ASSESSMENT AND EVALUATION OF SOIL HEALTH INDICATORS WITH CHANGES FROM BOREAL FOREST TO AGRICULTURE IN THUNDER BAY, ONTARIO

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The boreal forest is one of the most extensive biomes in Canada at an estimated 2.7 million ha. The requirement for a growing agricultural sector, along with a currently warming climate, has accelerated the conversion of boreal forest to agriculture. The resulting more intensive soil use may have a significant impact on the soil ecosystem, potentially degrading some ecosystem services, such as carbon sequestration, water purification, climate regulation, nutrient cycling, flood regulation, habitat for organisms, climate control, and the provision of food, fibre, and fuel. Analysis of soil health using a structured synthesis of physical, chemical, and biological indicators can provide information on the sustainability of soil use. Soil health as a metric of soil change can inform farm managers and policy-makers of the status of soil and increase awareness of sustainable farm practices in a region. In this dissertation, I assessed the impact of land conversion from boreal forest to agriculture near Thunder Bay, Ontario, Canada. I found that land conversion created declines mainly in biological soil health indicators, along with total nitrogen and carbon. Soil health indicators related to soil carbon were the most informative to land disturbance in plots < 10 years since land conversion; indicators associated with forage cropping systems on Thunder Bay dairy farms. Carbon and nitrogen mineralization increased with time since conversion from forest. Archaeal and fungal abundance increased after land conversion. In addition, land conversion altered the microbial functions related to carbon, nitrogen, and phosphorus cycling. New knowledge about the effects of land conversion in the boreal forest will play a critical role in developing and implementing environmental and agricultural policies supporting Canada's soil sustainability and soil health.

BIOGRAPHICAL SKETCH

Paul Benalcazar was born in Quito, Ecuador on January 25th, 1982 to his happy parents Luis Benalcazar and Nelly Vergara. His love for land, agriculture and landscape started when he had the opportunity to experience the countryside travelling to the Nabuzo community, near Penipe, Ecuador, where his grandfather "Papa Pepe" showed Paul how the family produced and harvested apples in the region. During holidays, Paul spent all of his time taking care of the plants and animals on his aunt Hilda Benalcazar's farm. Paul moved from Santo Domingo de los Colorados, where he studied his primary and secondary education. His secondary education was developed at the Instituto Agropecuario Calazacon, where in the morning, he and his classmates studied agricultural subjects and at noon, he studied basic sciences.

Paul obtained his bachelor's degree at the Polytechnic University of Chimborazo in 2007 in Agricultural Engineering. During his first year at the university, he met and married his wife, Cristina Ross, who has been on adventures in this crazy life of studying, travelling, parenting and working with Paul ever since. Paul is thankful to his daughter, Melany, for her understanding and sacrifice for being with two parents who love studying and know a little more about science. At university, Paul also had significant influences from professors who, apart from scientific knowledge, taught him how to manage social and political situations in real life. Paul finished his Master in Geomatic Science at Salzburg University.

Paul has worked with public, private and international organizations in different positions such as farm extension worker, trainer, project manager, consultant and assistant professor. In addition, Paul has had the opportunity to study in Australia and work as a visiting scholar at two universities, San Diego State University in California and Cornell University in New York. His projects involved studying the effects of the application of the cybernetic hydrologic model and the assessment of soil health indicators to boreal soils. Before coming to Canada, Paul was part of the first Irrigation Research Centre in Ecuador, in which he developed projects on soil-water use efficiency in conjunction with his mentor, Juan Leon. He joined the Forest Sciences PhD program at Lakehead University thanks to the Queen Elizabeth II Diamond Jubilee Advanced Scholars program managed by Brian McLaren and Fernando Romero of the Polytechnic University of Chimborazo.

DEDICATION

To my Wife,
Daughter,
Father,
Mother,
Sister and Brother.
Thank
you
every
day for your
support,
help,
encouragement
and everything
that helped
me come
this far…

I am so happy
to have
all
of you with me!

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My committee members Amanda Diochon and Tarlok Sakota are also to be thanked. Amanda allowed me to be part of her field trials and supported me during my first fieldwork. In addition, through little actions, Amanda provided me with valuable lessons on never giving up on a publication. Tarlok offered me his experience and practical knowledge about agriculture in the north and guided me during the first steps of my research, making contacts available to me and providing the historical context for the Thunder Bay agricultural community.

My thanks to the Queen Elizabeth II Diamond Jubilee Advanced Scholars Program, , made possible with financial support from the International Development Research Centre and the Social Sciences and Humanities Research Council, the Canadian Agri-Food Policy Institute, the Ontario Ministry of Agriculture, Food, and Rural Affairs, Agriculture and Agri-Food Canada (especially Lori Phillips), Mitacs, and the Soil Health Laboratory at Cornell University for providing supports for this research. Through this network, I have had the opportunity to expand my knowledge in soil health in rich ways. I am also grateful to Bob Schindelbeck for his expertise, professional advice, and willingness to receive an outsider into Cornell's Soil Health Laboratory. Thanks to all the team and the new friends made during my time at Agriculture and Agri-Food Canada and Cornell University.

Thanks to everyone at Lakehead University and the research network I spent long hours in the lab with Brent, Brianna, Joseph, Bomidae and his wife, Kristen, Nate, Steven, Moameth, and Zach. Thanks to all farmers in Thunder Bay agricultural areas who participated in this study and let me know more about dairy farm operations in northern Ontario.

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CHAPTER 1 INTRODUCTION

The boreal forest ecosystem is the most extensive biome in North America, spanning 270 million ha in Canada (Natural Resources Canada, 2018). The biome contains a significant portion of global forest carbon (1.9 ± kg m^{-2}), and it is a sink for atmospheric C (Botkin & Simpson, 1990; Kishchuk et al., 2016). In addition to playing an essential role in C dynamics (Seedre et al., 2011), the ecosystem services of the boreal forest also include provision of clean water and air (Price et al., 2013). However, due to the cumulative effects of natural and anthropogenic disturbances, the boreal forest has become a net C source (Kishchuk et al., 2016).

Boreal soils are formed by the combination of various factors related to climate, biotic activities, topography, parental material (Jenny, 1941), which all create a mosaic of soils across the boreal area (Maynard, 2002). In the Boreal Shield ecozone, soils support low to medium forest productivity of 0.6 -1.5 m^3 ha^{-1} $year^{-1}$ (Maynard, 2002). Here, Podzolic, Luvisol and Organic soils are the dominant orders (Soil Landscapes of Canada Working Group, 2010). Podzols are generally coarse-textured, less productive and more susceptible to nutrient depletion than the fine texture Luvisols (Lavkulich & Arocena, 2011; Sanborn et al., 2011). The natural soil formation process occurs slowly, naturally and for a prolonged time. Chemical properties and soil profile changes occur without notice (Jenny, 1941). However, soil nutrient cycles are disturbed by natural or anthropogenic interventions like wildfire, insect disease outbreaks, landslides, erosion, forestry operations, mining, agriculture, and other resource extraction.

Partial or complete removal of the above-soil biomass may have both positive or negative effects on soil health and ecosystem services (Maynard, 2002). Since the Industrial Revolution (1750-1850), human intervention has been changing the environment (Rockström et al., 2009). The Boreal region is considered relatively undisturbed by human activity, although it is

estimated that 3% of the Canadian Boreal zone has been converted to other land uses, where industrial development is the main driver of this change, creating 31% of the change (Anielski & Wilson, 2009). Agriculture, one of the significant contributions to the Canadian economy, has been growing as expected due to the demand for agricultural products nationally and internationally, where the market for cereals and meats continues to grow (Government of Canada, 2018). Ontario has more than half of the highest soil class for farming (Class 1) and makes up one-quarter of all farm revenues in Canada (Ontario Minister of Agriculture, 2019).

Agriculture has been one of the main causes of conversion of forested land, where historically, forests and shrublands have been seen as a potential source of agricultural land (Ramankutty & Foley, 1999). The Canadian government has long been developing projects for land clearing of forests to change mainly for agriculture, urban development, mining, and road building (Ripley et al., 1946). For example, development of the Boreal Shield ecozone expanded rapidly during the European pre-settlement stage in the late 18^{th} to mid-19^{th} centuries (DeFries et al., 2004). In the southern boundaries of the boreal forest, farmers have increased their production areas to meet the demand for food, fiber, and energy (Fitzsimmons, 2002). Since 1850, forest conversion to agriculture has contributed 35% of anthropogenic carbon dioxide (CO_2) emissions worldwide, also causing fragmentation and degradation of habitats, soil and water pollution, and overexploitation of native species (Foley et al., 2005). The environmental consequences of land-use changes also include impacts on global carbon cycles, atmospheric deposition, and lower fertility of soils (Wackernagel et al., 2002). From 1990 to 1998, agricultural expansion caused a net loss of 54,000 to 81,000 ha $year^{-1}$ of natural habitat in Canada (Robinson, et al., 1999), contributing to the loss of biodiversity that has undermined the sustainability of provision of food security, freshwater, and fresh air, and for regulation of the

climate (Foley et al., 2005). By 2016, the rate of deforestation in Canada declined to 37,000 ha $year^{-1}$, which now represents less than 1% of Canada's total forest area converted to other land uses (Natural Resources Canada, 2018). In 2017, total greenhouse gas (GHG) from the agriculture sector still accounted for 60 Mt, equivalent to 8.4% of total GHG emissions in Canada, where 30% represented CH_4; the agriculture sector emits 75% of N_2O of the total nationally. Since 2005, grazing livestock production has declined in Canada; as a result, total N_2O has a slightly higher proportion due to crop production than from livestock, and a decreasing C sink in agricultural soils has occurred due to a shift from perennial to annual crops (Government of Canada, 2019).

Clearing forest land, known as deforestation, is a process of converting forested areas to other land uses (Ramankutty & Foley, 1999). The Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA, 2019) defined deforestation as "permanent conversion of forest to other uses as a direct result of human activities such as agriculture, urban development, transportation and industry." Land clearing occurs when trees, stumps, brush, stones and other elements on the field are removed to acquire or add more land into crop production or pasture. This cleared land could be part of an existing farm or a new farm operation (NOFIA, 2017). Land clearing aims to increase land productivity and enhance the livelihoods of farmers by producing revenues for themselves and the country above those revenues from forest operations.

For national forest monitoring in Canada, a forest is defined as a minimum land area of 1 ha with tree crown cover of more than 25%, and with trees having the potential to reach a minimum height of 5 m at maturity in situ (UNFCCC, 2006); the Food and Agricultural Organization of the United Nations defines a forest as "land spanning more than 0.5 ha with trees higher than 5 m and a canopy cover of more than 10% or trees able to reach these thresholds in

situ. It does not include land that is under agriculture or land use" (Marklund & Schoene, 2006). The density of cover trees set at 10% or 25% is the main difference in the definition of forest between the two organizations. Below these densities, areas are defined as "other wooded land," which is often a class more relevant to monitoring in northern parts of the boreal forest (Dyk et al., 2015).

Even though clearing areas in the boreal forest can have a positive effect on the local and national economies, it involves recognized negative impacts on ecosystem services, specifically in carbon sequestration, provision of clean water, air purification, and soil aggregation (Acton & Gregorich, 1995). With the degradation of these ecosystem services, the agricultural sector is reducing its own resiliency (Walker et al., 2006). Once land is cleared, carbon can be released rapidly by fires or slowly with heightened decomposition by microorganisms (Houghton, 1995; Wei et al., 2014). The Boreal Shield ecozone has already shown signs of degradation, such as less soil organic matter, soil compaction, aggregate structure destruction, soil acidification, and decreases in water quality, including groundwater contamination by nitrate (Acton and Gregorich, 1995). It is estimated that agriculture causes a loss of 26 billion tons of topsoil per year globally, 2.6 times more than natural systems (AAAS, 2004). This loss is expected to be accelerated by climate change as soils warm and organic matter decomposes more rapidly, leading to lower aggregate stability (Kaiser, 2004).

