594	0.051	0.4464	9.577	152.70	20.60	2.268
4R-13-H	0-15	1.807	0.122	2.2091	6.464	170.80	18.65	0.503
	15-30	1.123	0.088	1.1878	5.637	96.96	19.12	2.926
	30-45	0.883	0.069	0.8528	6.442	164.00	23.72	17.030
	45-60	0.559	0.042	1.3097	10.790	277.90	38.94	0.142
4R-14-H	0-15	1.580	0.107	1.9405	4.593	111.10	17.84	0.614
	15-30	0.993	0.083	1.4383	9.267	361.30	65.29	0.465
	30-45	0.747	0.060	0.9532	3.143	44.26	11.68	0.121
	45-60	0.710	0.046	0.9787	8.779	405.50	70.09	0.106

A.2 Chapter 3 Tabasco, Mexico

Table A-2. Soil taxonomy and profile of sites

Site	Soil Tax.	Profile Description
Y. Palm (Gu)	Luvisol;	-Luvisol P4-6 (S); P7-9 (3.3) upland Horizon A: 0-20cm; pH 4.5 Horizon A_2 20-40cm; pH 4.0
	Gleyic Cambisol	Gleyic Cambisol; lower area, compact; Pts 1-3 (4.5m) Horizon A: 0-40 cm; pH 5.5 Horizon B: 40-90 cm Horizon Bt: 90 cm Gley Horizon; freckled black spots.
Y. Palm (Oc)	Luvisol	Dominant Grass sp. Paspanum (native) Horizon A: 0-25 cm loam granular structure; root zone Horizon AB: 25-35 cm transition from light brown to orange Horizon B: 35-80 cm yellow-orange clay w/ red mottle; blocky structure Horizon B_2: 80 cm… Red mottles
A. Palm (G9)	Luvisol (Gleyic)	Horizon A: 0-15 cm Horizon AB: 15-30 cm transition from light brown to darker brow Horizon Bt: 30-90 cm Horizon Bg: 90 cm
A. Palm (MM)	Luvisol	Horizon A 0-30 cm dark brown granular structure Horizon AB 30-45 cm dark brown with grayish hue-transition to heavier clay Horizon Bt 45 to >60 cm Yellowish orange with red mottles
A. Palm (Ed)	Luvisol (Gleyic)	Horizon A: 0-15 cm clayey loam; Horizon: A_2:15-45 cm subangular blocky structure; lighter hue Horizon Bt: 45-100 cm Yellow clay Horizon Bg: 100 cm
Pasture (Pr)	Luvisol	Horizon A: 0-25 cm Horizon AB: 25-40 cm; transition A to B; roots present down to 37 cm Horizon Bt: 40-80 cm; water table at 45 cm Bg: 80-100 cm. strong gley
Pasture (Ed)	Gleyisol	Horizon A: 0-15 cm; granular structure; roots Horizon AB: 15-40 cm Horizon B: 40-60 cm yellow clay with sections hues of gley Horizon Bg: starts at 60 cm
Pasture	Luvisol	Gleyic Black specs at 45-60 cm (possibly manganese)
Forest (Ro)	Luvisol	Horizon A: 0-25 cm dark brown; roots Horizon A_2: 25-45 cm dark brown; granular structure Horizon Bt: 45 to >60 yellowish clay; gray tongues and orange hues
Forest (Sa)	Luvisol	Horizon A: 0-30 cm Horizon AB: 30-60 cm transition from brown to yellow brown Steep slope; samples taken at upper, mid, and lower slope positions
Forest (FF)	Fluvisol	Horizon A 0-30 cm dark horizon AB 30-45 cm transition zone Horizon B 45-60 cm yellowish brown

Table A-3. Nutrient and metal values (Mg ha-1) of individual stands.

