

**THE EFFECT OF LAND CONVERSION FROM FOREST TO AGRICULTURE ON SOIL HEALTH  
INDICATORS IN RAINY RIVER, ONTARIO**

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## Abstract

Due to changing climate and shifting crop zones, there is an opportunity for agriculture to expand in northern regions. This will require conversion of forest and scrubland to agriculture. Conventional methods of land conversion from forest to agriculture have resulted in a decrease in soil organic matter (SOM), which contributes to the maintenance of soil structure, water holding capacity, and a diverse microbial community within the soil. This degradation to the soil may result in a decline in soil health. Integrating wood mulch, which is woody material that would otherwise be removed from the site, might help reduce the degradation of soil health by retaining more SOM on site.

The objectives of this study are to: 1. examine the effects land conversion has on soil health by measuring indicators identified as responsive to land management practices, and 2. determine if the integration of wood mulch during land conversion can mitigate any declines in soil health. Methods for measuring the soil health indicator 'wet aggregate stability' were also explored in terms of sensitivity, cost, training, and time to complete the analysis. In June 2024 agricultural fields (n=27) and forests (n=9) were sampled in Rainy River, ON, to investigate the effect of land conversion on soil health. Eighteen recently (<10 yr) converted fields (9 conventional and 9 integrated with wood mulch) and nine established fields (>50 yr; cleared conventionally) were sampled for this study.

Soil health declined with land conversion, but the clearing approaches did not differ significantly from each other. Land conversion, regardless of timing of conversion, significantly reduced wet aggregate stability, carbon mineralization rate and soil moisture in 27 sampled agricultural fields compared to 9 referenced forest sites. Quantitatively, automated wet sieving was found to have the lowest coefficient of variation at 11.2%. This approach for quantifying wet aggregate stability did not show any significant effect of land conversion on aggregate stability. Volumetric aggregate stability tests (VAST) had a coefficient of variation of 17.5% and did show significant effect of land conversion. The SLAKES mobile application had a coefficient of variation of 15.4% and showed a significant effect of land conversion. Post-hoc test results differed for VAST and SLAKES. Qualitatively SLAKES was the most economical method with low training and time requirements. VAST was more expensive than SLAKES and required a similar amount of training as SLAKES but data collection was faster. Automated wet sieving was the most expensive and required more training and time to produce results.

SLAKES is recommended for future wet aggregate stability measurements of agricultural soils due to its high sensitivity to changes in treatments of agricultural soils, affordability, speed in results, and low training requirements. Land conversion degraded soil health and the only difference between approaches for conversion was for potential carbon mineralization, which was lower in the mulched soils. Based on the

findings of this study, land conversion will degrade soil health regardless of the tested approach taken to mitigate negative effects.

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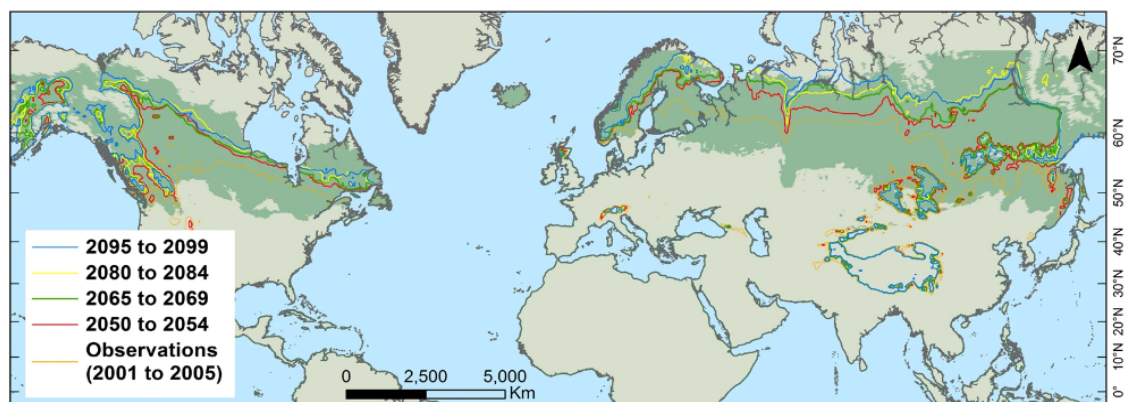
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## Introduction

The planet is warming at an alarming rate (Florides & Christodoulides, 2009), which presents challenges and opportunities for agriculture (Unc et al., 2021). The effects of climate change are already negatively affecting the yield of crops like wheat (Asseng, 2016), rice (expected to decrease by 5%-13.5% by 2060; Chen et al., 2020), and increased temperatures have been shown to increase water stress on canola (Quaderi et al., 2014). This is only a small set of examples of crops affected by climate change. At the same time, global warming is contributing to a northward shift in the world's climate zones, creating opportunities for agricultural expansion (Figure 1; Zabel et al., 2014; King et al., 2018). By 2099 the world's climate zones will shift northward by approximately four to six hundred kilometres; meaning that roughly 76% of the boreal forest will be farmable based on growing degree days (GDD) (King et al., 2018). In most systems, before the land can be used for production, the area must be cleared. In the case of the boreal forest, this involves the removal of woody biomass.



Projected advances of the  $GDD_5 \geq 1200$  boundary. The green area describes the current extent of the boreal region<sup>66,67</sup>.  $GDD_5$  values were estimated by averaging the projections of seven  $CO_2$  emission based GCMs (Extended Data Table 1). Each time step describes a five-year average of all estimates by the seven GCMs. The variability in projection among models is presented in Fig. 4 and Table 1. Map created using ArcGIS Desktop v. 10.4.1<sup>65</sup>.

*Figure 1 : Projected crop zone changes from 2001 to 2099 (source: King et al, 2018)*

The land in the boreal forest is currently perceived as marginal at best, low-intensity, and inadequate to support the needs of local communities (Unc et al., 2021). This may be changing with the shift in the global climate. The shift in global crop zones (Figure 1), shows that farmers can now plan their crops with changing crop zones in mind. Climate change driven agricultural frontiers (CCDAFs) reflect the potential for agricultural expansion in a changing climate (Hannah et al., 2020). It is yet unclear as to whether the CCDAFs will have an overall positive influence on economic development or ecosystem services (KC et al., 2020). Further study in this area is required to understand the full benefits that could be realized from the increase in food production. The increase in food production could have high potential for alleviating food insecurity in the north and could lead to a more stable future for those who live in northern communities.

Conventional land conversion practices in the USA involve removing the tree canopy, along with the top 15 cm of organic matter through a process called scarification (Cano et al., 2018). A similar process is commonly used in Ontario CA, first cutting and selling merchantable trees, followed by mechanically levelling the land as described in NOFIA, (2017). Removing the tree canopy and reducing organic matter inputs during conventional land conversion has been shown to reduce soil quality and soil health scores compared to that of pre-conversion conditions (Benalcazar et al., 2022). Soil organic matter (SOM) contributes to the maintenance of soil structure, water holding capacity, and a diverse microbial community (Wall et al., 2012; Cano et

al., 2018). Integrating shredded coarse woody debris (CWD), also known as wood mulch, into the soil during conversion could potentially mitigate the negative effects on soil health due to the loss of SOM during conventional land conversion. Wood mulch has been found to increase water conservation, help maintain soil temperature during temperature extremes and improve soil health when applied on top of soil (Kudinov, 1972; Einert et al., 1972; Fraedrich & Ham, 1982; Singh et al., 1991; Long et al., 2001; Chalker-Scott, 2007). Literature on integrated wood mulch into the soil as an additive is limited but shows evidence of increasing respiration and improving soil structure (Norlan, 2015; Barreiro et al., 2016).

Coarse woody debris can act as a nutrient storage releasing nutrients such as carbon, nitrogen, and phosphorus during decomposition (Mackensen & Bauhus, 2003). Bark and wood decompose rather slowly compared to leaves, needles and fine roots (Ganjugunte et al., 2004). This is due to several factors including but not necessarily limited to low nitrogen and phosphorus (Sinsabaugh et al., 1993), beta bonds between glucose and subunits in cellulose that are difficult to depolymerize (Wyman et al., 2005), large molecular weights (roughly 5.700-12.000 g/mol in hardwoods and 36.000-61.000 g/mol in softwoods)(Braten et al., 2003), high presence and aromaticity of lignins (Klotzbucher et al., 2011; Demuner et al., 2019; Villanova et al., 2023), and a large presence of tannins, causing them to provide a significant long-term release of organic matter into soil and nutrients (Ganjugunte et al., 2004). In boreal regions decomposition of CWD depends highly on the drainage and wood dominant species (Shorohova and Kapitsa, 2016). The timescale for complete decomposition of CWD in

boreal forests has been found to be between 40 and 100 years depending on vegetation zone, site conditions, tree species and size of CWD with less drained sites (higher moisture) reducing the decay rate of CWD (Shorohova and Kapitsa, 2016). The long decomposition time and nutrient storage shows a potential for CWD to be used as a long-term addition to the soil.

The need and opportunity due to climate change for conversion of forested land into agricultural fields and the effects conventional practices have on soil health, highlight the need to maintain the quality of the soil to insure productive and resilient agricultural systems. Assessing soil health requires a set of sensible and feasible soil health indicators (Karlen et al., 2013). There is no 'correct' set of soil health indicators, as seen by the differences in soil health indicators and methods used to measure soil health (Moebius-Clune, 2016; Fine et al., 2017; Bagnall et al., 2023). Bagnall et al., (2023) argue that wet aggregate stability, carbon mineralization rate, and soil organic carbon are the most sensitive indicators to use in measuring soil health.

Of the three indicators suggested in Bagnall et al., (2023), wet aggregate stability does not have a consistent means of measurement (Van Eerd et al., 2018). Automated wet sieving uses a size fraction of between 1mm and 2mm through a conservation of mass calculation (Kemper & Rosenau, 1986). Volumetric Aggregate Stability Testing (VAST) uses a size fraction of <2 mm and hand wet sieving method to compare the surface area dispersal of the aggregate through a manual wet sieving process. SLAKES, a mobile application developed at the University of Sydney, uses a size fraction between 4mm and 8mm to measure the wet aggregate stability based on the

change in surface area of wet aggregates over a ten-minute timespan (Fajardo et al., 2016). Automated sieving, VAST, and SLAKES have their own advantages and disadvantages, including varying accuracies with soil texture (Lado et al., 2004). Soil health indicator measurement should be both reliable, in terms of the results and variability between replicates within the method, and accessible (Hughes et al., 2023). Accessibility to soil health tests can be defined in terms of cost, time, and training required to perform the tests (Stott, 2019; Lehmann et al., 2020).

In this project, I ask three questions:

1. Does land conversion from forest to agriculture degrade soil health?
2. Does integrating wood mulch during land conversion result in better soil health outcomes than conventional clearing?
3. Do methods to measure wet aggregate stability differ in their sensitivity to detect changes from land conversion practices and their accessibility in terms of cost, time, complexity, and training required?

Chapter 1 will look into what effects, if any, land conversion has on soils converted from forest to agriculture over four treatments; F (reference forests), M (fields converted <10y to agriculture incorporating mulched coarse woody debris during conversion), C (fields <10y conventionally converted from forest), and CE (established >50y conventionally converted fields). Chapter 1 will also look at whether the addition of wood mulch resulted in better soil outcomes compared to

conventional clearing. Chapter 2 will investigate and compare quantitative and qualitative elements of three wet aggregate stability measurement methods.