As soon as the soil's surface is 'broken,' it starts to change (Wei et al., 2014). In Ontario and elsewhere, soils are at risk from many threats such as 1) forest clearing for new crop production because of population increases, 2) farm practices that have degraded soils, and 3) altered soil systems and soil health due to extreme weather events (OMAFRA, 2017). New agricultural areas are associated with changes in overall water balance, organic matter, nutrient

inputs and outputs, and exposure of soils to water and wind erosion. Conventional cropping methods cause a breakdown in soil structure, leading to compaction, erosion, and higher soil salinity (Wei et al., 2014). In January 1988, during the National Workshop on Soil Quality, Canadian scientists established a measure to reduce and mitigate soil degradation and improve productivity (Acton & Gregorich, 1995). As a result, Agriculture and Agri-Food Canada established plans to monitor and improve farm practices and create programmes to enhance soil organic matter and reduce erosion in Manitoba, Saskatchewan, Alberta, and some parts of Northern Ontario (Agriculture and Agri-Food Canada, 2017).

Soil quality and soil health are modern terms that are used interchangeably (Acton & Gregorich, 1995; Braimoh & Vlek, 2008; Moebius-Clune et al., 2017). Some scientists prefer the term 'soil quality,' whereas others prefer the term 'soil health,'which considers soil ecological services (Janzen et al., 2021; Karlen et al., 2021, pp. 24–37; Lehmann et al., 2020). Like human health, soil health gives an overall picture of the status of the soil. If a soil becomes degraded, more energy, money, and chemicals are needed to produce food, and it may degrade further (Acton and Gregorich, 1995). Soil quality is "the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health" (Doran & Parkin, 1994). On the other hand, Agriculture and Agri-food Canada define soil health as "its ability to support crop growth without becoming degraded or otherwise harming the environment" (Acton and Gregorich, 1995). According to the Soil Science Society of America committee, "soil health is the continued capacity of a soil to function under natural or a managed ecosystem to sustain plant, animals, and humans, maintaining or enhancing water and air quality" (Karlen et al., 1997). The concept of soil health is related to sustainability and management, which can sustain the life of living organisms (plants, animals, and humans),

and preserve the soil for future generations (USDA NRCS, 2018a). Good soil health is crucial for humanity's survival; sustained, productive, and environmental quality depends on integrating physical, chemical, and biological soil processes that are components of soil health (Moebius-Clune et al., 2017). In addition, the soil health concept is linked to feed and food quality and how it nurtures all organisms.

Understanding physical, chemical, and biological components of soil is essential to understanding soil processes (OMAFRA, 2016). Soil functions related to agriculture in terms of production and environmental quality include physical, chemical, and biological properties that promote plant growth, improve water storage, allow infiltration, sequester carbon, maintain nutrient cycles, detoxify dangerous chemicals, and control diseases, pests, and weeds to support the production of food, fibre, and fuel (Braimoh & Vlek, 2008; Moebius-Clune et al., 2017). Overall, soil health initiatives have evolved from the physical, biochemical assessment to molecular soil metrics (Bünemann et al., 2018). New advances in molecular techniques such as the quantitative PCR (qPCR) assays of functional and taxonomic communities allow a new understanding of soil functions. Functional classes include identification of taxonomic classification of bacteria, fungi, archaea, carbon cycling, nitrogen cycling, and phosphorous mineralization that comply with specific functions such as polyphenolic decomposition, breakdown of oligosaccharides, cellulose decomposition, nitrogen mineralization, nitrification, ammonification, and denitrification, as well as the C-P bond cleave, a co-factor in gluconic acid production and phosphoester and anhydride bond dephosphorylation (Nannipieri et al., 2014). Having healthy soil means having healthy biota with many soil organisms that sustain soil ecological functions. The critical component of soil health is a high range of microbial diversity that sustains soil ecological functions (Khatoon et al., 2020).

According to the USDA NRCS (2018), soil has inherent and dynamic qualities. Inherent soil quality is related to the origin of the soil and its natural ability to function (e.g., clayey soils drain more slowly than sandy soils, while deep soils have more roots than shallow soils). These fundamental characteristics are almost permanent and can change only with geologic time, so they cannot be changed easily by human intervention (Dick, 2018). In comparison, dynamic soil quality defines how soil changes according to soil management practices and land use over a decadal time scales, and how these in turn affect soil organic carbon, water holding capacity, soil structure, and nutrient retention (Moebius-Clune et al., 2017). Soil health indicators related to soil functions can evaluate the status of soil health affected by management or climate change (Allen et al., 2011). A soil health assessment identifies the disturbances and stresses that soils experience and assesses soil resilience. A soil health assessment should identify not only the quality, but also influence the implementation of available management practices (Schjonning et al., 2004). Soil health metrics are selected by identifying constraints to proper soil functioning and defining management goals for agricultural production (USDA NRCS, 2018b).

Soil evolution is a continual creation and destruction at all scales and may progress, stay the same, or retrogress, depending on the environmental circumstances. This dissertion uses a chronosequence approach, involving space-for-time substitutions to assess soil development considering time since conversion from forest to agriculture. A chronosequence is a set of sites formed from the same parental material or substrate that differ in the time since they were formed, and is a tool for knowing soil evolution where past events are registered in the soil profile, although not all the events are registered (Huggett, 1998).

Chronosequence studies depend on the temporal scale of factors or process of interest, and the lifespan of dominant factors. It could be a change in soil development or pedogenesis

over decades to millions of years in many landscapes (Huggett, 1998), or it could be a change in microbial succession in soil over short periods from just a few days to weeks or years (Bardgett & Walker, 2004). Therefore, a soil chronosequence helps to test two pedogenic theories, evolutionary vs. formation. Evolutionary soil development theory considers the inconstancy of the environmental phenomena and the multidirectional change and multiple steady changes as part of the non-linear dynamics. Therefore, in the dynamic system, there are additions, losses, transfers, and transformation of materials into the system, examples being additions of soil organic material (SOM), losses of soluble salts and carbonates, transfers of humus and sesquioxides, and transformation of mineral from primary elements to secondary elements (Simonson, 1959). The nature and amount of SOM depend upon the additions, transformation, and transfers from the past until the present, which is governed by the climate, the nature of flora and fauna, and the length of time for soil weathering. Two properties are part of the non-linear dynamic soil system: 1) the stable-periodic behaviour and, 2) the chaotic behaviour and both depend upon internal conditions and external forces. In addition, both properties may display self-organizing characteristics involving changes from spatially uniform (undifferentiated) to a non-uniform (differentiated or segregated) state in response to non-linear dynamic instability.

Soil formation theory posits that soil formation is progressive development under state factors until the soil reaches its equilibrium (mature soils) in a new environmental condition (Jenny, 1941). The soil formation theory porports that the nature and rate of the pedogenic process are the results of the interaction of five soil forming factors: parental material, climate, organisms, topography, and time. Most chronosequence studies refer to this theory. Equation 1 shows the soil formation factors as a functional equation where soil is a function of the five soil forming factors:

Soil= f[*parent material,climate,living organisms,topography,time*] [Eq 1]

However, studies indicate that human activities have altered soil formation with positive and negative impacts on the soil (Bidwell & Hole, 1965; Paul, 2014). Therefore, the anthropogenic factor should be considered as the sixth element in soil formation theory. The anthropogenic or human soil formation factor has been considered since 1964 (Bidwell & Hole, 1965). The human factor has affected the natural process of biotic and abiotic elements in the soil by accelerating changes, and this factor can shape the physical and chemical properties of the soil environment just as much or even more than the other soil forming factors (Arnold et al., 1990; Dudal, 2005).

A chronosequence is the most appropriate single, cycling, parrallel, initial convergence or continued convergence pathway, in which short interchages in permanent vegetation is analyzed, over 1 – 100 years, or in long term and retrospectrive searching for explanations for soil formation. In this regard, the fact that short-term changes in biological factors, and longer-term soil and vegetation development are linked and predictable, makes the chronosequence approach a reasonable tool to intrepret changes at different temporal scales (Walker et al., 2010).

This dissertation investigates soil health metrics of soil function through three interrelated research projects, all of which assess converted forest with forest references adjacent to farms in the Thunder Bay district of Northern Ontario. Chapter 2 determines the impact of land conversion from boreal forest to agriculture on soil health indicators. This study was published in the *Canadian Journal of Soil Science* (Benalcazar et al., 2022). This chapter uses the Comprehensive Assessment for soil health (CASH) framework to integrate indicators into a score to evaluate land conversion effects. In Chapter 3, predictive soil health indicators across a gradient from newly converted to long converted agricultural in boreal soils identifies the most influential soil health carbon indicators from the CASH overall score. In Chapter 4, the impact of

land conversion from boreal forest to agriculture using microbial community functional capacity (qPCR) and soil sequencing by operational taxonomic units. This study, which will be submitted for publication in the journal *Agronomy for Sustainable Development*, characterizes soil community diversity, abundance and functional genes by quantitative qPCR and soil sequencing. Each chapter is written as independent work with relevant background literature introducing prior methods and results, containing tables and figures to illustrate the works.

CHAPTER 2 THE IMPACT OF LAND CONVERSION FROM BOREAL FOREST TO AGRICULTURE ON SOIL HEALTH INDICATORS[1]

2.1 ABSTRACT

Climate change is creating opportunities for agricultural expansion northward into the boreal forest. Converting forested land to agricultural land generally results in significant losses of organic matter, which can impact soil health (SH). The objectives of this study were to assess the effects of land conversion on indicators of SH and to use the Comprehensive Assessment for Soil Health (CASH) framework to integrate measures of these indicators into a score to evaluate land conversion effects. Total carbon and nitrogen were also measured in this study. Soils (0-5 cm and 5-15 cm) were collected from six dairy farms near Thunder Bay, ON, that included a mature forest, a field converted from forest to agriculture <10 years ago and a field converted from forest to agriculture >50 years ago. Land conversion resulted in significant declines in permanganate oxidizable carbon, wet aggregate stability, soil respiration, and concentrations of organic matter, ACE protein, total nitrogen and total carbon. Lower CASH scores in the soils converted to agriculture are interpreted to represent a decline in soil health but the scores, along with soil organic matter concentrations, remain high (CASH=80; OM=6%). There was no effect of time since conversion, suggesting that any degradation to soil health happens quickly and is closely tied to declines in soil organic matter.

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[1] This chapter is a published paper in the *Canadian Journal of Soil Science*, *87*(2), 403–415.

2.2 INTRODUCTION

Changes in climate are creating opportunities for agricultural expansion northward into the boreal forest, which will require the conversion of scrub brush and forest to agricultural land (King et al., 2018; Unc et al., 2021; Bahadur et al., 2021). Land clearing removes the forest canopy, increasing soil temperatures and altering soil moisture, which favors the decomposition of soil organic matter (SOM) and the release of carbon and nutrients (Houghton, 1995; Wei et al., 2014). The significant losses of SOM documented after land conversion are often rapid because decomposition is occurring at a faster rate than organic matter is being returned to the soil and practices, such as tillage, destroy and disrupt the formation of aggregates. In Eastern Canada, land clearing to support agriculture has resulted in a 22 % decrease in soil carbon compared to uncleared adjacent areas (Angers et al., 1995) and in boreal regions, soil organic carbon stocks have decreased approximately 31 % where conversion has occurred (Wei et al., 2014).

In addition to being a storehouse of carbon and nutrients, SOM contributes to the maintenance of soil structure, water holding capacity, and a diverse microbial community (Wall et al., 2012; Cano et al., 2018). Significant losses of SOM are synonymous with soil degradation (e.g., Matson et al. 1999) and the deterioration of the soil's ability to naturally support the needs of humans, plants and animals (e.g., Karlen et al. 1997). Soil health has been defined by many but is inherently a metaphor that cannot be fully measured directly (Janzen et al. 2021). However, soil health assessments are useful tools to examine how land management practices are impacting soil functions in time and space beyond the typical chemical properties used to describe soil fertility (Karlen et al., 1997).