Stand (ID)	Starting Depth in cm	BD (g/cm^3)	C	N	Ca	K	Mg	P	Al	Fe
Y. Palm (ff)	0	0.87	36.21	3.46	3.33	0.12	0.48	0.56	0.0009	2.52E-05
	15	1.11	29.93	3.07	4.12	0.10	0.54	0.68	0.0008	0
	30	1.37	19.15	2.07	4.44	0.10	0.56	0.75	0.0009	0
	45	1.44	14.10	1.57	5.07	0.11	0.65	0.69	0.0009	0
Y. Palm (gu)	0	1.03	35.12	3.15	0.49	0.04	0.08	0.44	0.0451	2.82E-04
	15	1.14	26.66	2.47	0.41	0.04	0.06	0.36	0.0626	1.11E-04
	30	1.40	21.52	1.95	0.59	0.05	0.10	0.35	0.1181	1.05E-04
	45	1.40	15.10	1.58	0.63	0.06	0.11	0.18	0.3923	3.77E-04
Y. Palm (oc)	0	0.94	37.23	3.04	0.77	0.10	0.15	0.34	0.0566	7.28E-04
	15	1.18	36.33	2.73	0.79	0.07	0.14	0.33	0.0102	1.87E-03
	30	1.24	24.07	1.71	0.88	0.05	0.13	0.15	0.249	1.73E-03
	45	1.47	20.40	1.50	0.68	0.07	0.13	1.33	0.240	1.76E-04
A. Palm (Ed)	0	0.99	30.20	2.32	1.43	0.07	0.17	0.34	0.0401	1.91E-03
	15	1.05	21.73	1.67	1.45	0.07	0.21	0.26	0.0649	2.80E-03
	30	1.29	20.85	1.55	2.12	0.07	0.45	0.21	0.0511	1.07E-03
	45	1.35	18.62	1.36	3.02	0.09	0.78	0.11	0.0195	3.14E-04
A. Palm (G98)	0	1.10	25.39	2.17	2.27	0.07	0.35	0.51	4.00E-03	6.45E-04
	15	1.26	14.28	1.48	3.52	0.10	0.62	0.62	4.50E-03	1.62E-04
	30	1.37	8.53	0.99	4.61	0.10	0.74	0.70	1.86E-03	2.05E-05
	45	1.47	7.89	0.90	5.37	0.12	0.89	0.66	2.04E-03	0.00E+00
A Palm (MM)	0	0.95	54.19	3.86	0.78	0.05	0.14	0.32	0.0532	1.39E-03
	15	1.13	42.56	3.14	0.54	0.04	0.08	0.30	0.0759	1.62E-03
	30	1.39	25.64	1.76	0.74	0.05	0.14	0.23	0.115	2.31E-03
	45	1.53	15.62	1.35	0.78	0.06	0.27	0.15	0.246	2.12E-03

(Table continued on next page)

(Table A3 continued from previous page)