## **Chapter 1: A Comparison of Land Conversion Approaches on Soil Health Indicators**

### **Literature review**

#### **Land Conversion**

Changes in climate are creating opportunities for agriculture to expand northward. The expansion will likely require the conversion of forest to agriculture use, which typically involves forest clearing. The increase in usable agricultural land could bring many opportunities, from economic to food security, that could help benefit many northern countries (Parry et al., 1999). However, land conversion also negatively affects the environment. With conventional conversion practices in some regions, topsoil, which is rich in soil organic matter, is often removed from the site (Cano et al., 2018), making it unavailable for use in future agricultural production. Methods aimed at restoring or conserving organic matter during conversion are needed to offset the losses seen due to land conversion (Benalcazar et al., 2022).

Clearing the canopy created by the living forest allows for more light to penetrate to the soil surface, thereby increasing the relative temperature and altering the moisture balance (Benalcazar et al., 2022). Changes in climate conditions such as increased temperature, sunlight, and altered soil moisture allow for conditions to become favorable for decomposition of organic matter; thus, causing the release of stored carbon to the atmosphere and the potential loss of nutrients (Houghton, 1995; Wei et al., 2014). Land conversion of forested areas into agricultural lands in Eastern Canada decreased soil carbon by 22% when compared to adjacent uncleared lands

(Angers et al., 1995). In boreal soils the loss is often larger (31%) where land conversion has occurred (Wei et al., 2014). Globally the collection of 119 peer-reviewed publications reviewed by Wei et al., (2014) showed a global trend in carbon loss due to land conversion for agriculture use. Clearly, there is a need for research to mitigate soil carbon loss when land is converted to agricultural production.

Coarse woody debris plays a significant role in a forest ecosystem's material flow, energy flow and nutrient cycling (Zhao et al., 2007). Coarse woody debris can act as a natural nutrient source through the slow release of nutrients such as carbon, nitrogen, and phosphorus during decomposition (Mackensen & Bauhus, 2003). The green needles and leaves from leftover slash (i.e., logging debris such as branches, bark, needles, and leaves that is sometimes referred to as logging residue (Cook, 2018)) generally decompose within 2 years of harvest (Girisha et al., 2003). Bark and wood decompose rather slowly compared to leaves, needles and fine roots (Ganjegunte et al., 2004). This is due to several factors including but not necessarily limited to low nitrogen and phosphorus (Sinsabaugh et al., 1993), beta bonds between glucose and subunits in cellulose that are difficult to depolymerize (Wyman et al., 2005), large molecular weights (roughly 5.700-12.000 g/mol in hardwoods and 36.000-61.000 g/mol in softwoods)(Braten et al., 2003), high presence and aromaticity of lignin (Klotzbucher et al., 2011; Demuner et al., 2019; Villanova et al., 2023), and a large presence of tannins, causing them to provide a significant long-term release of organic matter into soil and nutrients (Ganjegunte et al., 2004). The timescale for complete decomposition of CWD in boreal forests has been found to vary between 40 and 100



years depending on vegetation zone, site conditions, tree species and size of CWD, with poorly drained sites (higher moisture) reducing the decay rate of CWD (Shorohova & Kapitsa, 2016), likely due to lower available oxygen. During the decomposition of woody debris, there is a decline in the carbon and nitrogen stocks of this pool (Yang et al., 2010), and nutrients are released with decomposition. The decomposition of woody debris does, however, often lead to the immobilization of nitrogen in the soil (Zimmerman et al., 1995). This occurs because the ratio of carbon to nitrogen in the wood is large, and nitrogen is limiting. The immobilization of nitrogen, at least in the short term, could cause the microbes in the soil to outcompete the plants for nitrogen, resulting in a decline in crop yield. CWD can potentially be a source of nutrients and organic matter to the soil, though it is unclear if these effects are short lived, in terms of soil health.

Soil organic matter, in addition to maintaining soil structure, moisture holding capacity, and microbial community, acts as a storage bank for carbon and soil nutrients, making it an important biological indicator of soil health and soil fertility (Wall et al., 2012; Cano et al., 2018). Soil organic matter is often lost during land conversion either due to decomposition or mechanically during the removal of woody debris. If the soils are not replenished with nutrients (i.e., fertilizers) the loss of the natural forests can lead to degradation of the soil and its eventual inability to ensure sustainable agricultural production (Tolimir et al., 2020). During the land conversion process in Northwestern Ontario, some farmers have mulched the woody debris and stumps in their fields and incorporated the mulch into the soils. This is being done with the goal

of retaining organic matter on site and to help limit the carbon, nutrient, and total soil health loss over time. It may also be a way to bring land into crop production more quickly.

## Soil Health

In addition to acting as a potential long-term fertilizer, mixing a layer of wood mulch into the soil could potentially help improve soil health. One of the most used and accepted definitions of soil health is by Doran and Zeiss, (2000); “The capacity of soil to function as a vital living system to sustain biological productivity, promote environmental quality, and maintain plant and animal health.” Soil health is generally quantified by integrating measures of the chemical, physical and biological properties of the soil (Nunes et al., 2021). The number of variables that could be measured is vast, and a key consideration is selecting indicators that are responsive, and easy to measure i.e., accessible. Bagnall et al., (2023) has suggested that the minimum set of indicators to assess soil health are soil organic carbon (SOC), wet aggregate stability, and carbon mineralization potential.

While many chemical, physical and biological factors of soil health have been identified, a keystone indicator is SOC (Doran and Parkin, 1996; Stott, 2019; Nunes et al., 2021). High SOC levels in soil have been associated with higher soil health scores and soil fertility, thereby helping increase agricultural production of the soil (Otsuki, 2021). High SOC levels have also been shown to influence wet aggregate stability, and the pH of the soil. The response of SOC to treatment in short and mid-term timespans

are difficult to detect due to the natural variability of soil and often high background levels (Purakayastha et al., 2008; Duval et al., 2018). Permanganate oxidizable carbon (POXC; synonymous with the term 'active carbon') has been proposed to estimate the trajectory of the SOC as soon as 2 years after treatment (Culman et al., 2013; Hurisso et al., 2016).

Carbon mineralization potential is an indicator of microbial activity in the soil, in turn indicating the potential for nutrients being made available for plant uptake from release during decomposition (Reike et al., 2022). Higher rates of potential carbon mineralization could indicate a larger or more efficient microbial community, potentially indicating good soil health, but also larger greenhouse gas emissions of the soil, which result from the decomposition process.

Soil aggregates, also known as 'soil structural units', are formed when soil organic matter is broken down, first by megafauna (organisms >20mm such as earthworms, snails, and small rodents) that process larger plant matter, and then by the microbial community (Jouquet et al., 2006; Coleman et al., 2017; Guhra et al., 2022). The microbial community secretes organic compounds that bind soil particles together forming aggregates. The stability and arrangement of aggregates help govern the flow of gas and water through the soil (Finn et al., 2017). The soil structure created by the aggregate units influences the microbial community's ability to transform organic matter to available nutrients. It is both time consuming and difficult to measure all the structural units throughout the soil, so wet aggregate stability on a fraction of

aggregates is often measured as an indicator. Wet aggregate stability is a measure of the ability of macroaggregates to resist dispersion (Amezketta, 1999).

Soil pH can be a powerful indicator of soil health, soil fertility, and soil disease resistance in conventional agricultural soils (Rengal, 2011). Too high or too low a soil pH can limit nutrient availability or cause phytotoxicity, while high pH is often associated with lower incidence of soil borne diseases. The soil microbial community has been shown to require a soil pH of 5.5-8.8, with higher concentrations of microbial biomass C and N above a pH of 7 (Pietri & Brooks, 2003; Fierer & Jackson, 2006; Pietri & Brooks, 2008; Neina, 2019). Below a pH of 7 the fungal community has been shown to outcompete the bacterial component of the microbial community, largely due to their adaptations to acidic environments (Anderson, 2003; Neina, 2019).

Another physical factor affecting soil health is the soil's ability to hold water against the force of gravity, the water holding capacity of the soil (Krull et al., 2004; Bordoloi et al., 2018). The water holding capacity of the soil is essentially an indicator of how much moisture the soil can hold, which is important for ensuring that plants can access water for growth. Soil water holding capacity is largely governed by soil texture, which can be obtained from measuring the sand, silt, and clay contents of the soil (Ritchie et al., 2015), and the SOM content (Bordoloi et al., 2018), using the calculations described in Bagnell et al., (2022). Loamy and intermediate textured soils with high OM and good structure provide the most ideal moisture conditions over time for crop plants (macropores allowing rapid drainage after rain/melt events when the available water to plants is highest) (Parikh and James, 2012). Pure smectite clays have a very-

high water holding capacity but would not be a “healthy” soil. This is due to the swelling and dispersion of smectites during rain/melt events, significantly decreasing infiltration rates as measured in several methods such as single, double, or marionette ring infiltrometers (Lili et al., 2008)) without special irrigation practices (Ried-Soukup and Ulery, 2018). Soils with low water availability due to low water holding capacity and low infiltration rates have been shown to result in lower soil health (Cano et al., 2018).

The objectives of this study are to determine if indicators of soil health, and overall soil health score, is affected by land conversion. I hypothesize that land conversion will degrade soil health and that this effect will be lower for sites that incorporated mulch during conversion compared to the sites that were cleared conventionally.

## Materials and Methods

### Study Area

Between June 19-20, 2024, soil samples were collected from 36 agricultural fields and forest sites in Rainy River, Ontario, Canada (48°42'N-48°48'N, 94°14'W-94°41'W; Figure 2). Rainy River has a mean annual temperature of 4°C and a mean annual precipitation of 577.9 mm per year, with 97.1 mm falling on average in June (CustomWeather, 2024).

## Rainy River Sites

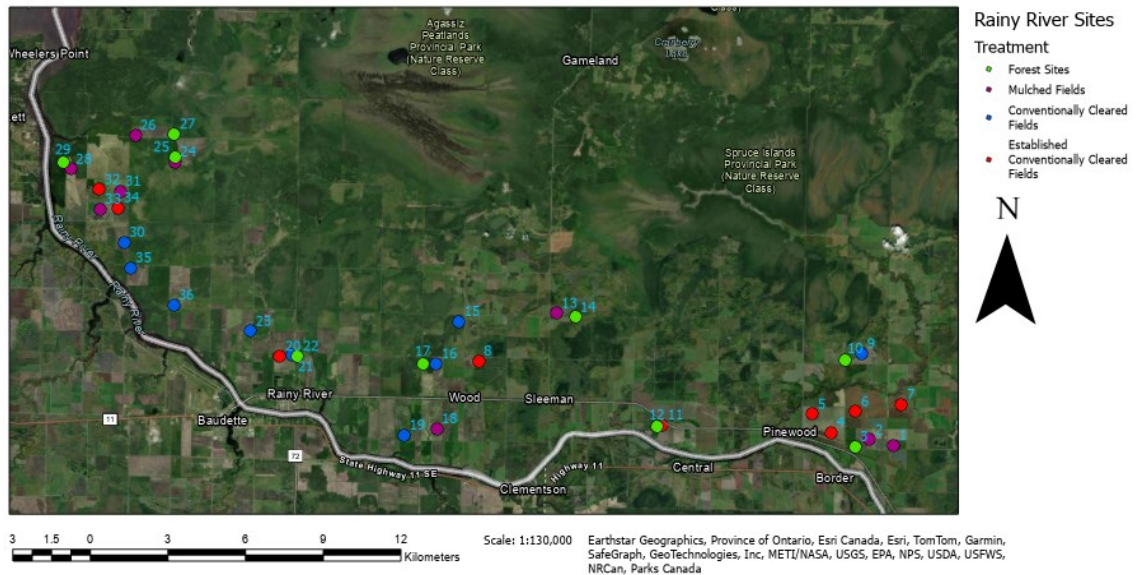


Figure 2 : Map of sites sampled in Rainy River, ON

Farmer participation was initiated through an email to area growers asking for participation in a study to better understand approaches for land conversion for agricultural expansion. All fields are owned by grain and oilseed producers and all are tile drained. Crop rotations include barley (*Hordeum vulgare*), canola (*Brassica napus*), soybean (*Glycine max*), and corn (*Zea mays*). Nine well established fields have been in agricultural production for over 50 years and were cleared conventionally (>50y CE), nine fields were cleared conventionally in the last ten years (<10y C), and nine fields were cleared and integrated with mulched coarse woody debris within the last ten years (<10y M). Nine mature mixed-wood forests (F) in the areas near the fields were sampled to establish pre-clear conditions. Brief site descriptions are available in Appendix 1. Sampling began one day following a weather event that took place June

18, 2024, that deposited an average of 76.2 mm, with extremes of 203.2 mm in some locations (NWS 2024).