Soil health assessments, such as the Comprehensive Assessment of Soil Health (CASH), integrate measurements of physical, chemical and biological properties of soils that are indicators of soil functions. CASH produces an overall score for a soil, along with scores for the individual indicators, that have been used to evaluate the effects of land management practices on soil functioning. Assessing soil health over time is viewed as being an indicator of sustainable management (Karlen et al., 1997) and the goal of the CASH score is to identify constraints to production at the site level to help increase land productivity while minimizing environmental impacts (Idowu et al., 2009). CASH was developed using soils and agroecosystems in the northeastern United States but has been used outside of the area, often with an assessment of the sensitivity of the measured indicators to the management practices of interest (e.g., Congreves et al., 2015).

In this study, we evaluated the effect of land conversion on soil physical, chemical and biological indicators using the CASH framework in two agricultural areas near Thunder Bay, Ontario. Soils were collected from forests, recently converted fields (<10 y agriculture) and established agricultural fields (>50 y agriculture) in the Murillo and Slate River areas. We hypothesize that: 1. the forests will have the highest SH scores and that land clearing and conversion will result in a decline in SH scores and detrimental changes in soil health indicators, 2. changes will be greatest in the >50 y agriculture sites, and 3. changes will be most pronounced near the soil surface.

2.3 MATERIALS AND METHODS

2.3.1 Study area and soil sampling

Six farms in the Thunder Bay area (45° 31' N--48° 17' N, 89° 30' W--89° 22' W; Figure 2.1) were sampled between July and August 2019 with 3 farms in the Murillo area and 3 farms in the Slate River area. Farmer participation was initiated through discussions at a rotational grazing workshop and extension work at the Lakehead University Agricultural Research Station. All farms are dairy operations and tile drained. Farmers use a combination of manure and mineral fertilizer at recommended rates to meet crop requirements and conservation tillage. The common N fertilizer included a blend of urea, ammonium sulphate, monoammonium phosphate, muriate of potash and zinc sulphate. The crop rotation for the region is alfalfa (Medicago sativa) , silage corn (Zea mays) , barley (Hordeum vulgare), or winter wheat (Triticum aestivum) Table (S5) . Each farm operation included a mature mixed-wood forest, fields that had been cultivated less than 10 years ago(<10 y agriculture), and fields that had been cultivated more than 50 years ago (>50 y agriculture).

The Murillo area is part of an end moraine, consisting of large deposits of till and boulders, with minor inclusions of water-laid alluvial silt, sand, and gravel deposited by glacial streams. End moraine layers include unsorted and unstratified materials of varying sizes and can both underlie and overlie sequences of layered silt, sand, and gravel. Also, end moraine water tables are generally low and have variable permeability and internal drainage, plus low compressibility and high bearing strength (Mollard & Mollard, 1983). The Slate River area is part of a glaciolacustrine plain consisting of varved and massive, fine-grained material deposited in glacial lakes. Amounts of clay, silt, and sand vary depending on basin and depth. Usually,

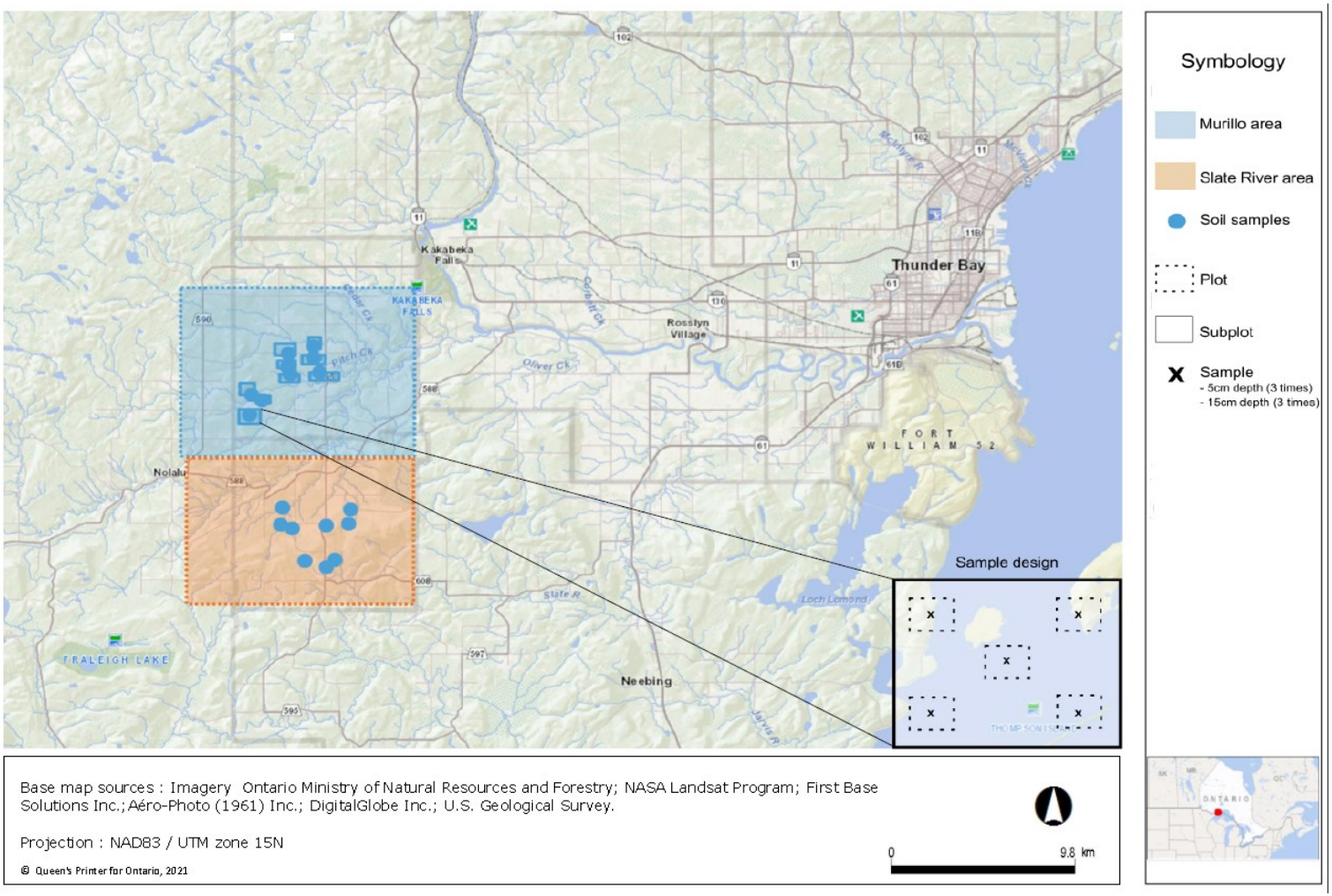

Figure 2.1. Murillo and Slate River areas and sample design located in the Thunder Bay area in Northern Ontario-Canada (base map source Ontario Ministry of Natural Resources and Forestry).

glaciolacustrine deposits consist of clay and silt with high water retention capacity, low permeability, and poor internal drainage. The Slate River area soils generally have low bearing strength and moderate to high compressibility (Mollard & Mollard, 1983).

Soils were collected using a soil split-core sampler (AMS Soil Samplers, Inc., American Falls, Idaho) at five locations in each cropped field and forest (at each corner and in the center). At each location, a total of three subsamples were collected to 15 cm in the mineral soil, divided by depth (0-5 and 5-15-cm) (Malone et al., 2009; Oertel et al., 2016) and composited. Forest soils samples were taken after removing the O horizon, which was largely plant litter. All samples were transported in a cooler to the laboratory at Lakehead University where they were air-dried and passed through an 8-mm and 2-mm sieve, as recommended by the CASH framework (Moebius-Clune et al., 2017). From each composite sample, a 1 L volume of soil was shipped to the Cornell Soil Health Laboratory in Ithaca, NY, and the USDA Forest Service Northern Research Station in Grand Rapids, MN, for analysis.

2.3.2 Laboratory analyses

2.3.2.1 *Physical soil health indicators*

The physical soil indicators measured in this study were texture, wet aggregate stability (WAS), and surface and subsurface penetration resistance. Texture was assessed at Cornell using the Kettler method (Kettler et al., 2001) to determine particle size distribution by sieving and sedimentation. WAS was assessed at Cornell using a rainfall simulator to measure the soil aggregate's resistance to disaggregation with moisture and raindrop impact. A force of 0.5 J was applied for 5 min to soils in a sieve that contained a known weight of soil aggregates ranging in size from 0.25 to 2.00 mm. The Cornell rainfall simulator delivers 12.5 mm of water in 5 min (Moebius et al., 2007). Using the Sjoerd (2002) procedure, penetration resistance was measured

in the field over the 0-15 cm depth (surface) and 15-30 cm (subsurface) depth using a Dickey-John penetrometer.

2.3.2.2 *Chemical soil health indicators*

The chemical soil properties measured in this study were pH, phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), total C and total N. All the measurements were completed at the USDA Forest Service Northern Research Station in Grand Rapids, MN. Soil pH was measured in a suspension of two parts water to one part soil determined by a Lignin pH robot (LIGNIN LLC) (Moebius-Clune et al., 2017). Phosphorus, K, Mg, Fe, Mn, and Zn were extracted using a modified Morgan's solution, an ammonium acetate plus acetic acid solution, buffered at pH 4.8. The extracted slurry was filtered through filter paper and analyzed with an inductively coupled plasma emission spectrometer (ICP Arcos, Spectro Analytical Instruments, Kleve, Germany; Moebius-Clune et al., 2017). Total N and total C were analyzed using 0.5 g of soil combusted in a LECO CHN 628 Series total elemental analyzer (LECO Corporation, St. Joseph, Michigan), using the CHN1 (stock) method, at a temperature of 950°C in the furnace, and 850°C after burned.

2.3.2.3 *Biological soil health indicators*

The biological soil properties in this study included OM, ACE-Protein, soil respiration (Resp), and permanganate oxidizable C (POXC) and were measured at Cornell. OM was determined by using loss on ignition (Broadbent, 1965). Ten grams of soil were weighed and heated to 500°C in a furnace. The exposure to higher temperature removed the carbonaceous material while retaining mineral materials in the sample. The resulting difference loss is the OM. The Autoclave Citrate Extractable (ACE) Protein Index, adapted from Wright and Upadhyaya (1996), was equivalent to ACE-Protein in the OM (Moebius-Clune et al., 2017). Three grams of

soil were weighed and placed in a test tube with 24 mL of extractable sodium (0.02 M, pH 7), then stirred for 5 min at 180 rpm and placed in the autoclave at 121°C and 14.50 psi above atmospheric pressure for 30 min. Next, 2 mL of extract was clarified by centrifuging at 10,000 rpm to remove soil particles. A small subsample of this clarified solution was used in a standard colorimetric protein quantification assay (Bicinchonic Acid Assay), and the results were compared to a serum albumin standard curve of soil protein, using a BioTek (Winooski, Vermont) spectrophotometric plate reader (Moebius-Clune et al., 2017).