Site	Starting Depth	BD	C	N	Ca	K	Mg	P	Al	Fe
Pasture	0	0.99	49.95	3.95	0.94	0.22	0.18	0.28	6.33E-02	1.01E-03
(Ed)	15	1.12	36.00	2.61	1.05	0.17	0.18	0.26	1.65E-01	1.81E-03
	30	1.33	29.63	2.10	1.25	0.11	0.28	0.22	2.79E-01	1.58E-03
	45	1.47	23.46	1.85	1.46	0.13	0.41	0.15	3.58E-01	1.60E-03
Pasture	0	0.83	45.07	3.56	0.87	0.07	0.08	0.26	7.37E-02	1.81E-03
(Pr)	15	1.03	36.28	2.70	1.11	0.05	0.07	0.23	1.39E-01	2.31E-03
	30	1.29	26.86	1.98	1.29	0.06	0.11	0.15	2.18E-01	2.51E-03
	45	1.16	17.72	1.38	1.02	0.05	0.13	0.08	2.41E-01	2.08E-03
Pasture	0	1.15	27.65	3.01	3.49	0.15	0.74	0.59	9.18E-03	4.82E-04
(G98)	15	1.28	19.49	2.28	3.73	0.16	0.85	0.65	1.02E-02	3.26E-04
	30	1.47	11.49	1.35	5.94	0.22	1.54	0.43	4.79E-03	0
	45	1.56	9.73	1.17	6.80	0.25	1.76	0.52	2.99E-03	0
Forest Rep	0	0.91	32.42	3.11	0.67	0.09	0.13	0.26	1.21E-01	3.05E-03
(Sa)	15	1.18	28.70	2.95	0.85	0.10	0.13	0.28	2.80E-01	3.57E-03
	30	1.19	22.40	2.17	1.33	0.10	0.14	0.18	4.84E-01	3.56E-03
	45	1.37	19.85	2.02	1.35	0.11	0.13	0.12	6.71E-01	2.97E-03
Forest Rep	0	0.74	28.34	2.90	7.04	0.19	0.80	0.40	1.02E-03	3.31E-05
(FF)	15	0.91	31.75	3.25	9.49	0.20	0.91	0.51	1.50E-03	0
	30	1.20	28.10	2.75	13.79	0.28	1.26	0.77	2.53E-03	3.84E-03
	45	1.24	25.78	2.08	14.11	0.27	1.11	0.06	2.63E-03	1.11E-04
Forest Rep	0	0.72	46.32	3.44	1.21	0.09	0.24	0.29	1.93E-02	1.73E-03
(Ro)	15	0.92	40.83	3.01	0.93	0.07	0.18	0.19	1.01E-01	4.91E-03
	30	1.20	26.44	1.93	0.56	0.06	0.17	0.27	3.34E-01	4.79E-03
	45	1.52	18.00	1.44	0.63	0.11	0.40	0.45	5.19E-01	1.59E-03

A.3 Chapter 4 Wisconsin, USA

Table A-4. Top: the forest floor C (Mg/ha) with standard error in parenthesis based on 50% mass. Bottom: C:N ratios for each depth across rotations with standard error in parenthesis

	10-yr	**20-yr**	**30-yr**	**45-yr**
Forest Fl.	1.62 (0.09)	0.96 (0.22)	1.27 (0.38)	1.10 (0.50)
C:N				
0-15 cm	20.36 (1.96)	20.75 (1.89)	23.47 (1.60)	20.20 (0.98)
15-30 cm	12.59 (2.18)	14.92 (1.32)	21.49 (1.32)	20.75 (1.09)
30-45 cm	13.19 (2.73)	17.20 (3.41)	23.06 (2.65)	21.54 (1.23)
45-60 cm	15.16 (5.24)	15.09 (1.54)	20.53 (1.15)	17.26 (1.42)

Table A-5. Nutrient Index based on biomass guidelines and soil series based on NRCS Web Survey

Site ID	**Age (Year)**	**Nutrient Index**	**Soil Series**	**Slope**
CtyY	11	Poor	Sayner, Vilas	6-15%
SHP	10	Moderate	Padus Pence	0-6%; 6-15%
JRRD	11	Poor	Rubicon	0-6%
CtyD	17	Poor	Siskiwit-Vilas	0-6%
Spruce	21	Moderate	Pence	0-6%
TC23	23	Moderate	Padus Pence	0-6%
FWN	20	Poor	Keweenaw Vilas	0-6%
POSK	32	Moderate	Padus Pence	0-6%
SPDR	32	Poor	Padus	0-6%
WDLK	33	Poor	Rubicon	0-6%
GRSE	33	Moderate	Padus Pence	0-6%
RAIN	44	Poor	Padus Pence	0-6%
TC40	40	Moderate	Padus Pence	0-6%
TC43	43	Moderate	Padus Pence	0-6%
TC56	56	Moderate	Keweenaw	15-25%

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Figure 2-1: Argentina, Concordia region was annotated and exported from Google Earth Pro and is used according to the policies outlined in their guidelines for non-commercial use, available at: https://www.google.com/permissions/geoguidelines/

Figure 4-1: Wisconsin inset map was modified from the original source: Soils of Wisconsin compiled by F.D. Hole, 1973; Wisconsin Geological and Natural History Survey Map.