## Sampling Method

A minimum of 20 soil cores were collected from the top 15 cm of mineral soil in two representative areas of each site using a 1.9cm diameter model LS Oakfield push probe (Oakfield Apparatus, Oakfield, WI, USA). Samples were collected by walking across a representative area of the field or forest in a “W” pattern and homogenized in a labelled Ziplock bag. Samples were stored in cooler bags and transported to Lakehead University where they were stored cool prior to drying and analysis.

## Soil Moisture

Immediately after compositing the soils from each site, a  $40 \text{ g} \pm .05 \text{ g}$  of each sample was weighed, recorded, and placed on a weighed and labelled tin pan. The pan for each site was then put in an oven at  $105^{\circ}\text{C}$  until the weight became stable ( $\sim 2 \text{ d}$ ). The tin of dried soil was then reweighed. Soil moisture (SM) was then calculated as:

$$= 100 * \left[ \frac{\text{Field Moist Sample Weight (g)}}{\text{Field Moist Sample Weight (g)} - \text{Oven Dry Sample Weight (g)}} \right]$$

## Preparing samples

Composites samples were air dried at room temperature ( $\sim 22^{\circ}\text{C}$ ). Periodically (every 2-3 d) samples were rotated until the soil was dry. Soils were initially passed through a 4 mm sieve with the remaining aggregates larger than 4 mm saved for the SLAKES wet aggregate stability approach. Soils were then passed through a 2 mm sieve,

with rocks and debris larger than 2 mm discarded. Unless stated otherwise, all analysis was conducted on air-dried, <2 mm soil.

## Texture

A subsample of <2 mm soil was sent to A&L Laboratories in London, ON, for soil texture determination using the hydrometer method (Gee and Bauder, 1986). The texture type was determined using the CASH soil texture triangle (Figure 3) classifying each sample as coarse, medium, or fine textured depending on the clay, silt, and sand ratio.

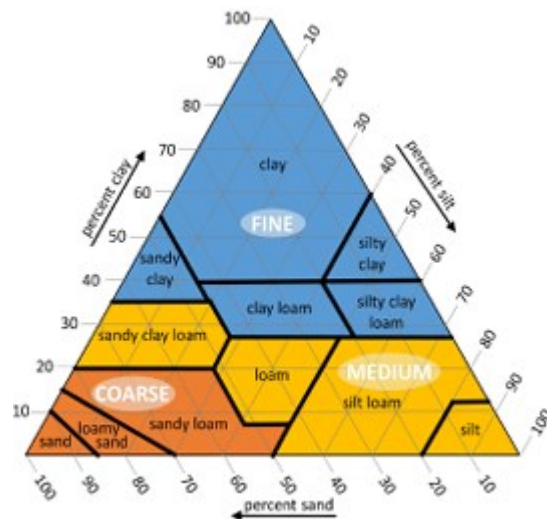


Figure 3 : Cornell assessment of soil health (CASH) soil texture triangle (Moebius-Clune et al., 2016)



## Biological Indicator: 24 h Potential Carbon Mineralization

Following Zibilske (2018), potential C mineralization was determined using a 24h incubation. Dried and sieved (<2mm) samples were weighed to  $40 \text{ g} \pm .05 \text{ g}$  and placed in 50 mL perforated plastic beakers with a fiberglass filter at the bottom to keep the sample from falling through the perforations. The beakers were placed in 250 mL mason jars with a septa in the lid to allow for headspace sampling. Twenty mL of distilled water was added to the bottom of each jar and the jar was sealed. A sample of the headspace was immediately collected in a labelled syringe and the time the sample was taken recorded. Blank samples (no soil added) were created and sampled after every 7<sup>th</sup> sample. Jars were placed in an incubator at 24°C for 24 h. After  $24 \text{ h} \pm 1 \text{ min}$  samples were removed from the incubator and a labelled syringe was used to collect a gas sample from the headspace. Samples were analyzed the same day they were collected using a SRI gas chromatograph with a FID (SRI 3103, SRI GC) to measure the concentrations of CO<sub>2</sub> in the headspace. Three gas standards (100 ppm, 1000 ppm, 10000 ppm) were used to create a calibration curve for each sampling date. The quantity of CO<sub>2</sub>-C produced was calculated using the difference in concentration between the initial concentration and the concentration after 24h. The rate of C mineralization was calculated by converting the concentration of CO<sub>2</sub> to mass units using the Ideal Gas Law and dividing it by the mass of the sample and the time.

## Biological Indicator: Permanganate Oxidizable Carbon (POXC)

Permanganate oxidizable carbon is conceptualized as a labile carbon fraction that is readily available for decomposition by the microbial community. Its quantity is determined by the rate of oxidation of a fraction of organic matter by a dilute potassium permanganate solution ( $\text{KMnO}_4$ ; Wei et al., 2003). Stock  $\text{KMnO}_4$  standard solutions of molarities 0.005, 0.01, 0.015, and 0.02 were produced in accordance with the procedures outlined in OSU Soil Fertility Lab, (2019) and stored in the dark until samples were ready for analysis (~ 24 hr).

A subsample of  $2.5 \text{ g} \pm 0.05\text{g}$  of each sample was weighed into a 50 mL labelled centrifuge tube. A set of dilution tubes were prefilled with 49.5 mL of distilled water. Working in batches of ten, 18 mL of distilled water and 2 mL of 0.2M  $\text{KMnO}_4$  stock solution was added to each sample tube. These were then capped and placed in 2 boxes (5 per box) and placed horizontally on a shaker at 180 oscillations per minute for 2 min. After 2 min, each tube was inverted and given a vigorous shake. The tubes were then uncapped and left to settle for 10 min. At 10 min, 0.5 mL of each sample was pipetted into the prefilled dilution tubes. The tubes were then capped and placed in low light until all samples were processed.

Standards and samples were transferred to a 96-well plate and absorbance at 550 nm was measured using a BioTek 8000TS plate reader. The standards were used to produce a standard curve with the intercept (a) and slope (b), and POXC (mg/kg soil) was calculated as:

$$= \left[ 0.02 \text{ mol L}^{-1} - (a + b * Abs) * (9000 \text{ mg mol}^{-1}) * \left( \frac{0.02L}{\text{Sample weight (kg)}} \right) \right]$$

Where 0.02 mol/L represents the initial  $\text{KMnO}_4$  solution concentration, a is the intercept of the standard curve, b is the slope of the standard curve, Abs is the absorbance of the sample, 9000 is the mg of C oxidized by 1 mole of  $\text{MnO}_4$  and sample weight is the weight of the sample in kg.

### Biological Indicator: Soil Organic Matter

Soil organic matter was measured by loss on ignition as described by (Ball, 1964) at the Lakehead University Environmental Laboratory at Lakehead University. Soils were weighed into porcelain crucibles and heated to  $550^\circ\text{C}$  in a muffle furnace, which removes organic matter while maintaining mineral materials. The mass lost is organic matter and is calculated as the difference in weight pre- and post-combustion divided by the pre-combustion weight.

### Chemical Indicator: pH

The pH of the soil is considered a master variable (Penn and Camberto, 2019). Soil pH was determined following FAO, (2021). A pH probe was calibrated by measuring standard solutions at pH 4, 7, and 10. Ten g of each sample was weighed into a labelled 50 mL centrifuge tubes. Twenty-five mL of distilled water was then added to each tube and capped. Samples were then placed horizontally on a reciprocal shaker set on low for 60 min. Samples were left to rest for another 60 min. Working one sample at a time,

each sample was stirred for 10 s with a glass rod before inserting the pH probe into the suspension. Once the pH reading became stable it was recorded for each sample.

### Chemical Indicators: Total C and Total N

Total C and N were determined on <2 mm subsamples by flash combustion using an Elementar Vario EL Cube in the Lakehead University Instrumentation Laboratory after samples were homogenized for 3 minutes in a Spex 8000D ball mill.

### Physical Indicator: Wet Aggregate Stability

SLAKES is a free mobile application developed by the University of Sydney that uses image recognition software to record slaking (the breakdown of large (>2mm) soil aggregates into smaller (<.25mm) microaggregates from sudden emersion in water (USDA Natural Resource Conservation Service, 2008)) over a 10 min period (Fajardo et al., 2016). Three non-sieved air-dried aggregates (4-10 cm diameter) were placed in an empty 90 mm diameter petri dish on a A4 LED light table. A smart phone was suspended 15 cm above the petri dish. A reference image of the aggregates was taken with a timestamp and then the aggregates were transferred to a petri dish containing distilled water. Aggregates were placed into the water in the same position as the reference image. The software recorded the area of the aggregates after 10 min, also with a timestamp. The times and aggregate area data in the app were exported to an Excel file, where the initial area was divided by area at the end of the ten minutes. The value is a proportion. The test was done in triplicate for each sample.

## Physical Indicators: Permanent Wilting Point, Field Capacity and Plant

### Available Water Holding Capacity

Increasing soil organic matter/soil organic carbon content may increase a soil's resilience to moisture deficits. That is, its capacity to hold water could be increased with increasing organic matter content. Quantifying a soil's wilting point, field capacity and water holding capacity requires costly and time-consuming methods but pedotransfer functions can be used to calculate these properties using variables like soil organic carbon content and texture (Saxton and Rawls 2006; Bagnall et al., 2022). Using the pedotransfer functions developed by Bagnall et al., (2022) from soils collected across North America, permanent wilting point ( $\theta_{PWP}$ ) is calculated as

$$= 7.222 + 0.296 * clay - 0.074 * sand - 0.309 * SOC + 0.022(sand * SOC) + 0.022 * (clay * SOC)$$

and field capacity ( $\theta_{FC}$ ) is calculated as

$$= 37.217 - 0.170 * clay - 0.304 * sand - 0.222 * SOC + 0.051 * (sand * SOC) + 0.085 * (clay * SOC) + 0.002 * (clay * sand)$$

where the units for clay, sand, and SOC contents are in 10 g kg<sup>-1</sup>. Plant available water holding capacity (AWC) is then calculated as the difference between field capacity and the permanent wilting point.