Soil respiration is a measure of microbial community activity and an indicator of diverse soil functions, such as nutrient transformation, mineralization and solubilization (Krishnan et al., 2020). Microbial activity also contributes to stabilizing soil aggregates, facilitating soil aeration, infiltration and carbon sequestration (Moebius-Clune et al., 2017). The heterotrophic soil respiration method is adapted from Zibilske (1994) and indicates the microbial metabolic activity of the soil (Allen et al., 2011). The laboratory methodology quantifies the CO2 released from a re-wetted air-dried soil after four days. Twenty grams of air-dried soil were weighed in an aluminum boat with perforations, and the boat was placed over two small filter papers in a glass jar. A pipette trap filled with 9 mL of 0.5M KOH was placed into the jar to trap the CO2 during the four days of incubation. Using a pipette, distilled water (7.5 mL) was added to the jar to rewet the soil sample so that capillary action could raise water into the soil. The jars were then sealed for four days at room temperature of 23.5oC. The CO2 respiration measurements were determined by observing the electrical conductivity in the KOH trap with a WTW ProfiLineCond 3310 electrical conductivity meter. Greater CO2 indicates a more active microbial community in the soil (Moebius-Clune et al., 2017). Permanganate oxidizable C, here described as POXC, is a small part of the OM pool that is readily available as a food source for

soil microbes (Moebius-Clune et al., 2017); it is also known as the labile fraction of soil C (Weil et al., 2003). POXC is a function of the rate at which the soil reacts with dilute potassium permanganate (KMnO4; Weil et al., 2003). A hand-held colorimeter was used to determine the absorbance of the soil potassium permanganate solution at 550 nm. The colorimetry reading has an inverse linear relationship with POXC.

2.3.2.4 *Overall soil health score*

The measured biological, chemical and physical soil properties were integrated using the CASH framework to calculate a SH score for each category of conversion on each farm (Andrews et al., 2004). The SH score is calculated on a scale of 0-100 and scores are interpreted as very low (<40), low (40-55), medium (55-70), high (70-85), and very high (>85) (Moebius-Clune et al., 2017). Soil health scores were calculated for both soil depths, acknowledging that the framework was designed to represent soil health in the top 0-15 cm of soil.

2.3.3 Statistical analysis

A Type III marginal linear mixed effects model was used to determine if the soil health indicators and scores were affected by land conversion, soil depth and if there was an interaction effect. Fixed effects included time since conversion and soil depth. In the case of surface and subsurface resistance, the fixed effect was time since conversion. Time since conversion nested in farm was included a random effect for all indicators. Post-hoc examinations of significant conversion effects were conducted using orthogonal contrasts to determine if 1. the forest soils differed from the agricultural soils and 2. if there was an effect of time since conversion on the measured indicators and scores. Pearson correlations coefficients for the physical, biological, and chemical indicators were represented using the full dataset. All analyses were conducted using SPSS 25 (IBM Corp, 2019). Data are represented as means with standard errors.

2.4 RESULTS

The concentration of Mg (589 ± 323 mg kg-1), and proportions of silt and clay in the soils did not differ significantly with time since conversion or soil depth ($p>0.05$; Table S1). The proportion of sand in the soil did not differ significantly with time since conversion ($p<0.05$; Table S1) but there was a significantly greater proportion of sand in the 5-15 cm depth interval (24%) compared to 0-5 cm (20%) ($p<0.05$; Table S1). Regardless of the subtle differences in sand content, soils in the area have a silt loam texture [sand (22% ± 21%); silt (53% ± 16%); clay (25% ± 11%)]. There was a significant land conversion by depth interaction effect for WAS, OM, ACE-Protein, Resp, TN and TC ($p<0.05$; Table S1). In the 5-15 cm depth, there was no effect of land conversion on any of these indicators ($p>0.05$; Table S2). In the 0-5 cm soils, land conversion had a significant effect on OM, ACE-Protein, Resp, TN and TC ($p<0.05$); the effect on WAS was only significant at $p<0.10$ (Table S2). The forest soils (0-5 cm) had significantly greater WAS (137%), OM (210%), ACE-Protein (192 %), Resp (159%), TN (190%), and TC (220%) than the agricultural soils and there was no difference in the time since conversion on these indicators (Figure 2.2-2.3).

Concentrations of POXC and P, and overall CASH scores were significantly affected by land conversion irrespective of depth ($p<0.05$; Table S1) and were consistently higher in the forest compared to the agricultural soils (Figure 2.4). Time since conversion had no effect on concentrations of P and POXC, or CASH scores.

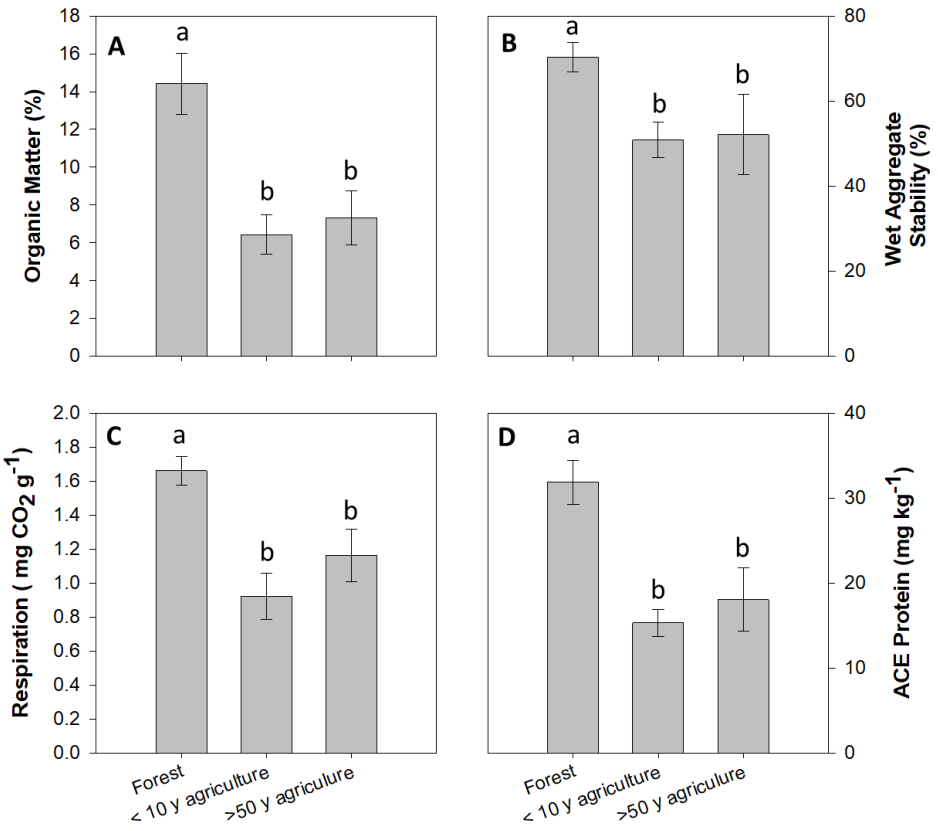

Figure 2.2. Organic matter (A), wet aggregate stability (B), soil respiration (C), and ACE-Protein concentrations (D) in mineral soils (0-5 cm) collected at forest and <10 y and >50 y agricultural sites. Bars are means +/- standard error. Significant differences ($p<0.05$) are denoted by different letters.

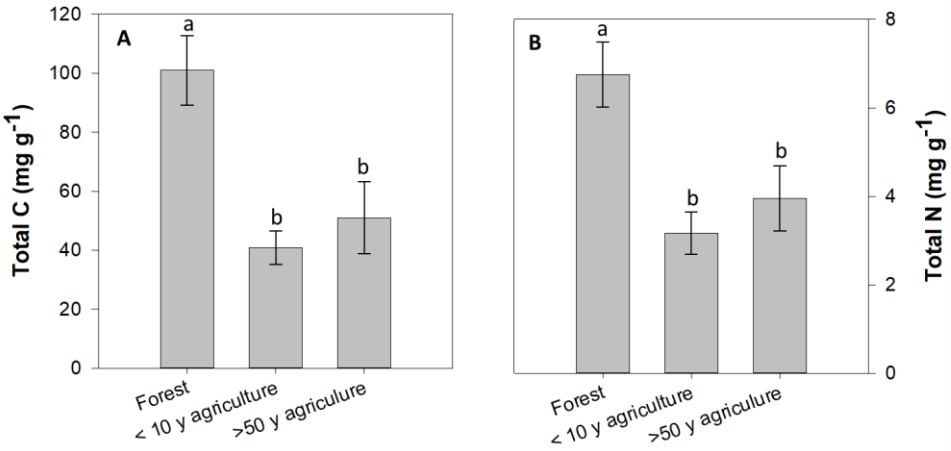

Figure 2.3. Total C (A) and total N (B) in mineral soils (0-5 cm) collected at forest sites and <10 y and >50 y agricultural sites. Bars are means +/- standard error. Significant differences ($p<0.05$) are denoted by different letters.

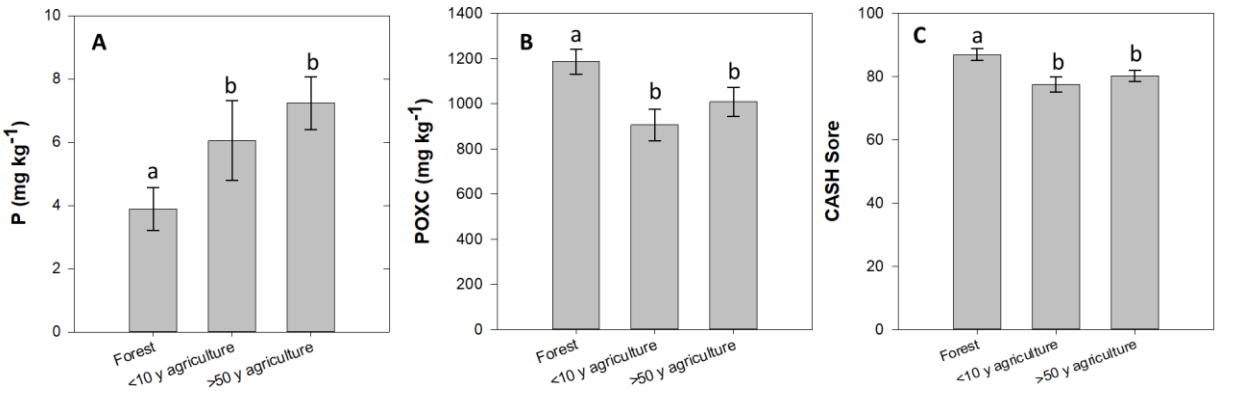

Figure 2.4. Concentrations of P (A), POXC (B) and CASH scores (C) in mineral soils (0-15 cm) collected at forest sites and <10 y and >50 y agricultural sites. Bars are means +/- standard error. Significant differences ($p<0.05$) are denoted by different letters.

Pearson product-moment correlation coefficients were calculated for the indicators to create a correlation matrix (Table S4). Of the 171 pairs, only 54 were significantly correlated to each other ($p<0.05$) and strong correlations ($r>0.50$) were observed for only 28 pairs. The majority (24) of these pairs were observed between WAS, Mn, TN, TC, OM, ACE-Protein , Resp and POXC.

2.5 DISCUSSION

Land conversion resulted in a degradation of soil health, as indicated by the changes in the indicators and overall scores but the changes were largely in the surface (0-5 cm) and occurred within the first 10 years following conversion. Acton & Gregorich (1995) reported that between 15-30 % of soil C stocks are lost after the first 10 years following conversion from forest to agriculture in Canada, while Guo & Gifford (2002) indicated that conversion from native forest to cropland declined soil carbon stocks by 42%. Other data suggest that during the first 30 years after the conversion to agriculture, 30-35% of the total soil carbon stored is lost in the top 7 cm, and even after 30 years these soils continue to be sources of greenhouse gasses (Oertel et al., 2016). Organic matter declined from 144 mg g-1 in the forest to 69 mg g-1 in the

agricultural soils. Declines were also evident in the 5-15 cm depth interval (85 to 58 mg g-1), but the difference was not statistically significant.

The loss of OM is consistent with declines in aboveground and belowground vegetation inputs that accompany most conversions to agriculture (Moebius-Clune et al., 2011). In a forest, belowground dead roots are the primary sources of soil C (Guo, Wang, & Gifford, 2007), and rapid declines of OM are partly attributable to the loss of OM inputs and partly due to the increased rates of decomposition of existing OM. This is evident in the decline in light fraction SOM (Post & Kwon, 2000). Also, tillage breaks down soil macroaggregates exposing organo-mineral surfaces and gives decomposers access to intra-aggregate carbon that lead to high rates of decomposition when combined with increases in soil temperature with forest clearing (Pennock & Van Kessel, 1997). Despite these declines, SOM concentrations are higher than in southern Ontario, where SOM concentrations range from 16 to 43 mg g-1 in agricultural soils (Congreves et al., 2015).

Organic matter influences many soil functions, including the number and species of microorganisms, nutrient cycling, soil structure, soil aggregation, water storage, and infiltration rates (Cano et al., 2018; King et al., 2020; Wall et al., 2012). In this study OM concentrations were highly correlated with WAS, Resp, and concentrations of total N, total C, ACE-Protein, and POXC, which is consistent with Graham et al., (2021) and Fine et al. (2017). Therefore, it is not surprising that all these indicators also declined significantly with land conversion. Wet aggregate stability is an indicator of the soil's ability to resist erosion. Wet aggregate stability declined by 27% in the 0-5 cm depth interval with land conversion. Graham et al., (2021) reported declines in WAS of 7 and 19% in grasslands converted to row crops using no-till and conventional tillage systems, respectively. Tillage breaks up aggregates and exposes OM to

oxygen and microbial decomposition (Helfrich et al., 2006), and though there was no significant effect of long-term cultivation on WAS, it was lowest in the agricultural soils that were converted more than 50 years ago. Of note is that surface and subsurface hardness were not affected by land conversion, suggesting that the conservation tillage systems used in the area are not leading to significant soil compaction but that it may be affecting aggregate stability. Land conversion from forest to agriculture led to significant increases in soil P concentrations (~ 59%). These increases are likely driven by organic and inorganic fertilizer inputs but may also reflect the release and retention of P from the rapid decomposition of OM. No other chemical indicators (i.e. pH, K, Fe, Mg, Mn, Zn) were significantly affected by land conversion.

Most of the significant effects of land conversion were detected in the biological soil health indicators. Soil proteins are the largest pool of organic N (Weintraub and Schimel, 2005) and the ACE-Protein measurement used in this study is an indicator of potentially available N (Hurisso et al., 2018). ACE-Protein declined by 48% in the near surface (0-5 cm) with land conversion, while total N declined by 38%. Similarly, POXC is used as an indicator of labile carbon availability and is viewed as being a highly sensitive indicator of management induced change. It only declined by 18% while total C declined by 46%. This result may suggest that losses of SOM may be more concentrated in the stable fraction of the SOM pool and that the microbial community may be mining SOM stores for N, which is typically limiting in the soil.

Soil respiration (Resp) was also lower in the < 10 y agricultural soils than in the forest soils and was most strongly correlated with total N, consistent with the tight coupling between the N and C cycles. Other studies show that continued loss in OM leads to lower Resp over time (Moebius-Clune et al., 2016; Yiqi & Zhou, 2010) but this study indicated that Resp was comparable between the forest soils and sites that had been in agriculture more than 50 years

ago. Litter removal and cultivation usually decreased soil respiration and increased with input additions (Jonasson, Castro, & Michelsen, 2004), with the influences of an abiotic process such as temperature, precipitation, and evapotranspiration (Yiqi & Zhou, 2010, p. 105).

2.5.1 CASH scores and the sensitivity of indicators

CASH scores were significantly higher in the forest (86) than in the agricultural sites (79) but there was no significant effect of time since conversion suggesting that any deterioration to soil health happened quickly but then stabilized. In all cases these scores are very high to high. None of the chemical indicators showed significant changes with land conversion. CASH scores were developed for agricultural soils (Moebius-Clune et al., 2016); however, its application in non-agricultural soil also provides an overview of forest sustainability linked to soil health. The relevance of the soil indicators in undisturbed soil provides an indicator of soil ecosystem integrity and the ecological functions provided by those ecosystems (FAO, 2020). In addition, determining a benchmark for soil health by comparing forests and agricultural fields can be used to support decision-making to improve soil health (Maharjan et al., 2020).

2.6 CONCLUSIONS

Increased knowledge of land-use change after the conversion from forest to agriculture on soil health indicators is important to understand how soil functions. Our study found that land conversion has detrimental effects on physical and biological indicators of soil health and CASH scores. Most of these differences were detected at the surface (0-5 cm). Land conversion generally resulted in declines in soil health indicators and an overall decline in CASH scores. There were no negative effects of land conversion on the suite of chemical indicators measured in this study, aside from P, but there were detrimental effects on soil physical and biological indicators that are closely tied to declines in SOM. Most of the differences were detected at the

surface (0-5 cm) but we acknowledge that this may not be the case for agricultural areas with conventional tillage systems.

CHAPTER 3 PREDICTIVE SOIL HEALTH INDICATORS ACROSS A GRADIENT FROM FORESTS AND NEWLY CONVERTED TO LONG-CONVERTED AGRICULTURAL BOREAL SOILS

3.1 ABSTRACT

Changing climate offers new opportunities to expand agriculture in northern latitudes and understanding the impacts of land conversion from forests to agriculture on soil health is critical for soil disturbance impacts. One method is to assess soil disturbance impacts using soil health indicators that relate to soil functions. Soil health indicators have been used extensively in predominately long-term agricultural settings but rarely following forest to agricultural conversion and are untested in northwestern Ontario boreal soils. One common soil health assessment method is the Comprehensive Assessment of Soil Health (CASH) framework. We found that many of the CASH indicators are highly correlated, so we optimized the number of indicators to a suite that reflect an efficient approach to assess boreal soil health. We evaluated 16 soil health indicators, taking soil samples from two sites in the Thunder Bay region, at two depths (0-5 and 5-15 cm) and within four conversion categories, defined by time since agricultural conversion to dairy farm forage cropping (forest, <10 y agriculture, >10 to <50 y agriculture, and >50 y agriculture,). Indicators related to soil carbon were the most highly correlated. Biological soil health indicators (organic matter, soil respiration, soil protein and permanganate oxidizable C), physical indicators (wet aggregate stability and surface hardness) and one chemical indicator (Mg) were the most predictive indicators of overall soil health using the k-fold cross-validation analysis. The set of indicators defined by our study offers the most efficient suite for understanding boreal soil health impacts following short (<10 y agriculture)- and long-term (>50 y agriculture) conversion of forests to agriculture.

3.2 INTRODUCTION

New opportunities to expand agriculture into northern latitudes has created a need to evaluate soil disturbance for short and long-term agricultural sustainability in the North. Changing climate has prompted policies that support land-use conversion to expand food production in Canada's north, despite limited understanding of the impacts of land conversion on soil disturbance indicators (Altdorff et al., 2021). Although there is no universal indicator for evaluating soil functions, using a combination of metrics related to soil health is a common approach to assess soil disturbance (Doran & Parkin, 1994; Karlen et al., 2019; Lehmann et al., 2020). An ongoing debate questions how we can most effectively evaluate soil health, given that indicators must be sufficiently sensitive to reflect management changes and history, yet not so sensitive (e.g., parent material, and precipitation regime), along with those of sampling logistics (Lazicki et al., 2021).

Physical indicators (e.g., aggregate stability, water infiltration, and bulk density), chemical indicators (e.g., pH, and N, P, and K concentrations) and biological indicators (e.g., organic matter, active carbon, microbial biomass, and microbial activity) have been identified as reliable indicators of soil function (Allen, Singh, & Dalal, 2011; Andrews, Karlen, & Cambardella, 2004; Idowu et al., 2009; Wade et al., 2022). Other studies found that penetration resistance (physical indicator), soil respiration, ACE-Protein, and permanganate oxidizable C (biological indicators), and pH (chemical indicator) were the most responsive metrics of management and land-use changes (Doran & Parkin, 1994; Fine et al., 2017; Sadegh Askari & Holden, 2015).

There are soil health indicators that are more sensitive than others. For example, short-term evaluation from one season to another, could use soil respiration or POXC, while for

evaluation over 10-30 years, soil organic matter could be used. Having both evaluations is necessary to assess changes in soil functions related to land use conversion and changes in management practices (Andrews et al., 2004). Several soil health frameworks have been developed (Chu et al., 2019; Moebius-Clune et al., 2017; Nunes et al., 2021), and efforts are ongoing to standardize soil health assessment protocols for North America (Norris et al., 2020). Norris et al. (2020) developed a framework to identify widely applicable soil health indicators that are sensitive across management practices, soil inherent properties and their location. This framework has not yet been applied and does not account for recently converted boreal soils that provides unique characteristics to consider in evaluating soil health including climate, parent material, textural class, and management practices (Amsili et al., 2021; Congreves et al., 2015; Lehmann et al., 2020). Of significance to boreal soil health is that there is greater accumulation of organic matter compared to other soils due to low temperatures that reducing microbial decomposition (Gauthier et al., 2015; Group et al., 1998).

To reduce redundancy and multicollinearity, minimum data sets (MDS) identify soil health indicators that are the most sensitive to change by identifying those indicators most influential on soil health scores (Yemefack et al., 2006). Several statistical models have been developed to obtain MDS using descriptive, univariate and multivariate analysis, factor analysis, regression analysis, and machine learning techniques (Askari & Holden, 2014; Chahal & Van Eerd, 2019b; DuPont et al., 2021; Kalcsits, & Kogan, 2021; Raiesi & Beheshti, 2014; Rekik et al., 2018; Yemefack et al., 2006; Wade et al., 2022).

3.2.1 Previous research and hypotheses

In a previous study using the Comprehensive Assessment of Soil Health (CASH) system, Benalcazar et al., (2022) reported that permanganate oxidizable C, wet aggregate stability, soil

respiration, organic matter, soil protein, total N and total C predicted soil health changes following conversion to agriculture in Thunder Bay- Ontario. Using the same study sites, our goal was to develop a MDS that are the best measures of the overall soil health scores across a forest to agricultural conversion gradient with time since conversion as a factor.

Dairy crop systems dominate agriculture in the Thunder Bay region. Field that were converted from forest to agriculture more than fifty years ago had lower overall soil health scores than adjacent forests from 84.7/100 to 79.8/100 (Chapter 2). In fields <10 y since agricultural conversion, the overall soil health score was 76.7/100. In this paper, we will develop a MDS for across the conversion gradient including baseline forest conditions, on sites <10 y in agriculture, sites >10 to <50 y in agriculture, sites >50 y in agriculture, and among all agricultural sites combined. We hypothesize that carbon-related indicators will be the most influential on overall soil health scores due to SOM is a key indicator of soil health functions in boreal soils as well. To test this hypothesis, we applied the best subset regression model, using the overall soil health score to identify the variables most influenced by time since conversion to develop a MDS. We conducted a k-fold cross-validation analysis to identify the best suite of soil functional indicators across the gradient of time since conversion.

3.3 MATERIALS AND METHODS

3.3.1 Study area

Northwestern Ontario is part of the Boreal Shield Ecozone with dominant soil orders that include podzols, brunisols, luvisols and organics (Schut et al., 2011). The climate is dry and warm in summer, cold and wet in winter, mostly covered by snow, and a short growing season with no snow cover from 6 to 8 months (Climate Atlas, 2019; Kishchuk et al., 2016). The Thunder Bay region has a mean annual temperature of 2.3 °C, annual precipitation of 726 mm,

and a frost-free season of 120.5 days (Climate Atlas, 2019). The altitude at Thunder Bay is about 249 above msl and the region lies at around 48° 27' N latitude and 89° 27' W longitude. The region is part of a glaciolacustrine plain with fine-grained deposits laid down among ancient lakes.