## Soil Health Scoring

Scoring functions were determined for each soil health indicator used to determine the soil health score using a “more is better” approach and the following procedure. Data outliers were removed using the interquartile range (IQR) technique. For each indicator, the IQR was calculated as the difference between the 25th and 75th percentiles. If data points fell outside of the upper limit (75th percentile +  $1.5 \times \text{IQR}$ ) or lower limit (25th percentile -  $1.5 \times \text{IQR}$ ), they were removed. Once outliers were removed, the data were grouped into texture classes as outlined in Moebius-Clune et al., (2016). The mean and standard deviations for wet aggregate stability were calculated from the North American Project to Evaluate Soil Health Measurements Dataset (Soil Health Institute, 2024). The mean and standard deviations for 24h potential C mineralization and active carbon were calculated by Chahal et al., (2023) and Fine et al., (2017) respectively. The mean and standard deviations for each indicator differed based texture (coarse, medium, and fine). These values were used to assign a score for each indicator using a cumulative normal distribution function (Moebius-Clune et al., 2016). The overall soil health score was calculated as the average of the soil health scores for wet aggregate stability, 24h potential C mineralization, and active carbon and is assigned a value between 0 and 100.

*Table 1 : Mean, standard deviations (SD), and sample size (N) by texture class for the soil health indicators used to calculate an overall soil health score (AC=active carbon, WAS=wet aggregate stability, CMR=potential carbon mineralization)*

Variable	Unit	Coarse			Medium			Fine		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
AC	mg/kg	486.1	241.1	1844	531.2	182.2	3531	1.68	0.7	396
WAS	unitless ratio	0.53	0.2	300	0.36	0.15	735	0.32	0.13	390
CMR	mg C-CO <sub>2</sub> /kg soil/d	60.2	23	138	72.5	25	280	82	82	339

## Statistical analysis

To test for the effects of land conversion on soil properties, indicators and soil health scores one-way ANOVAs and t-tests (comparing M and C treatments) were carried out in RStudio. A Tukey post-hoc test was used to identify treatment differences (R Core Team, 2023; POSIT Team 2025) at  $P < 0.05$  and a trend at  $P < 0.1$ . Figures and standard deviations were produced using the dplyr and ggplot2 functions in the tidyverse package (Wickam et al., 2019).

## Results

Site 14 was removed from the analysis because it is an organic soil at >60% organic matter and all other soils were mineral soils. Sites 10 and 30 were removed from the soil health scoring following IQR analysis (Appendix 5). Sites 10 and 30 were removed from wet aggregate stability indicator analysis following IQR analysis. The full dataset of IQR analysis and site indicator values is available in appendices 3 and 4 respectively.

## Does land conversion degrade soil health compared to unconverted forests?

Soil health scores were lower in all treatments than reference forests (Figure 4), though the differences were not significant (Table 2) with a p-value >0.05.

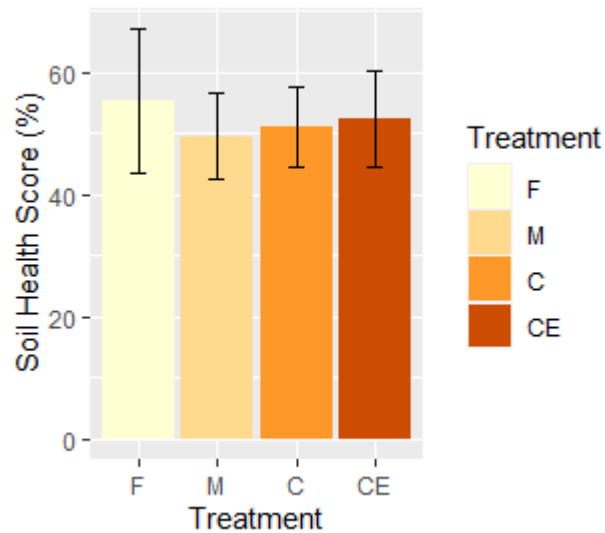


Figure 4 : Soil health score averaged by treatment (F=forest, M=mulch, C=conventional, CE=conventional established) with standard deviation represented by error bars.

Table 2 : ANOVA results for soil health (SH) score and indicator values (Soil moisture (SM), wet aggregate stability (WAS), percent nitrogen (N%), percent carbon (C%), carbon mineralization rate (CMR), pH, organic matter (OM), water holding capacity (WHC), and active carbon (POXC)).

Indicator	F value	P value
SM	5.698	0.003
WAS	4.763	0.008
N%	0.225	0.878
C%	2.395	0.087
CMR	4.082	0.015
pH	2.059	0.126
OM	1.303	0.291
WHC	1.219	0.319
POXC	0.332	0.802
SH Score	0.668	0.578



Treatment had a significant effect on wet aggregate stability, carbon mineralization rate and soil moisture (Table 2).

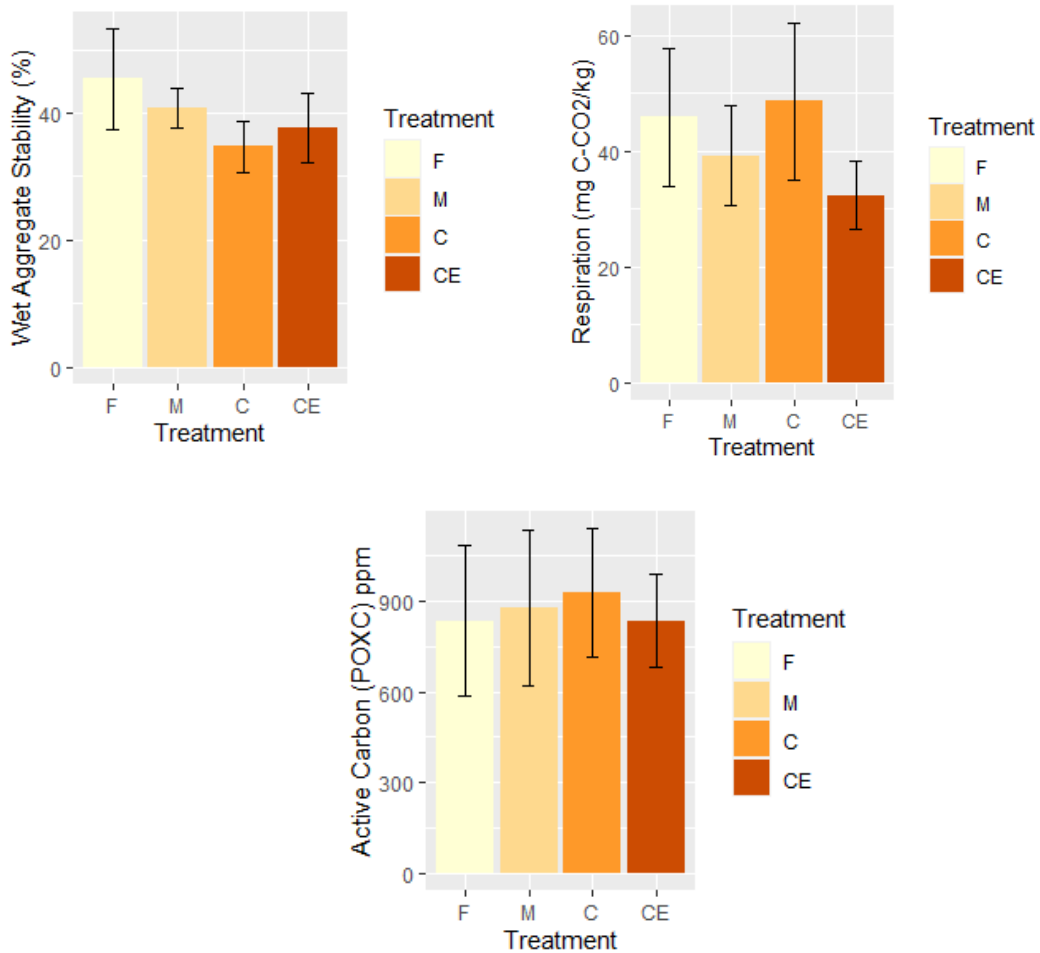


Figure 5 : Individual values of soil health indicators : wet aggregate stability (top left), carbon mineralization rate (respiration) (top right), and active carbon (bottom) averaged by treatment (F=forest, M=mulch, C=conventional, CE=conventional established) with standard deviation represented by error bars.

Table 3 : Tukey test pair-wise comparison P values of wet aggregate stability, percent carbon (C%), carbon mineralization rate (CMR) and soil moisture (F=forest, M=mulch, C=conventional, CE=conventional established) ( \*\* denotes significance at  $P < 0.05$ ; \* denotes significance at  $P < 0.1$ ).

Tukey Test	M-F	F-C	F-CE	M-C	M-CE	CE-C
Wet Aggregate Stability	0.4772	0.0065**	0.0651	0.1220	0.6143	0.6888
C%	0.5485	0.9982	0.1196	0.6310	0.7526	0.1465
CMR	0.5003	0.9901	0.0529*	0.2979	0.5262	0.0199**

Soil Moisture	0.0104**	0.0077**	0.0115**	0.9963	0.9999	0.9939
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Wet aggregate stability in C was significantly lower than in F at  $P < 0.05$  (Table 3; Figure 5). To a lesser extent, wet aggregate stability was significantly lower in CE sites at  $P < 0.1$  than F. Mulched fields were not found to be significantly different from other treatments. Potential carbon mineralization was significantly higher on average in F and C compared to CE at  $P < 0.1$  and  $P < 0.05$  respectively. The increase in C treatments was not significantly different from F. Mulched fields did show a reduced carbon mineralization rate compared to F, though the difference was not significant. There was no effect of treatment found on active carbon concentrations (Table 2). Soil moisture was significantly lower in all treatments compared to F (Table 3; Figure 6). Soil moisture was not significantly different between the M, C, and CE treatments. Carbon, nitrogen, organic matter, pH, and water holding capacity were not affected by treatment (Table 2; Figure 6).