3.3.2 Soil sampling

All agricultural fields for this study have been under conventional dairy farm management, using tillage, synthetic fertilizer, fall and spring manure applications and rotations among alfalfa, forage corn, grain and forage barley and spring wheat (Benalcazar et al., 2022). We defined a total of 60 plots, with 16 samples from forest sites, 12 samples from <10 y agricultural sites, 16 samples from >50 y agricultural sites and 16 samples from sites >10 to <50 y following forest to agriculture conversion (Figure 3.1). Soil samples were collected using a split-core sampler (AMS Soil sampler, Inc., American Falls, Idaho) at five locations in each agricultural field and forest area (at each corner and in the center). Three subsamples were collected down to 15 cm from the mineral soil at 0-5 and 5-15 cm depth and composited separately from each depth. In forest areas the forest floor O horizons were not sampled, so that upper soil layers across the conversion gradient were comparable. All soil samples were prepared at the Lakehead University soils laboratory by air drying and passing samples through 8-mm, then 2-mm sieves. Soil water content was standardized by oven-drying for 24 h at 105°C. Detailed protocols for the remaining procedures are available in Moebius-Clune et al. (2017).

3.3.3 Measurement of soil health indicators

Physical indicators included wet aggregate stability (WAS) and surface and subsurface hardness. Chemical indicators included pH and extractable P, K, Mg, Fe, Mn, and Zn. We also measured total C and total N. We used organic matter (OM), autoclaved-citrate extractable soil

protein (ACE-Protein), soil respiration (Resp) and active carbon (measured as permanganate oxidizable C or POXC) as biological indicators. We used the rain simulator to test wet aggregate stability, generating precipitation with a drop size of 0.6 mm in an adjustable Mariotte tube that controls the hydraulic pressure. A thin layer of soil previously screened at 0.25 mm was placed on a sieve of the same size. After the simulated rainfall event, the materials that fall through, versus the materials that remain on the sieve, are collected, dried and weighed, and the fraction of stable soil aggregate is calculated as the difference in weight between the stable aggregates that remain on the sieve (Moebius-Clune et al., 2017). Surface hardness (SurH) and subsurface hardness (SubH) were measured with a standard probe inserted into the soil from 0-15 cm and 15-30 cm (Sjoerd W, 2002).

Soil pH was measured at a ratio of 1:1 soil to water, and plant available soil macro- and micronutrients (P, K, Mg, Fe, Mn, and Zn) were measured using inductively coupled plasma optical emission spectrometry analysis (SPECTRO Analytical Instrument Inc.), after extraction with a modified Morgan solution (acetate plus acetic acid; pH 4.8). Organic matter was determined after oven drying at 105°C and weighing followed by ignition for 2 h in a muffle furnace at 500°C. The soil sample was then weighed again and the percentage of mass lost (i.e., loss on ignition) was calculated after weighing again (Moebius-Clune et al., 2017). Autoclave-citrate extractable soil protein (ACE-Protein) was extracted from a 0.02-M subsample of sieved, air-dried soil with sodium citrate (pH 7), and the concentrated sample was run through a series of centrifuge and autoclave steps (Wright & Upadhyaya, 1996). The soil's protein content was estimated using a bicinchoninic acid assay with a bovine serum albumin standard curve and a BioTek spectrophotometric plate reader. Soil respiration was measured in duplicate to improve the quantification of CO_2 after a 4-day incubation using the methodology modified by Haney and

Haney (2010). Sieved soil was placed in glass vials with a KHO-based CO_2 trap. After four days, the amount of CO_2 was determined by measuring the solution's electrical conductivity with an OrionTM DuraProbe and 4-Electrode Conductivity Cell. To account for headspace CO_2 corrections, glass vials with no soil as comparative blanks were also run. Active carbon (POXC) was estimated by measuring absorbance using a handheld spectrophotometer. A 2.5-g sieved subsample was mixed with 20 ml of 0.02M $KMnO_4$ (pH 7.2) in duplicate. Total C and total N was estimated using the CASH framework. For that determination, 0.5 g of sieved soil were analyzed following complete combustion using a LECO CHN 628 analyzer and the CHN1 method (LECO, 2020). The overall soil health score has an scale of 0-100 and score categories are defined as very high (>85), high (70-85), medium (55-70), low (40-55), and very low (<40).

3.3.4 Statistical analysis

The overall soil health score obtained using the CASH framework was used to apply a best subset regression (BSR). The BSR approach consists of testing all possible combinations of predictor variables and selecting the best model according to a criterion. In this case, our predictor variables were the 16 soil health indicators (inclusive of total C and N) and we selected the best model by the BSR exhaustive method, examining three subsets for each variable. We chose the optimal model using adjusted R^2 (Miller, 2002) for the forest (A), <10 y agriculture (B), >10 y to <50 y agriculture (C), and >50 y agriculture (D) soil samples. In addition, all

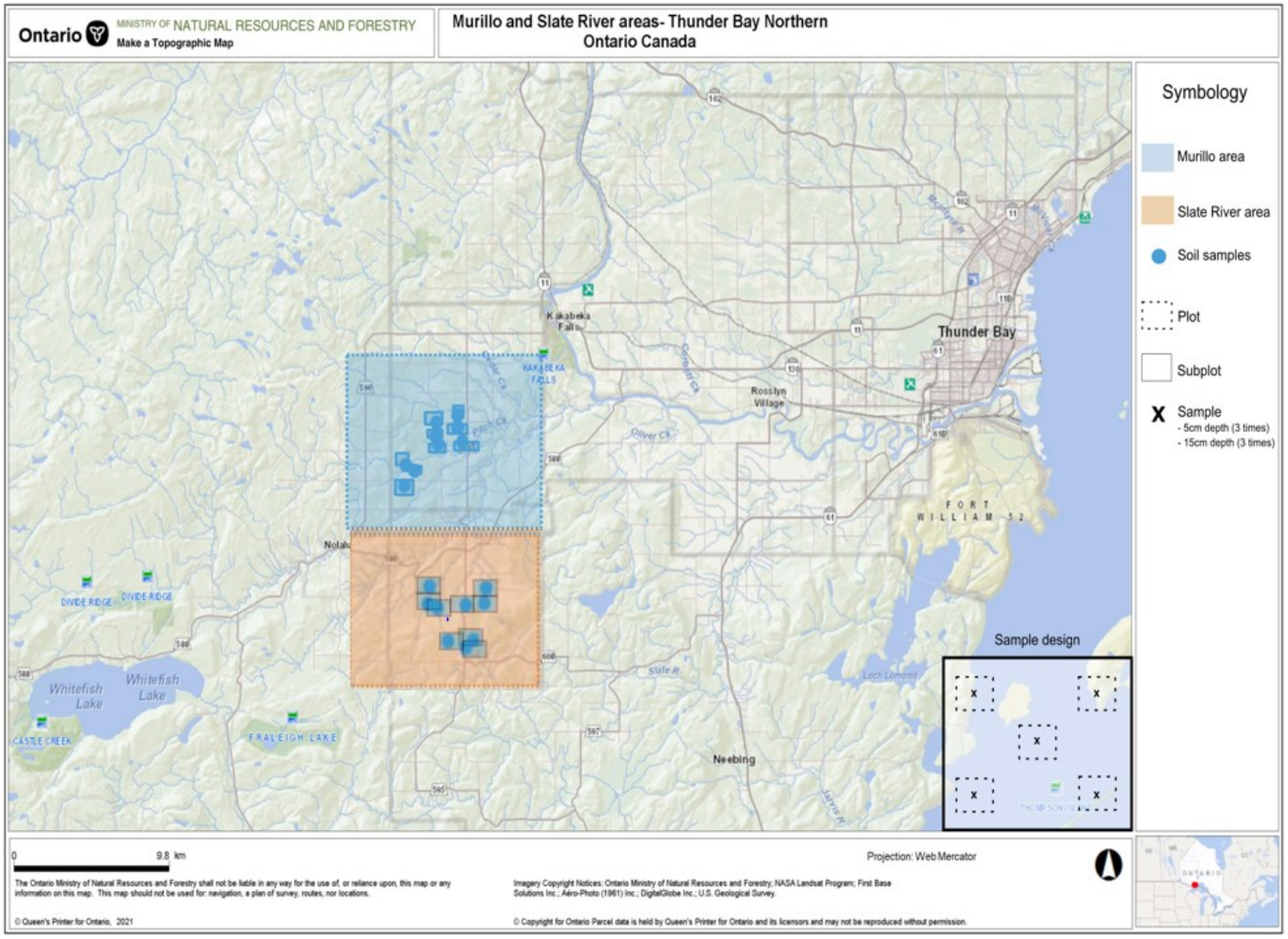

Figure 3.1. Soil samples collection in Thunder Bay from Murillo and Slate River agricultural areas in Northern Ontario, Canada. Base map source Ontario Ministry of Natural Resources and Forestry).

agricultural areas were merged, creating an all agriculture (BCD) category, and finally a dataset merging all categories included forest (ABCD). We did not consider differences in depth intervals since we had identified no significant depth effect in a prior study using the same dataset (Benalcazar et al., 2022); therefore, the results were averaged over the levels of depths include in each category.

Our final step was to select a model based on the prediction errors computed on new test data using the k-fold cross-validation technique that splits out test data to evaluate a model (Gareth at al., 2013). These analyses were conducted using R software (R Core Team, 2020), specifically the "leap" package (Lumley, 2020). Best models of different sizes were selected by using the "nvmax" option, which defines the maximum number of predictors to incorporate in a model. For our initial analysis, "nvmax = 3" was the setting. This function will return models of up to the best three variables: the best 1-variable model, the best 2-variable model, and the best 3-variable model. While this process offers simplicity for ease of interpretation, an optimal model with a more rigorous approach was also identified for confirmation. In this case, the k-fold cross-validation technique (k =10) refers to the number of groups that a given sample is to be split into a new test data to evaluate model performance (Refaeilzadeh et al., 2009). The k-fold cross-validation has a lower bias than other methods (Brownlee, 2018). The function "train" in the "caret" package in R software was used to identify the best optimal model (Kuhn, 2014).

3.4 RESULTS

In forest areas the CASH overall soil health score determined by Benalcazar et al. (2022) was 85.6/100 (Table 3.1). According to the BSR approach, the single variable that was most influential on the overall soil health score was total C (Table 3.2). Considering the best two-

variable model, we found that respiration and subsurface hardness were the most predictive of overall health score. The best three-variable model was organic matter, Mg, and total C. In fields <10 y in agriculture, the overall soil health score was 76.9Table 3.1) with the BSR analysis identifying POXC as the best predictor of the overall soil health score. In the two-variable model, POXC and P were the best predictors, while ACE-Protein, K, and Mg were the best predictors in a three-variable model (Table 3.2). In fields >10 to <50 y since agricultural conversion, the overall CASH soil health score was 79.4 (Table 1). The best predictors were organic matter for a single-variable model, Zn and total N for a two-variable model, and wet aggregate stability, surface hardness and POXC for the three-variable BSR model (Table 3.2). In fields >50 y in agriculture the overall CASH soil health score was 79.4 (Table 1) and Mn was the best predictor of soil health score, while wet aggregate stability and surface hardness were in the best two-variable BSR model. In the three-variable model, the combination of respiration, wet aggregate stability and surface hardness was the most predictive (Table 3.2).

Table 3.1 Overall soil health score for field samples by time since conversion (Benalcazar et al., 2022) including new scores for >10 y but <50 agriculture, combined scores for agriculture, and combined scores for all agriculture and forest.

Time since conversion	Overall soil health score
A	85.6
B	76.9
C	79.4
D	79.4
BCD	79.3
ABCD	80.6

Forest = A, sites <10 y in agriculture = B, sites >10 to < 50 y agriculture = C, sites >50 y agriculture = D

Table 3.2 Best subset regression (BSR) model identified for forest, <10 y in agriculture, >10 to <50 y in agriculture, >50 y in agriculture, and all agriculture composite using the Comprehensive Assessment of Soil Health (CASH) framework as predictors of the overall health score.

Time since conversion	Vars	R^2 adj	WAS	Sur_H	Sub_H	OM	Ace-Protein	Resp	POXC	pH	P	K	Mg	Mn	Zn	Total C	Total N
Forest	1	0.58														*	
	2	0.68			*			*									
	3	0.75				*							*			*	
< 10 y agriculture	1	0.57							*								
	2	0.75							*		*						
	3	0.85					*					*	*				
> 10 to< 50 y agriculture	1	0.61				*											
	2	0.77													*		*
	3	0.87	*	*					*								
> 50 y agriculture	1	0.52												*			
	2	0.65	*	*													
	3	0.74	*	*				*									
All agriculture	1	0.46						*									
	2	0.57		*				*									
	3	0.61		*				*		*							
All dataset	1	0.52						*									
	2	0.66			*			*									
	3	0.68			*			*		*							

± WAS, Wet aggregate stability; Sur_PR, Surface hardness; Sub_SPR, Subsurface hardness; OM, organic matter; Prot, ACE-Protein; Resp, soil respiration; POXC, active carbon; Total C, total carbon; total nitrogen.

With k-fold cross-validation, the optimal best model for the forest included organic matter and POXC; for <10 y in agriculture, the variables were ACE-Protein and Mg; for >10 to <50 y in agriculture, the best predictor was organic matter; and for >50 y in agriculture, surface hardness and wet aggregate stability were the best predictors. Finally, when all sites were combined, surface hardness and soil respiration were the best predictors of the overall soil health

score (Table 3.3, S6). To aseess how well our regresion models fits the dataset, we found that all agriculture dataset (BCD) had a mean square error (RMSE) of 4.5, while <10 y agriculture (B) had at RMSE of 498.0 (Table S7).

Table 3.3 The most predictive indicators of soil health in boreal soils at the local level using k-fold cross-validation analysis.

Soil indicators	Soil function	Categories						References
		A	B	C	D	BDC	ABCD	
OM	Nutrient cycling/soil resistance/ soil resilience/water cycling/carbon sequestration/habitat provision	*		*				(Allen et al., 2011; Lal, 2016, 2021; Lehmann et al., 2020)
POXC	crop production/water quality / climate control/human health/ habitat provision	*						(Allen et al., 2011; Brady & Weil, 2016; Lehmann et al., 2020; Reicosky, 2018, Lucas & Weil, 2021)
ACE-Protein	crop production/ water quality/climate control		*					(Hurisso et al., 2018; Lehmann et al., 2020)
Soil respiration	crop production/ water quality/ climate control/human health					*	*	(Laganière, Paré, Bergeron, & Chen, 2012; Lehmann et al., 2020; Yiqi & Zhou, 2010)
Aggregate stability	crop production/water quality/climate control				*			(Allen et al., 2011; Brady & Weil, 2016; Lehmann et al., 2020; Mikha & Wills, 2021)
Hardness (0-15 cm and 15-30cm)	crop production/water quality/habitat provision				*	*	*	(A. S. Gregory et al., 2007; Lehmann et al., 2020)
Mg	crop production/ water quality/human health		*					(Jones Jr, 2012; Lehmann et al., 2020; Strawn, Bohn, & O'Connor, 2019)

Forest = A, <10 y agriculture = B, >10 y to <50=C, >50 y agriculture =D

3.5 DISCUSSION

Soil disturbance associated with land conversion drives changes in biological, physical and chemical soil properties (Karlen et al., 2021). Of the 16 CASH soil health indicators analyzed, we found that seven indicators were the most predictive of overall soil health in the boreal soils of Thunder Bay- Ontario. Four biological indicators (organic matter, soil respiration, ACE-Protein, POXC), two physical indicators (wet aggregate stability and surface hardness) and one chemical indicator (Mg) were the best predictors of soil health, according to the k-fold cross-validation optimal model. Models with a maximum of three variables with relatively high predictability are efficient in assessing soil health scores with minimum analyses.

After land conversion, soil organic matter belowground decreased from levels observed in intact forest soils. Tillage and conventional farm management practices accelerate soil organic matter decomposition (Guo & Gifford, 2002). In addition, environmental factors such as wind and runoff cause increasing soil erosion as vegetation cover decreases (Lal, 2009, 2016). Here and elsewhere, soil organic matter has been identified as a key indicator for evaluating soil health functions (Cantone & Schmidt, 2011; Lehmann et al., 2020). Several studies have shown a positive correlation between organic matter and other biological soil health indicators such as soil respiration, ACE-Protein and POXC (Amsili et al., 2021; Congreves et al., 2015; Nunes et al., 2021).

Soil respiration, ACE-Protein, and POXC are usually used to detect seasonal changes (Nunes et al., 2018; van Es & Karlen, 2019), and they exhibit the same pattern as organic matter (Table 3.2). Soil respiration measures microbial metabolic activity, which releases labile carbon by mineralization (Zibilske, 1994). Soil respiration rates are generally a function of autotrophic and heterotrophic soil biota (Vargas et al., 2011), whose activity influences vital soil ecological functions. In concert with ongoing climate change, rates of soil organic matter turnover and mineralization are predicted to significantly increase in boreal soils. Therefore, determination of soil microbial community response to changes in temperature, moisture and aeration will become increasingly important for evaluating soil health (Hirsch et al., 2002; Laganière et al., 2012). In agricultural soils, soil respiration is an effective indicator because it increases when residues, amendments, reduced tillage, and increased crop diversity are implemented to agricultural fields (Amsili et al., 2021; NCRS, 2014). Also reduced disturbance like no-tillage and crop diversity increased soil respiration (Mitchell et al., 2017), stimulating microbial community activity (Moebius-Clune et al., 2017).

Active carbon (POXC), identified as a labile fraction of total organic carbon (Culman et al., 2012; NCRS, 2014a; Weil et al., 2003), is sensitive to management induced change in soil carbon by cultural practices such as tillage, crop rotation, cover crops, and is correlated with other indicators like wet aggregate stability, microbial biomass, and basal soil respiration (Lucas & Weil, 2021). Furthermore, POXC is an indicator of soil microbiology activity, itself related to a soil's ability to decompose organic residues (Weil et al., 2003). Therefore, in boreal soils, active carbon could be an indicator used to predict changes to management practices where soil organic amendments might enhance soil functions (Weil et al., 2003).

ACE-Protein contains much of the organically bound soil nitrogen. ACE-Protein becomes available in the soil solution as NH_4^+ and NO_3^- through mineralization leading to optimal moisture and temperature conditions for plant uptake (Gil-Sotres et al., 2005; Moebius-Clune et al., 2017). ACE-Protein is related to several soil functions (Table 3.2), reflecting potential N supply for subsequent crop production, and it is negatively impacted by management practices like intensive tillage (Hurisso et al., 2018). In an agricultural context, the potential for organic matter mineralization can be evaluated with the C:N ratio that is adjusted with plant residues and manure application (Mullen, 2011). Wide C:N ratios can be indicative of greater rates of immobilization. When compared to other soils, boreal soils have wider C:N (Cleveland & Liptzin, 2007; Tipping, Somerville, & Luster, 2016).

Wet soil aggregate stability has decreased since forest conversion to agriculture (Table 3.2). Aggregate stability, the soil's ability to resist dispersion and erosion, can be affected by tillage practices, exposing soil organic matter to oxidation and accelerated microbial decomposition (Helfrich et al., 2006). Tillage influences other hydrological functions that mediate soil water content, detention and transmission, soil organic matter and soil biota

(Reicosky, 2018). Evaluation of soil aggregate stability provides an indicator of C sequestration, revealing changes in the structure and composition of the microbial community, pore surface area, and soil development (Guo et al., 2020). In agricultural areas, increases in soil aggregation increase resilience to extreme weather conditions (Brady & Weil, 2016). They can be the best indicator of soil drainage and sufficient soil aeration (O'Neill et al., 2021). Surface and sub-surface hardness are related to the degree of compaction and increases with heavy equipment operation. As a result of soil compaction, water and plant roots are restricted, impacting soil hydrological and ecological functions. Studies in the boreal region indicate that high surface hardness provides evidence of poor root system development and aeration, rendering crops more susceptible to disease. Soil infiltration, percolation and soil water content will change with increased precipitation rates and quantities associated with climate change in the boreal region (Climate Atlas, 2019).

As an essential plant macronutrient, soil Mg can affect nutrient availability (Moebius-Clune et al., 2017) and increased following conversion of forest to agriculture (Benalcazar et al., 2022). Magnesium is positively correlated with total N, total C, respiration, and POXC in northwestern Ontario boreal soils (Benalcazar et al., 2022). Greater Mg can be explained by interactions between mineral soil ions and those of soil organic matter (Congreves et al., 2015), and may reflect soil pH changes after land conversion that enhance available Mg. Magnesium can be used in combination with other factors such as temperature, water availability and land use to determine carbon stock (Wiesmeier et al., 2019) which is likely to decrease following conversion of forests to agriculture. Across the gradient of age since land conversion and two scenarios, seven soil health indicators are found the most predictive of overall soil health scores on agricultural and forest areas in northwestern Ontario. Forest conversion to agriculture and

length of time in agriculture play an important role in overall soil health score. The important soil health variables in Thunder Bay- Ontario include organic matter, soil respiration, ACE-Protein, active carbon, wet aggregate stability, surface hardness, and Mg, which together span the range of biological, physical, and chemical indicators.

3.6 CONCLUSIONS

Soil disturbance is an inevitable consequence of agricultural conversion from forests. After more than 50 years in boreal dairy farm systems, a region dominated by short growing seasons and long winters, the indicators most predictive of overall soil health score in northwestern Ontario are related to soil carbon. Our results have implications for agriculture beyond this region, suggesting a list of indicators that account for long- and short-term soil sustainability, including tracking the implications of climate change on soil health. This new set of variables is an example of a minimum dataset of soil health that could efficiently be used to monitor soil health with relatively high predictability.

CHAPTER 4 THE IMPACT OF LAND CONVERSION FROM BOREAL FOREST TO AGRICULTURE BY ASSESSED MICROBIAL COMMUNITY FUNCTIONAL CAPACITY BY qPCR AND SOIL SEQUENCING BY OTU [2]

4.1 ABSTRACT

Healthy soils result from a healthy ecosystem where microorganisms play an essential role. Climate trends suggest that new agricultural opportunities continuously arise in boreal soils, posing an urgent need to understand the impacts of land conversion to agriculture. The characterization of soil community diversity, abundance and functional genes by quantitative qPCR and soil sequencing offer metrics for assessing soil functional changes after land conversion. In this study, we sampled soil in a four-time series (forest, <10 y, >10 and <50 y, and >50 y in agriculture) and at two depths (0-5 and 5-15 cm). We assessed microbial community functional capacity by qPCR of key genes associated with C, N cycling and P mineralization and communities for archaeal, bacterial and fungal microorganisms. In addition, soil sequencing by the operational Taxonomic Unit (OTU), bacterial and fungal taxonomic abundance. We observed impacts on soil microbial abundance and composition. Microbial biomass decreased after land conversion affecting archaeal and fungal community abundance. In addition, land conversion altered the microbial functions related to carbon, nitrogen, and phosphorus cycling in ng-1 DNA soil. In soil sequencing by OTU, bacterial and fungal taxonomic abundance and composition changed, and bacterial communities showed greater abundance after land conversion. The five most abundant bacterial 16S phyla were *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, and *Gemmatimonadetes*. In general, our results reveal some of the impacts of land conversion on soil health in the Boreal region and add insight into a potential future with more sustainable practices in agriculture.

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[2] This chapter will be submitted to the journal *Agronomy for Sustainable Development.*

4.2 INTRODUCTION

Climate change is driving shifts in temperature and precipitation patterns that have accelerated land-use shifts and promoted new agricultural areas. As a result, boreal areas have become the new agriculture frontier (Altdorff et al., 2021). Healthy soils are influenced by interactions between physical, chemical, and biological soil attributes, management practices, climate, and other interactions that define soil functions (Lehmann et al., 2020; Zwetsloot et al., 2021). Soil functions include those related to carbon transformation, habitat provision, carbon sequestration, and biological population regulation (Kibblewhite et al., 2007). When land conversion changes natural vegetation cover to a cropping system, soil compaction, soil erosion, and salinization typically increase, whereas soil biomass, soil biotic abundance and diversity, and soil organic carbon decrease (Reicosky, 2018).