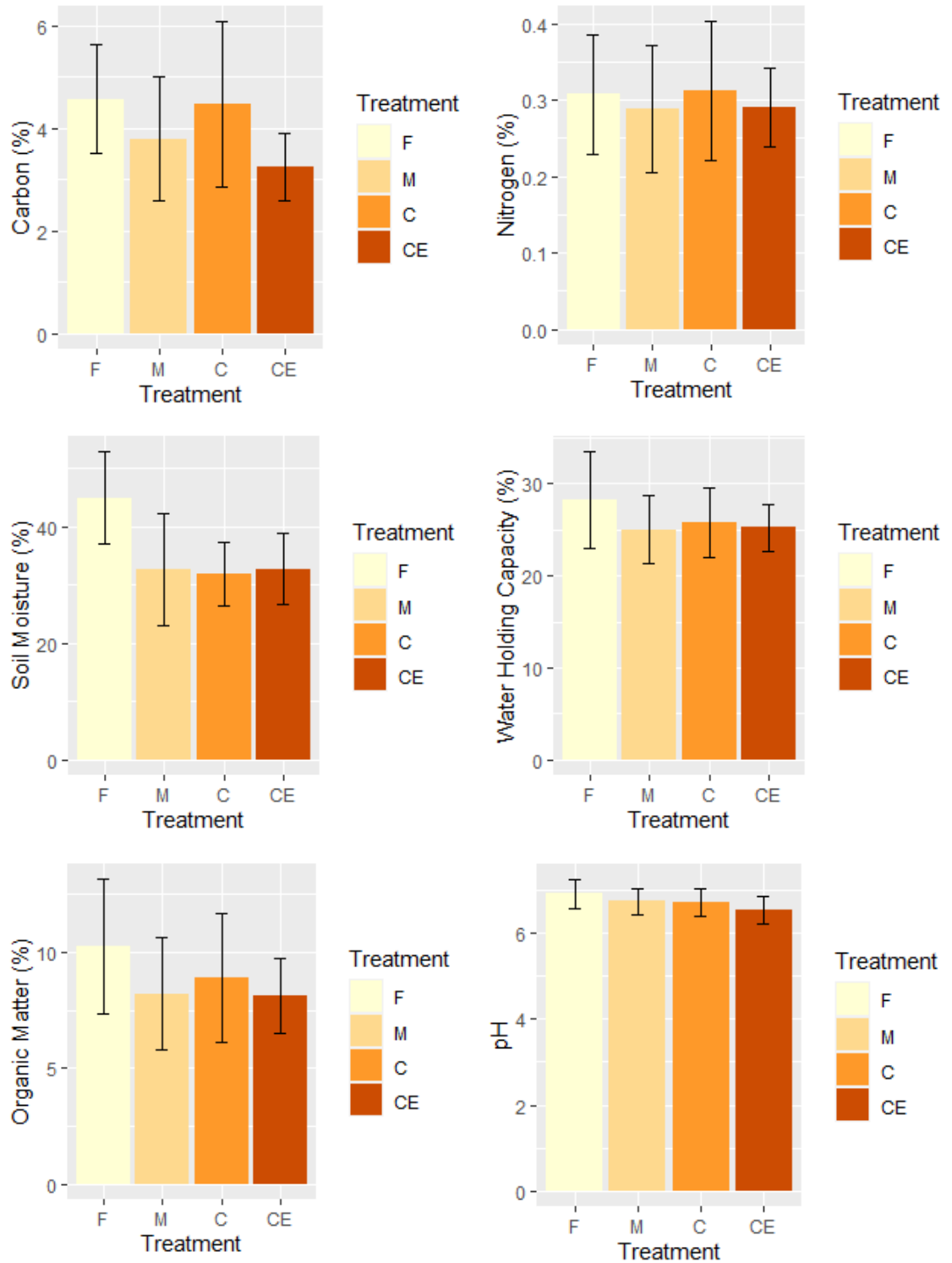


Figure 6 : Soil health indicators (carbon %, nitrogen %, soil moisture, water holding capacity, organic matter, and pH averaged by treatment (F=forest, M=mulch, C=conventional, CE=conventional established) with standard deviation represented by error bars.

## Does integrating wood mulch during land conversion produce better soil health outcomes than conventional clearing?

Though average soil health scores were lower for M compared to C, the differences were not significant at  $P < 0.05$  (Figure 4: Table 4).

*Table 44 : T- tests comparing soil health indicators (SLAKES wet aggregate stability (SAS), carbon mineralization rate (CMR), active carbon (AC), soil moisture (SM), carbon (C%), nitrogen (N%), water holding capacity (WHC), and pH) and overall soil health score (SH Score) for mulched (M) and recently conventionally cleared (C) treatments. (( \*) denotes significance at  $P < 0.05$ )*

Indicator	Units	t	df	p-value	C mean	M mean
SH score	unitless	0.442	15	0.665	51.04	49.57
SAS	%	-3.478	15	0.003*	34.71	40.78
CMR	mg C-CO <sub>2</sub> /kg/d	1.749	16	0.099	48.63	39.26
AC	mg/kg	0.458	16	0.653	928.61	877.58
SM	%	-0.205	15	0.840	31.84	32.63
C	%	1.002	16	0.331	4.46	3.79
N	%	0.596	16	0.560	0.31	0.29
WHC	%	0.438	16	0.668	25.78	25.01
OM	%	0.574	16	0.574	8.91	8.2
pH	unitless	-0.186	16	0.855	6.71	6.74

Wet aggregate stability (SLAKES) was significantly higher in M compared to C. Carbon mineralization rate, active carbon, soil moisture, carbon, nitrogen, water holding capacity, organic matter, and pH were not significantly affected by treatment (Table 4).

## Discussion

Though not found to be significant at  $P < 0.05$ , soil health scores declined with land conversion with forest sites having a mean soil health score of 55.4 and converted sited averaging scores below 52. The observed trend is consistent with the findings of Benalcazar et al., (2022), suggesting declines in soil health happen quickly after conversion. The scores reported here are lower compared to Benalcazar et al., (2022)

with F having a score of 55.4 (86 in Benalcazar et al., (2022)), while scores in the converted treatments ranged between 49-52 (compared to 79 in Benalcazar et al., (2022)). Though the data in this study produced lower overall soil health scores from removing less sensitive indicators as suggested by Bagnall et al., (2023), the reductions themselves were similar to Benalcazar et al., (2022) with converted land having scores 3-6% on average below referenced forest sites (7% in Benalcazar et al., (2022)). The alignment of the results is encouraging because Benalcazar et al., (2022) used the Comprehensive Assessment of Soil Health (CASH) suite for calculating the soil health score, which includes 17 response variables (Moebius-Clune, 2016), whereas this study used three indicators (wet aggregate stability (SLAKES), 24hr potential C mineralization, and active carbon (POXC)).

Though only three indicators were used to calculate a soil health score, additional response variables were included in the current study. Of the other eight indicators measured or calculated, only two showed a significant effect of land conversion treatment and both are related to the carbon concentration or changes to the concentration. Soil moisture at time of sample collection was lower in the agricultural soils and the C to N ratio was narrower, consistent with the observed decline in soil carbon concentration. This trend was also evident between the recently- and well-established fields that had been cleared conventionally. Though there was no difference in the overall soil health scores, the carbon concentration declined and with it, there was a decline in soil moisture and a narrowing of the C to N ratio. These trends are useful for interpretation, and were produced with reduced suite of indicators,

providing encouragement for reducing needed indicators, particularly if budget and time are limited, though further correlation analysis is needed to support this statement.

Mulching and incorporating coarse woody debris into the soil during land conversion did not significantly affect the concentrations of soil organic matter/carbon. Concentrations of organic matter and carbon (mean  $\pm$  standard error) were  $8.20 \pm 0.80\%$  and  $3.79 \pm 0.40\%$  in the mulched soils and  $8.91 \pm 0.93\%$  and  $4.46 \pm 0.54\%$  in the conventionally cleared soils, with a slightly narrower C to N ratio in the mulched soils ( $13.1 \pm 0.4$  vs  $14.2 \pm 0.4$ ). This study does not have estimates on the amounts of coarse woody debris incorporated or removed at each site but the changes in the physicochemical environment may have resulted in a rapid decomposition of the wood, such that an increase in soil organic matter/content was not detectable. Gregorich et al., (2016) showed, using an isotope label, that sampled plant litter decays rapidly (i.e., 40% of the initial mass is lost within 6 months) and that decay rate increases significantly with temperature. When the forest canopy is removed, it changes the temperature and moisture regime in a soil. Typically, temperatures are higher, and soils are wetter following a clearcut forest harvest (Finer et al., 2016). In the case of agriculture lands where moisture isn't limiting, soils are often tile drained to promote a moisture content that allows spring seeding and to promote optimal crop growth. Finer et al., (2016) show that decomposition of wood in a clearcut, particularly when it was incorporated into the mineral soil, was faster because of higher temperatures and that in the intact forest, decomposition was limited by available N. In a system where forest

is converted to agriculture, temperatures will be higher, mineral nitrogen is applied to meet crop requirements, and moisture, weather permitting, will promote higher rates of decomposition, and notably wood decomposition. These conditions and the aeration of the soil from tillage incorporate debris (Khan, 1996) foster an ideal environment for increasing the rate of decay of existing soil organic matter (Wei et al., 2014) and coarse woody debris at a faster rate than the 40 years suggested by Shorohova and Kapitsua, (2016). Even though it might visually appear that the pool of mulched coarse woody debris is large as incorporation is occurring, its net contribution to soil organic matter may not be high enough to detect a change against the backdrop of the overall decline in the total pool or may have been too large to pass through the 2mm sieve. In tropical regions, decomposition is rapid, and change in soil organic matter due to land conversion is not always apparent (Girisha et al., 2003). Consistent with Benalcazar et al., (2022), declines in the soil health indicators happened quickly after conversion, and likely occurred because the soil was physically disturbed by equipment and the temperature and moisture regime shifted which enhanced the rate of decomposition of existing soil organic matter.

Wet aggregate stability was significantly lower in agricultural soils compared to forest soils and, alongside the declines in carbon concentration, is consistent with the disruption of aggregates by tillage systems that releases soil organic matter previously physically protected from decomposition (Kasper et al., 2009; Paul et al., 2013). There was no statistical difference in the 24h potential carbon mineralization rates between forest and agricultural soils. This could suggest that the microbial community is

consuming soil carbon more efficiently in the agricultural soils or that the pool of labile carbon is similar among the soils, which was what was observed for POXC. The 24h potential carbon mineralization was lower in the established agricultural soils compared to the recently conventionally converted soils, which mirrored the declines in soil carbon concentration and a narrowing of the C to N ratio.

The only significant difference between the mulched and conventionally cleared sites at a  $P < 0.05$  was for the wet aggregate stability measured by the SLAKES method, where the mulched sites were higher. In a companion study (chapter 2) comparing approaches for assessing wet aggregate stability, the volumetric aggregate stability test (VAST) showed the same trend. The significant increase in wet aggregate stability of mulched soils compared to conventionally cleared soils could be attributable to decomposition and incorporation of wood into the soil organic matter pool. A high initial decay rate of organic matter, and particularly wood, after conversion could have led to a greater production of organic compounds by the microbial community that bind aggregates together (Jouquet et al., 2006; Guhra et al., 2022) compared to conventionally cleared fields that have most of their organic matter removed upon conversion (Cano et al., 2018). Thus, integration of mulched coarse woody debris during land conversion can potentially be used to increase the wet aggregate stability of soil, though additional research is required to test this hypothesis.

At  $P < 0.1$  mulched sites had a significantly lower carbon mineralization rate compared to recently converted sites suggesting that the initial high decay rate of



organic matter is slowing down in mulched sites <10years since conversion while the decay rate is still high in conventionally cleared sites <10years since conversion.

## Conclusion

Though soil health was not significantly affected by land conversion in this study, there was a trend of a decline in soil health that happened within 10y of conversion that is consistent with findings in Northwestern Ontario. Land conversion significantly reduced soil moisture, potential carbon mineralization, and wet aggregate stability. Reductions in these variables could be explained by the removal of the canopy, tiling and tillage, which alters the temperature and moisture regime in the soil, creating conditions that enhance decomposition of soil organic matter/carbon.

Soil health was not significantly affected by the integration of mulched coarse woody debris. The integration of mulched coarse woody debris during land conversion resulted in higher wet aggregate stability, which could be explained by a rapid decay of organic matter into organic compounds that act as a glue to increase wet aggregate stability of the soil. This high rate of decay of organic matter is slowing down compared to conventionally cleared sites that still show a higher rate of decay. Based on the findings in this study, integration of mulched woody debris during land conversion would be recommended for improving the wet aggregate stability of soils, due to the significant increase in aggregate stability without detriments to other soil health indicators seen in this study. The amount of coarse woody debris that should be integrated into the soil to cause a lasting effect on the overall soil health score requires further study.

## Chapter 2: A Comparison of Wet Aggregate Stability Measurement Methods

### Introduction

Aggregates are soil structural units, their arrangement and stability influence the movement of gases and liquids in the soil (Amézketa, 2008). Aggregates are formed when mineral particles adhere to binding agents (Aksakal et al., 2020). These units can organize into larger units, forming a hierarchy that controls the structure and the stability of the soil to resist external stresses (Six et al., 2000), while also affecting other factors, which include but are not limited to infiltration, root development, and accessibility to organic matter (Amézketa, 2008; Aksakal et al., 2020). Quantifying aggregate structure and stability is time consuming and laborious but measuring the stability of macroaggregates to resist dispersion in water is a well-established indicator of soil structure (Amézketa, 2008). Because of the influence of soil structure on the soil's ability to function, quantifying macro-aggregate stability is routinely included in evaluations of soil health/quality (Moncada et al., 2015; Rieke et al. 2022) and has been shown to be responsive to change(s) in a land management system (Van Eerd et al., 2018; Rieke et al., 2022).

There is no method that is universally applied to measure macro-aggregate stability and its response to changes in the environment (Moncada et al. 2015; Van Eerd et al., 2018; Rieke et al., 2022). The aggregate size range targeted often differs between methods, as do the type and magnitude of disruptive forces applied in the approach. Each method also differs in its accessibility to the research community in terms of set-

up costs, training, time to complete the analyses, space, etc. (Van Eerd et al., 2018), as well as in its sensitivity to detect changes in response to disturbance (Moncada et al., 2015).

Automated Wet Sieving (AWS), the traditional method of estimating wet aggregate stability, described by Kemper and Rosenau (1986), uses an aggregate size fraction of 0.5 mm to 2.0 mm to estimate the wet aggregate stability of soils. The method involves comparing the mass of aggregate loss in 2 rounds of sieving 4.0 g of soil in solution (1 in distilled water for 3 min, 1 in a sodium hexametaphosphate solution for 5 min). This method requires use of an automated wet sieving machine, an oven, and a laboratory scale to complete analysis.

Volumetric aggregate stability test (VAST) is a hand sieving method of estimating wet aggregate stability described by Brinton, (2016) and available for purchase for ~\$1000 CAD. The purchase provides a kit containing a 0.5 mm mesh sieve, a container for dipping the sieve into distilled water, 2 standard soils with predetermined wet aggregate stabilities, a 1 CC measuring spoon, a Solvita test card, paper instruction, and a USB thumb drive containing the excel file used to enter in test results, and an instructional video. This method uses a <2mm aggregate size fraction and involves dipping 1 CC of soil into water and comparing the dispersion surface area change of soil in the mesh sieve to a reference card. The results are then entered into the excel file producing the wet aggregate stability of the sample.

SLAKES, the method of choice for chapter 1 of this study, is a free mobile application developed by the University of Sydney that uses an aggregate size fraction between 3 mm and 10 mm to estimate the wet aggregate stability of soils. The SLAKES method involves taking photos of aggregate dispersion in water over 10 minutes and uses image recognition to estimate the surface area change and calculate the wet aggregate stability of the sample. This method requires a light table, petri dish, and smartphone to complete analysis.

This study evaluates the effect of land conversion from forest to agriculture on soil wet aggregate stability via three methods using soils collected around Rainy River, ON, Canada and compares these methods in terms of the responsiveness of aggregates to disturbance and method accessibility.

## Materials and Methods

Soils were collected in the area of Rainy River, ON, between June 19-20, 2024 from nine mixed wood forests (F), nine established agricultural fields cleared conventionally more than 50 years ago (CE), nine agricultural fields converted from forest to agriculture less than 10 years ago by removing all residual biomass and some topsoil (conventional; C), and nine agricultural fields converted from forest to agriculture less than 10 years ago that incorporated wood residues (mulch; M). Sites are owned by farmers in the area that volunteered their properties for sampling. All agricultural fields were cropped in rotations of barley, oats, canola, and maize and fertilized following soil test recommendations. One composite sample of a minimum of 50 soil cores were collected from the 0-15 cm depth of mineral soil using a 3.5 cm

diameter soil probe at each site. Soils were air-dried at Lakehead University in Thunder Bay and subsampled for the wet aggregate stability analyses and background characterization, which included soil texture determined using the hydrometer method. Clay content ranged from 5-32%, with a mean and median content of 18%.

### Automated Wet-Sieving

Automated wet-sieving, as described by Kemper and Rosenau (1986), slakes sand-sized particles through oscillation in water. Four-gram subsamples of 1-2 mm size macroaggregates were placed in 0.25 mm sieve cups that are a component of an automated wet sieving apparatus (Eijkelkamp Agrisearch Equipment 08.13. Giesbeek, Netherlands). Samples were gently rewetted using a spray bottle with distilled water for 5 minutes. The samples were then submerged repeatedly over 3 min into 100 mL of distilled water to isolate the unstable fraction. Following this, samples were repeatedly submerged into 100 mL of sodium hexametaphosphate for 5 minutes, after gentle disruption with a glass rod with a rubber end to isolate the stable fraction. Soil slaked into the solutions was oven-dried at 105°C to a constant weight. The weight of the containers was subtracted from the dry weights, and in the case of the stable fraction, 0.2 g was subtracted to account for the weight of the sodium hexametaphosphate. Wet aggregate stability (%) was calculated by dividing the stable fraction weight into the combined weight of the stable and unstable fractions and multiplying the quotient by 100. The test was done in triplicate for each sample and averaged for statistical analysis.

## Volumetric Aggregate Stability Test (VAST)

Woods End Laboratories volumetric aggregate stability test, VAST, is a wet sieving method that slakes aggregates through oscillation in water (Brinton, 2016). A standardized volume (1 cc) of <2 mm sieved soil was placed on a 35 mesh (0.5 mm) sieve and repeatedly dipped 30 times in room temperature distilled water for 30 sec. The sieve was carefully placed on paper towel to wick excess moisture and then the area (Area 1) was quantified using the Solvita VAST card (Woods End Laboratories, Maine, USA). The sieve was placed back in the water and aggregates were disrupted by gently rubbing the soil in a circular motion. The sieve is then dipped 10 times before being removed and blotted, as described. The area occupied by sand size particles (Area 2) was quantified using the VAST card. Wet aggregate stability (%) was calculated as difference between area 1 and 2, divided by the area of circle on the VAST card ( $11.04 \text{ cm}^2$ ), multiplied by 100. The test was done in duplicate and averaged for each sample, as prescribed in the protocol.

## SLAKES

SLAKES is a free mobile application created by the University of Sydney that uses image recognition software to record slaking over a 10 min period (Fajardo et al., 2016). Three non-sieved air-dried aggregates (3-10 cm diameter) were placed in an empty 90 mm diameter petri dish on a A4 LED light table. Aggregates that were most representative of each sample (avoiding flattened or unique looking aggregates) were selected (introducing possible subjectivity into the results) A smart phone was

suspended 15 cm above the petri dish. A reference image of the aggregates was taken with a timestamp and then the aggregates were transferred to a petri dish containing distilled water. Aggregates were placed into the water in the same position as the reference image. The software recorded the area of the aggregates after 10 minutes, also with a timestamp. The times and aggregate area data in the app were exported to an Excel file, where the initial area was divided by area at the end of the ten minutes. The value is a proportion. The test was done in triplicate and averaged for each sample.

### Statistical and Qualitative Analyses

For each method, the effect of land conversion on wet aggregate stability was tested using a one-way ANCOVA using the GLM univariate procedure in SPSS (IBM SPSS Statistics for Windows, Version 29.0.2.0, Armonk, NY, USA). Clay was included as the covariate because its content is often correlated with wet aggregate stability and its content varies across the sites. Analyses of residuals, the Shapiro-Wilk's test for normality and Levene's test for homogeneity of variance were used to confirm the assumptions of the ANCOVAs. Orthogonal contrasts were used to examine the effects of conversion ( $LC_1 = \lambda_{\text{forest}} - 1/3 (\lambda_{<10y\ M} + \lambda_{<10y\ C} + \lambda_{>50y\ C})$ ), time since conversion ( $LC_2 = \lambda_{<10y\ C} - \lambda_{>50y\ C}$ ) and mode of conversion ( $LC_3 = \lambda_{<10y\ C} - \lambda_{<10y\ M}$ ) on wet aggregate stability. The relationship between methods was characterized using the Pearson's correlation coefficient, as was the relationship between clay content and the methods.

An overview of each method and its accessibility in terms of cost, complexity, training requirements, storage and space requirements, and time for data acquisition

was compiled as part of this study. Approximate costs are provided in Canadian dollars. Costs of consumables for each sample and method was less than one dollar, which I classified as low. Complexity was assessed in terms of the equipment set up and the number of steps required to conduct the analysis. Training was assessed in terms of the amount of time required to train someone to produce repeatable results in a safe working environment, which includes WHIMIS if chemicals are involved. Storage and space requirements considered the footprint of the equipment and its storage between projects. Finally, time for data acquisition for a sample was estimated based on workflows for this project.

## Results and Discussion

There was no effect of land conversion treatment on wet aggregate stability for the automated wet sieving approach ( $P=0.787$ ) but wet aggregate stability was affected by clay content ( $P=0.013$ ). Wet aggregate stability, determined using automated wet sieving, was positively correlated with clay content ( $r=0.462$ ;  $P=0.005$ ), which is consistent with what has been reported in the literature (Aksakal et al., 2020). For VAST and SLAKES, clay had no effect on the outcome ( $P=0.751$  and  $P=0.871$  respectively) but treatment did affect wet aggregate stability. The treatment effect was more significant for SLAKES ( $P=0.047$ ) than VAST ( $P=0.071$ ), which was only significant at  $P<0.1$ . For VAST, there was no difference between the forests and agricultural fields ( $P=0.761$ ) or between young and old conventionally cleared fields ( $P=0.236$ ). The fields that had been mulched during conversion had a significantly higher wet aggregate stability than the fields that were cleared conventionally ( $P=0.011$ ; Figure 6A). For SLAKES, forests



soils had a significantly higher wet aggregate stability than agricultural soils ( $P=0.008$ ; Figure 6B) and there were no differences based on time of conversion ( $P=0.623$ ) or conversion approach ( $P=0.318$ ).

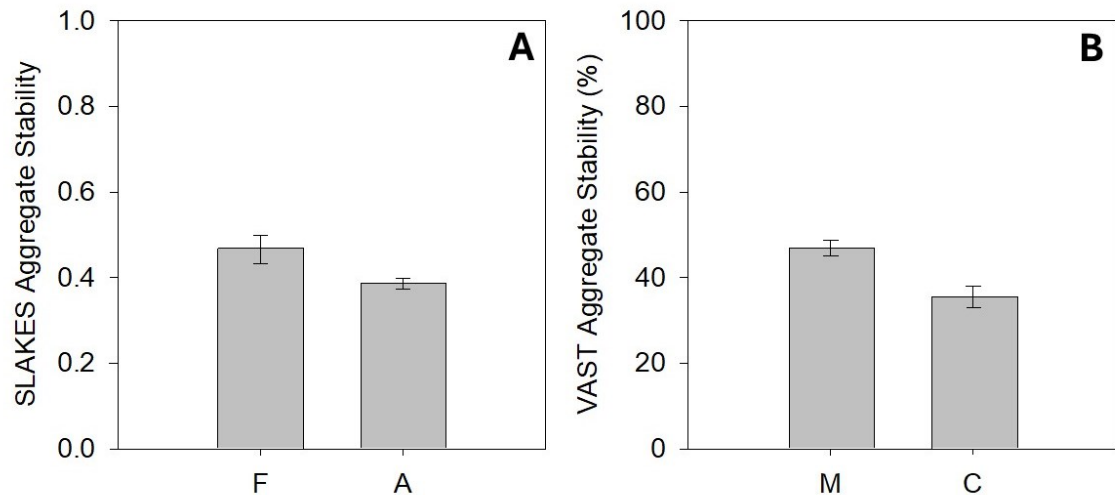


Figure 7 : Orthogonal contrasts (means and standard error of the mean) for wet aggregate stability quantified using the SLAKES and VAST methods. Panel A shows the difference between forested (F) and agricultural soils (A) and panel B shows the difference between soils where woody biomass was mulched and incorporated into the soil (M) and soils that had the biomass removed from the field prior to cultivation (C). (Note only statistically significant contrasts shown.)

Wet aggregate stability (mean  $\pm$  standard error) was highest for automated wet sieving ( $56.6\% \pm 1.4$ ), and lower but similar for VAST ( $43.3\% \pm 1.7$ ) and SLAKES ( $40.4\% \pm 1.3$ ). There was no correlation between the methods, even at  $P=0.1$ , but each method targets a different aggregate size range (Table 5). Aksakal et al., (2020) showed that wet aggregate stability varies with dry aggregate size, with stability increasing with increasing size of the aggregate. This trend was not observed in this study; however, Aksakal et al., (2020) first separated their soils into a spectrum of size fractions and then assessed their stability to consider both the structure and stability. This is

Table 5 : Summary and qualitative comparison of wet aggregate stability methods

	Automated Wet Sieve	Volumetric Aggregate Stability Test (VAST)	Slaking Image Analysis (SLAKES)
Aggregate Diameter	1-2 mm	0.5-2 mm	3-10 mm
Sieve mesh size	0.25 mm	0.5 mm	NA
Sample	4 g	standard volume (1 cm <sup>3</sup> )	Three air-dried aggregates
Output	% water stable aggregates	% water stable aggregates	10 min ratio of aggregate image areas
Mean CV (%) between samples	15.1	23.5	19.1
Mean CV (%) within samples	11.2	17.5	15.4
Cost of equipment	~\$7,000	~\$1,000	~\$100
Cost of consumables	Low	Low	Low
Complexity	Moderate	Moderate	Low
Training	Moderate	Moderate	Low
Storage and space	High	Low	Low
Time required for data acquisition	>1 d	5 min	15 min

obviously a much more laborious approach, but it does allow for a more complete interpretation of the effect of a disturbance on aggregation. Human bias was also a potential factor in the differences seen between the methods with VAST requiring human judgement on reading the indicator card, and with the selection of tested aggregates in the SLAKES method. Ultimately applying an approach consistently over time will provide the evidence required for decision making around a management practice, and the approach chosen may be determined by method accessibility (Rieke et al., 2022).

The SLAKES app has the lowest capital investment cost, was the least complex, and required the least amount of training of the three methods (Table 5). There are no chemicals involved so WHIMIS training is not required. The application is free and easy to use, with caveat that the user has a cellphone, though the user can use a digital camera. As long as the user places the aggregates in the same location with the same orientation between the wet and dry petri dishes and taking the pictures at the correct time, there is little room for human error. Automated wet sieving has a clear advantage with respect to human error (Van Eerd et al., 2018) but it does have a significantly higher capital investment cost, requires a drying oven, scale, chemicals, more time to complete, and more training than the other methods. VAST, like SLAKES, does not require chemicals but it does require a commitment of time to be able to obtain reproducible results and there is potential for error between users because there is some subjectivity in determining areas. Standard soils are included with the test kit, which facilitates quality assurance and control. A training manual and videos are

provided, along with a Microsoft Excel spreadsheet with macros that calculate wet aggregate stability and its coefficient of variation between replicates.

SLAKES was responsive to land conversion and was an intermediate among the methods in terms of its within sample and treatment variability. Data can be acquired relatively quickly and overall; it was the most accessible and most responsive method included in this study.

## Conclusion

In this project, I asked three questions:

1. Does land conversion degrade soil health?

Though soil health scores were not significantly affected by land conversion in this study at  $P < 0.05$  the average soil health decline seen was consistent with Benalcazar et al., (2022) and that soil health declines with land conversion and the decline happened within 10y. Soil health scores were lower on average compared to Benalcazar et al, (2022) due fewer soil health indicators included in the soil health score calculation as suggested by Bagnell at al., (2023). Land conversion significantly reduced soil moisture, 24h potential carbon mineralization, and wet aggregate stability. Reductions in these variables could be explained by the removal of the Oh soil horizon, the removal of the canopy, tiling and tillage, which alters the temperature and moisture regime in the soil, creating conditions that enhance decomposition of soil organic matter/carbon.

2. Does integrating wood mulch during land conversion result in better soil health outcomes than conventional clearing?

Soil health scores were not significantly affected by the integration of mulched coarse woody debris. However, the integration of mulched coarse woody debris during land conversion resulted in higher wet aggregate stability, which could be explained by a rapid decay of organic matter into organic compounds that act as a glue to increase wet aggregate stability of the soil. At  $P < 0.1$  the integration of wood mulch resulted in a

significantly lower carbon mineralization rate compared to conventionally cleared sites indicating that the initial high rate of decay in mulched sites have slowed sooner than conventionally cleared sites.

3. Do methods to measure wet aggregate stability differ in their sensitivity to detect changes from land conversion practices and their accessibility in terms of cost, time, complexity, and training required?

Wet aggregate stability (mean  $\pm$  standard error) was highest for automated wet sieving ( $56.6\% \pm 1.4$ ), and lower but similar for VAST ( $43.3\% \pm 1.7$ ) and SLAKES ( $40.4\% \pm 1.3$ ). There was no correlation between the methods, even at  $P=0.1$ , but each method targets a different aggregate size range. Ultimately, applying an approach consistently over time will provide the evidence required for decision making around a management practice, and the approach chosen may be determined by method accessibility.

The SLAKES app has the lowest capital investment cost, was the least complex, required the least amount of training of the three methods, was responsive to land conversion and despite bias in aggregate selection was an intermediate among the methods in terms of its within sample and treatment variability. VAST required similar materials, training, and complexity as SLAKES at a larger capital investment cost, was sensitive to land conversion, but showed the most within sample and treatment variability of the three methods. While the automated wet sieving approach showed the least within sample and

treatment variability among the three methods it was not responsive to land conversion. This was largely correlated with the clay content of the samples. Automated wet sieving was also the most complex, required the most training, and was the highest capital investment cost of the three methods. Qualitatively and quantitatively SLAKES is the recommended method of the three tested, though consistency of method use will lead to informed land management decisions.

## Limitations of the study

The scope of this study only included 9 homogenized samples of each treatment, all from the same region (Rainy River, ON). There are no exact estimates or uniformity of the amount of coarse woody debris that was implemented into the 9 mulched fields. We do not know the exact time since conversion only that recently converted fields are ~ 10 years since conversion and that established fields are > 50 years since conversion. The sensitivity of indicators averaged for soil health in this study were not directly compared to other methods of estimating soil health such as CASH. I also only included 4 treatments (reference forests, fields converted <10y to agriculture incorporating mulched coarse woody debris during conversion, fields <10y conventionally converted from forest, established >50y conventionally converted fields). The SLAKES method of measuring wet aggregate stability contains risk of bias due to selection of representative aggregates used. The VAST method of measuring aggregate stability contains potential for user error and bias both in performing

the hand sieving and in reading the result. Only 3 methods of measuring wet aggregate stability were compared in this study, while others exist.

## Future Research

Future soil health studies should compare the sensitivity of soil health scores averaged using different sets of indicators, to confirm the indicators that produce the most sensitive soil health score to changes in land management. Future studies should apply the methods used in this study to varying regions and soil types to confirm the conclusions made in this study. The correlation of clay content, and reliability of aggregate stability measurement method should be investigated due to the correlation seen in the automated wet sieving method in this study.



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## Appendices

### Appendix 1: Site Location Information

Table 6 : Site location information

Site No.	Clearing Practice	Soil Name	Notes
1	mulched	Dilke sandy loam	<10 years; mulched in the east; south of clearing was pasture
2	mulched	Dilke sandy loam	<10 years; mulched behind the solar panels
3	forest	Emo clay	next to VanZwol
4	conventional	Emo clay	well established
5	conventional	Emo clay	well established
6	conventional	Emo clay	well established; good producing
7	conventional	Emo clay	well established
8	conventional	Emo clay	well established
9	conventional	Arbor vitae clay loam	<10 years (east is older, west is a little messy)
10	forest	Arbor vitae clay loam	around Advent
11	conventional	Emo clay; stay close to church	well established
12	forest	Emo clay	by Mennonite Church
13	mulched	Crozier clay	<10 years
14	forest	Crozier clay	next to Blue Rd
15	conventional	Crozier clay	<10 years (very recent)
16	conventional	Crozier clay/orson fine sandy loam/bergland loam	(<10 years)
17	forest	Bergland loam	
18	mulched	Woodyatt loam	<10 years
19	conventional	Bluett clay	recent (<10 years)

20	conventional	Wood fine sandy loam south and crozier clay loam north	established
21	conventional	Wood fine sandy loam south and crozier clay loam north	recent (<10 years)
22	forest	Wood fine sandy loam south and crozier clay loam north	
23	conventional	Devlin sandy loam	<10 years; very recent
24	mulched	Innes Lake	recent
25	forest	Innes Lake	
26	mulched	Innes Lake/Fort Frances clay	recent
27	forest	Innes Lake/Fort Frances clay	
28	mulched	Siflet silty clay loam	recent
29	forest	Siflet silty clay loam	
30	conventional	Bluett clay loam	<10 years
31	mulch	Innes lake	grain
32	conventional	Fort Frances clay loam	established; grain
33	mulch	Innes lake	grain
34	conventional	Innes lake	established; grain but originally pasture
35	conventional	Crozier clay loam	<10 years
36	conventional	Crozier clay loam	<10 years

## Appendix 2: Texture Information by site

Table 7 : Texture Table (M=mulched fields, F=forest, C=recently converted conventionally cleared fields, CE= established conventionally cleared fields)

Site No.	Treatment	Sand (%)	Silt (%)	Clay (%)	Texture	Texture class
1	M	43	37	19	loam	Medium
2	M	36	38	25	loam	Medium
3	F	43	36	21	loam	Medium
4	CE	28	40	32	clay loam	Fine
5	CE	49	33	18	loam	Medium
6	CE	49	33	19	loam	Medium
7	CE	35	39	26	loam	Medium
8	CE	29	40	31	clay loam	Fine
9	C	35	40	25	loam	Medium
10	F	49	32	19	loam	Medium
11	CE	32	41	28	clay loam	Fine
12	F	40	36	24	loam	Medium
13	M	51	33	16	loam	Medium
14	C	80	17	4	loamy sand	Coarse
15	C	80	15	5	loamy sand	Coarse
16	F	74	20	6	sandy loam	Coarse
17	M	73	17	10	sandy loam	Coarse
18	C	68	24	8	sandy loam	Coarse
19	CE	66	25	9	sandy loam	Coarse
20	C	48	34	18	loam	Medium
21	F	49	33	18	loam	Medium
22	C	55	28	17	sandy loam	Coarse
23	M	64	25	11	sandy loam	Coarse

24	F	61	27	12	sandy loam	Coarse
25	M	50	30	20	loam	Medium
26	F	42	37	20	loam	Medium
27	M	34	35	31	clay loam	Fine
28	F	56	32	12	sandy loam	Coarse
29	C	69	24	7	sandy loam	Coarse
30	M	49	33	19	loam	Medium
31	CE	61	27	12	sandy loam	Coarse
32	M	53	31	16	sandy loam	Coarse
33	CE	58	30	13	sandy loam	Coarse
34	C	41	36	22	loam	Medium
35	C	46	37	17	loam	Medium
36	C	44	36	20	loam	Medium

## Appendix 3: IQR Analysis

Table 8 : IQR Analysis

			Percentiles				
		25	50	75	Difference (75-25)	Lower limit	Upper limit
Weighted Average	Soil Moisture	28.43	34.43	42.24	13.81	7.72	62.96
	Wet Aggregate stability	50.63	57.10	62.71	12.08	32.50	80.83
	Vast Aggregate Stability	34.25	43.00	51.75	17.50	8.00	78.00
	App Aggregate Stability	33.92	40.17	43.92	10.00	18.92	58.92
	N%	0.24	0.29	0.36	0.12	0.06	0.53
	C%	3.12	3.58	5.14	2.02	0.09	8.17
	Carbon Min Rate	0.13	0.15	0.20	0.06	0.04	0.29
	pH	6.42	6.80	6.99	0.57	5.58	7.84
	Organic Matter %	6.72	8.74	11.22	4.50	-0.03	17.98
	Permanent wilting point %	12.97	15.34	17.15	4.18	6.70	23.42
	Field Capacity %	35.06	42.13	45.44	10.38	19.48	61.02
	Water Holding Capacity %	22.10	26.32	29.42	7.32	11.13	40.39
	POXC (mg/kg)	660.48	868.63	1067.12	406.64	50.52	1677.07
	SOC	3.90	5.07	6.51	2.61	-0.02	10.43
Tukey's Hinges	Soil Moisture	28.64	34.43	41.81	13.15	8.89	61.56
	Wet Aggregate stability	50.69	57.10	62.67	11.98	32.71	80.65
	VAST Aggregate Stability	35.50	43.00	51.50	16.00	11.50	75.50
	App Aggregate Stability	34.17	40.17	43.83	9.67	19.67	58.33
	N%	0.24	0.29	0.36	0.12	0.07	0.53
	C%	3.13	3.58	5.03	1.90	0.28	7.88
	Carbon Min Rate	0.13	0.15	0.20	0.06	0.04	0.29
	pH	6.43	6.80	6.99	0.56	5.59	7.83

	Organic Matter %	6.73	8.74	11.12	4.39	0.15	17.69
	Permanent wilting point %	13.00	15.34	17.12	4.12	6.83	23.30
	Field Capacity %	35.32	42.13	45.36	10.04	20.26	60.42
	Water Holding Capacity %	22.11	26.32	29.29	7.18	11.33	40.06
	POXC (mg/kg)	677.55	868.62	1061.10	383.55	102.22	1636.43
	SOC	3.90	5.07	6.45	2.54	0.09	10.26

## Appendix 4: Soil Health Indicator Values with Outliers Highlighted

Table 9 : Soil health indicators (soil moisture (SM), Automated wet sieving wet aggregate stability (AAS), VAST wet aggregate stability (VAS), SLAKES wet aggregate stability (SAS), nitrogen % (N), carbon percent (C), pH, organic matter (OM), soil organic carbon (SOC), permanent wilting point (PWP), field capacity (FC), water holding capacity (WHC), and active carbon (POXC)) with outliers highlighted (yellow)

Site No.	Treatment	SM (%)	WAS (%)	VAS (%)	SAS (%)	N (%)	C (%)	CMR (mg C-CO <sub>2</sub> /kg)	pH	OM (%)	SOC (%)	PWP (%)	FC (%)	WHC (%)	POXC (mg/kg)
1	M	27.37	50.81	47.00	42.33	0.24	2.96	48.49	6.11	6.71	3.89	13.77	37.08	23.31	638.42
2	M	38.28	62.35	51.00	38.67	0.29	3.14	36.35	6.70	8.72	5.06	17.18	43.48	26.30	630.59
3	F	45.37	43.07	48.00	49.33	0.35	4.80	42.40	7.31	10.90	6.32	17.20	46.76	29.55	755.27
4	CE	34.12	56.64	38.00	46.67	0.26	2.62	27.28	5.97	7.49	4.34	19.01	43.07	24.06	1049.07
5	CE	33.75	52.32	60.00	40.67	0.34	4.06	24.39	6.40	9.40	5.45	15.28	42.32	27.05	1046.12
6	CE	33.75	50.24	57.00	37.67	0.34	3.86	25.14	6.42	8.97	5.20	15.40	41.77	26.38	767.63
7	CE	35.00	70.15	40.00	38.67	0.28	3.02	36.70	6.43	8.75	5.08	17.57	43.91	26.34	746.61
8	CE	39.18	69.58	38.00	27.33	0.32	3.15	37.69	6.36	9.73	5.64	19.96	47.82	27.87	767.54
9	C	28.22	56.76	44.00	30.67	0.30	3.20	71.15	6.20	7.52	4.36	16.44	40.91	24.47	770.53
10	F	45.33	63.62	32.00	60.00	0.27	4.01	39.21	6.52	9.31	5.40	15.63	42.54	26.91	885.59



11	CE	31.21	62.75	54.00	39.67	0.25	2.76	33.61	6.38	7.54	4.37	17.56	41.94	24.37	643.41
12	F	40.78	58.75	62.00	33.67	0.23	3.50	58.11	6.51	8.94	5.19	17.06	43.62	26.56	610.38
13	M	33.02	62.21	45.00	42.00	0.36	5.46	31.29	6.42	11.33	6.57	15.84	45.68	29.84	1100.41
14	F	223.62	64.94	17.00	49.33	1.26	29.17	95.07	6.53	61.46	35.65	57.35	162.63	105.28	2715.00
15	C	35.54	62.92	21.00	34.67	0.50	6.76	68.45	6.46	11.58	6.72	13.27	41.77	28.50	851.66
16	C	34.74	61.61	40.00	43.00	0.25	3.66	36.72	6.27	7.17	4.16	9.56	31.66	22.11	607.11
17	F	37.22	57.30	28.00	51.67	0.23	4.04	35.55	6.53	6.80	3.94	10.76	32.25	21.48	543.69
18	M	29.45	43.88	43.00	42.00	0.24	3.29	49.36	6.83	6.58	3.82	9.76	31.50	21.74	958.86
19	C	68.10	42.87	40.00	33.00	0.40	7.22	49.84	6.64	13.85	8.03	15.78	48.48	32.71	1188.52
20	CE	22.53	56.80	31.00	40.33	0.23	3.02	39.20	6.84	5.95	3.45	12.94	34.80	21.85	711.69
21	C	30.61	49.38	38.00	38.33	0.23	3.44	45.78	7.10	7.26	4.21	13.83	37.60	23.77	1073.13
22	F	53.82	50.56	55.00	56.33	0.41	5.53	56.07	7.11	11.72	6.80	16.85	47.37	30.52	1277.74
23	C	42.68	49.54	31.00	33.00	0.35	5.62	45.76	6.83	11.95	6.93	15.04	45.20	30.16	1265.42
24	M	54.24	34.45	52.00	33.67	0.46	5.92	53.65	6.77	11.65	6.76	15.02	44.87	29.85	1195.22
25	F	56.92	65.14	56.00	46.00	0.38	6.21	34.61	7.12	13.81	8.01	19.30	53.48	34.18	1079.58

26	M	36.57	57.01	44.00	44.00	0.33	4.56	33.52	7.04	10.57	6.13	16.50	45.52	29.02	1211.12
27	F	47.10	74.67	30.00	40.00	0.37	5.25	64.88	7.03	14.08	8.17	23.04	58.52	35.48	887.26
28	M	24.61	57.18	57.00	43.67	0.19	2.66	35.28	6.99	5.41	3.14	10.35	31.32	20.97	936.54
29	F	33.14	54.09	47.00	36.33	0.22	3.16	36.37	7.15	6.36	3.69	9.22	30.58	21.37	641.29
30	C	29.06	65.53	43.00	58.67	0.29	3.67	49.43	6.87	8.04	4.66	14.76	39.67	24.92	922.48
31	M	27.65	53.40	44.00	40.33	0.24	3.11	32.52	6.89	6.53	3.79	11.17	33.26	22.09	623.57
32	CE	24.53	47.03	55.00	32.33	0.23	2.40	29.34	6.98	5.45	3.16	11.86	32.70	20.85	775.56
33	M	22.49	59.43	39.00	40.33	0.24	3.03	32.91	6.89	6.31	3.66	11.36	33.33	21.97	603.48
34	CE	40.94	62.60	42.00	35.33	0.36	4.22	38.05	6.92	9.92	5.75	16.90	44.99	28.09	1002.91
35	C	26.07	59.46	33.00	32.67	0.27	3.21	40.07	7.04	6.75	3.92	13.07	36.39	23.32	914.63
36	C	27.78	56.09	29.00	32.33	0.22	3.40	30.47	6.98	6.04	3.50	13.74	35.84	22.10	764.03

## Appendix 5: Soil Health indicator Standard Deviation Information

*Table 10 : Chapter 1 soil Health indicators : (soil moisture (SM), SLAKES wet aggregate stability (SAS), nitrogen % (N), carbon percent (C), pH, organic matter (OM), water holding capacity (WHC), active carbon represented by POXC, and overall soil health score (SH Score) and chapter 2 aggregate stability methods : automated wet sieving (AWS) and volumetric aggregate stability test (VAST), standard deviation and mean by treatment*

Treatment	SM (%)		SAS (%)		N (%)		C (%)		CMR (mg C-CO <sub>2</sub> /kg)		AWS (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
F	45.00	7.96	45.00	7.96	0.31	0.08	4.56	1.06	45.90	11.90	57.70	10.20
M	32.60	9.65	32.60	9.65	0.29	0.08	3.79	1.20	39.30	8.67	53.40	9.21
C	31.80	5.50	31.80	5.50	0.31	0.09	4.46	1.61	48.60	13.50	54.80	6.96
CE	32.80	6.04	32.80	6.04	0.29	0.05	3.23	0.66	32.40	5.90	58.70	8.20

Treatment	pH		OM (%)		WHC (%)		POXC (mg/kg)		SH Score (%)		VAST (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
F	6.91	0.33	10.20	2.92	28.30	5.24	835	251	61.40	8.91	46.60	13.00
M	6.74	0.30	8.20	2.41	25.00	3.74	878	257	58.80	5.99	46.90	5.51
C	6.71	0.33	8.91	2.79	25.80	3.78	929	213	59.90	7.01	34.50	7.46
CE	6.52	0.33	8.13	1.63	25.20	2.59	835	154	54.80	5.05	46.10	10.40