Changes in one soil function will inevitably modify other soil functions (Kibblewhite et al., 2007). For example, carbon transformation, regulated by primary agents (fungi and bacteria), is affected when the decomposition rate of organic matter changes. In addition, the diversity and composition of one soil community type may shift to another depending on the imposed agricultural management practices (Bevivino et al., 2014); these changes may be beneficial or detrimental depending on the imposed management practices (Merloti et al., 2019; Peltoniemi et al., 2021). Practices such as tillage can alter organic matter decomposition rates and subsequent nutrient release to different microbial populations, as well as modify microbial habitats and disrupt their functions (Kibblewhite et al., 2007).

Land conversion from native systems to agriculture is known to affect soil microbiology and their functions (Fierer et al., 2021) Forest conversion for agricultural purposes will impact microbial communities (including archaea, bacteria, and fungi). Fully predicting the impacts of

land conversion on soil health requires understanding how soil microbial communities respond. For example, soil microbial communities related to N-cycle functions were altered after land conversion in one study based in Brazil, with denitrification and nitrification functional potential increasing in agricultural fields (Merloti et al., 2019). A global meta-analysis found that land conversion decreased the relative abundance of *Acidobacteria*, and *Actinobacteria*, but overall impacts on *Proteobacteria* and *Bactereoidetes* varied according to land-use change (Zhou et al., 2018). In addition, forest degradation decreased overall bacterial and fungal abundance, with total microbial carbon and nitrogen decreasing up to 56.7% and 54.5% (respectively) from forest to cropland. Another study comparing land-use changes in grassland, arable and permanent bare fallow found that *Verrucomicrobia* were more abundant in grassland, while *Gemmatimonadetes* and *Nitrospirae* were more abundant in bare fallow and arable soil (Hirsch et al., 2017). Shifts in microbial community structure alter soil function; different communities have a dominant impact on soil functions associated with soil carbon-decomposition (*Acidobacteria*, *Proteobacteria*, *Actinobacteria* , *Gemmatimonadetes*, *Bacteroides*) (Merloti et al., 2019; Pankhurst & Doube, 1997; Reicosky, 2018) and nitrogen cycling (*Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Nitrospira* active involved in the nitrogen fixation, while *Alphaproteobacteria* is part of the denitrification process) (Merloti et al., 2019; Pankhurst & Doube, 1997; Reicosky, 2018).

Several microbial-based soil health indicators can be used to characterize soil community composition and functions. Along with other indicators, these soil assessments measure overall biomass, the presence or absence of specific microorganisms, organic matter decomposition, and other aspects of carbon, nitrogen, and phosphorus cycling (Pankhurst & Doube, 1997). Pankhurst & Doube (1997) and Fierer et al. (2021) described a complete list of the common microbial soil

health indicators. Molecular analyses of microbial communities and functions, using sequencing and quantitative PCR (qPCR) approach, are considered emerging soil health metrics (Norris et al., 2020). Amplicon sequencing targeting ribosomal or internal transcribed spacer regions (16S rDNA and ITS, respectively) provides information on the relative abundance of different bacterial, archaeal, fungal or protist taxa and is used to understand how microbial community composition and diversity change. Quantitative PCR of the same gene regions can estimate the overall abundance of these different taxa. Similarly, shotgun metagenomic approaches and qPCR can provide information on the relative abundance of functional genes of interest, such as those involved in C and N cycling (Fierer et al., 2021).

Climate change has rendered northern regions a new area for food production; however, a broad and comprehensive scientific understanding of land conversion to agriculture on soil functions and microbial communities is currently incomplete and poorly understood (Li et al., 2019). Information on microbial diversity, structure and abundances of soil archaeal, bacterial and fungal communities in boreal soils is required. In this study, we investigated the microbiome in soils, comparing baseline mature forest conditions with those of fields converted from forest to agriculture <10 years ago, fields converted >10 years ago but <50 years ago, and fields converted from forest to agriculture >50 years ago. We used qPCR to assess changes in microbial nutrient cycling functions and amplicon sequencing to examine microbial community composition. We aimed to detect post-land conversion changes in various microbial community descriptors; i) functional potential, ii) diversity, and iii) community composition according to time since conversion. We hypothesized that (a) after land conversion functions associated with C cycling would decrease while those associated with N cycling would increase, (b) as the age of the agricultural fields increased the overall abundance of fungi would decrease while that of

bacteria would increase, and (c) overall diversity would increase with increased land-use diversity.

4.3 MATERIALS AND METHODS

4.3.1 Study sites and soil sampling

Sixty soil samples were collected from dairy farms in the Thunder Bay, Ontario region were sampled from July through August 2019 (45° 31' to 48° 17' N, 89° 30' to 89° 22' W) within the Murillo and Slate River area. Murillo and Slate River are formed from water-laid alluvial silt, sand, and gravel deposits that vary depending on the basin and depth (Mollard & Mollard, 1983). Thunder is part of the ecoregion 3W (Lake Nipigon), located on the Precambrian Shield with substantial basalt and volcanic rock formations with low to moderate buffering capacity (Wester, Henson, Crins, Uhlig, & Gray, 2018). Soils include high water retention capacity, low permeability and poor drainage (Mollard & Mollard, 1983). Each farm operation in the sample included a mature mixed-wood forest, fields that have been cultivated for less than ten years (<10 y in agriculture), fields between >10 and <50 y in agriculture, and fields that have been cultivated for more than 50 years (>50 y agriculture). Cleared sites have been under conventional management systems with tillage, synthetic fertilizers, spring and fall manure applications, and crop rotations of alfalfa, barley, corn, canola, and spring and winter wheat (Chapter 2, Benalcazar et al., 2022).

Our sampling strategy included soil depths of 0-5 cm and 5-15 cm in two areas (Murillo and Slate River). We collected soil samples using a soil split-core sampler (AMS Soil Sampler, Inc., American Fall, Idaho) at each cropped field and adjacent forests considering time since conversion to agriculture (soil samples were collected at each corner in the center). The O horizon, largely plant litter, was removed in forest areas. At each location, we collected a total of

three sub-samples: sampling three times in the mineral soil, divided by depth (0-5 and 5-15 cm) and composited. We kept the soil samples cool (4 °C) while transporting them to the laboratory, then we sieved each sample first through 8 mm and then through 4 mm sieves to homogenize. We froze the soil samples at -40°C and analyzed composite soil samples of equal depth from each site at the Agriculture and Agri-Food Canada (AAFC), Harrow Research Center, Microbial Ecology Laboratory, in Harrow, Ontario.

4.3.2 Microbial community and functions via qPCR

DNA was extracted in duplicate (2 x 0.25 g) from soil samples using Qiagen DNeasy PowerSoil kits (Qiagen Inc., Canada), except for an added 10-min incubation at 65°C before bead-beating. DNA quality was visualized on a 1.4% agarose gel; quantity and quality were further evaluated using spectrophotometry (NanoDrop One, ThermoScientific). Once extraction reproducibility was confirmed, replicate extractions for each soil sample were pooled, and DNA was re-quantified using fluorometric approaches (Quant-iT™ dsDNA BR kits; Life Technologies), normalized to an initial working concentration of 10 ng μL^{-1}. For qPCR analyses, DNA was further diluted to a working concentration of 3.0 ng μL^{-1} and then re-quantified using a high-sensitivity fluorometric assay (Quant-iT™ dsDNA HS kits; Life Technologies, USA).

We assessed microbial community functional capacity by qPCR of key genes associated with C cycling, N cycling, and P mineralization (Table 4.1; Table S10). In addition, we estimated the relative abundance of bacteria, archaea, and fungi using 16S rRNA and 18S gene regions. We performed assays in triplicate using the Bio-Rad CFX384 Touch Real-Time PCR Detection System (Bio-Rad, USA) in 5 µl reaction volumes containing 3.0 ng DNA (functional genes) or 0.3 ng DNA (taxonomic genes), and 0.12 µg of UltraPure™ BSA (Life Technologies, USA), with primer concentrations and commercial master mixes and primer concentration as listed in

Table 4.1. Gene abundance was calculated with respect to a plasmid-based standard curve with a concentration range from 2 to 2×10^7 gene copies μl^{-1} DNA. Reaction efficiency ranged from 85 to 100%. Plasmids were generated by amplifying soil DNA in 50 µL PCR reactions using the appropriate primers (Table 4.1) and GoTaq master mix (Promega, USA), subcloning the cleaned (QIAquick PCR Purification Kit, Qiagen Inc., USA) PCR products into competent TOP10F' One Shot *E. coli* (Invitrogen, USA), and extracting plasmids from positive clones (QIAprep Spin Miniprep Kit, Qiagen Inc., USA). Plasmids were sequenced (AAC, University of Guelph) and analyzed by *blastn* to confirm plasmid insert identity.

4.3.3 Sequencing microbial community

The amplicon sequencing strategy we used follows the approach developed under the Government of Canada's Genomics Research and Development Initiative (GRDI), EcoBiomics Project (Edge et al., 2020). Briefly, the primers for bacteria and archaea target the V4-V5 hypervariable region of the 16S rDNA gene 515FY/926R (Parada et al., 2016; Thompson et al., 2017). The primers for fungi target the ITS2 (ITS9F/ITS4R) region (Ihrmark al., 2012; White et al., 1990). A 10 ng µL-1 aliquot of each composited DNA sample was shipped on ice to Genome Quebec for library preparation and paired-end sequencing (2 x 250 bp) on an Illumina NovaSeq6000 platform. Bioinformatic analysis was performed on the General Purpose Science Cluster in Dorval, Quebec (Edge et al., 2020), using a custom workflow as outlined in Pérez-Guzmán et al. (2021).

4.3.4 Statistical analysis

4.3.4.1 *Microbial community and functions via qPCR*

To evaluate the differences between land conversion, we conducted Shapiro-Wilk and Levene tests to check for data distribution and homoscedasticity. qPCR data were analyzed on a

copy number ng^{-1} DNA basis, placed in Supplementary materials (Tables S11-S20). As fixed factors, we used time since conversion (A= forest, B = cleared <10 y in agriculture, C = >10 and <50 y in agriculture and D = >50 y in agriculture); and two depth intervals (0-5 cm, and 5-15 cm). Since depth was a significant factor for qPCR data, we further applied one-way ANOVA followed by the Tukey *post hoc test* ($p < 0.05$). We applied the Kruskal-Wallis test for non-normal Gaussian distribution data, followed by the Wilcoxon test using the p-value adjustment Bonferroni method. The package used was "pgirmess" (Giraudoux, 2021) in R software (R Core Team, 2020).

4.3.4.2 *Sequencing microbial community*

Bacterial 16S rDNA and fungal ITS datasets were summarized at the class level. We used the non-parametric multidimensional scaling (NMDS) method for the complete dataset using time since conversion and depth intervals. We used the Wisconsin double standardization to generate the distance matrix reflecting the distance between each site's pairs. Then we used the Bray-Curtis dissimilarity index to quantify the composition dissimilarity between time since conversion of the bacterial 16S rDNA and fungi ITS. We determined dissimilarities for factor variables using the Adonis function in the Vegan package, R software. Additionally, we performed univariate community analysis using the Shannon diversity and Simpson Index for fungal and bacterial diversity, using ANOVA, or Kruskal-Wallis test, followed by *post hoc* test using Tukey HSD or pairwise Wilcoxon test using the p-value adjustment Bonferroni method. Finally, we conducted a one-way ANOVA for normal data or a Kruskal-Wallis test for non-parametric tests, followed by a *post hoc* test.

4.4 RESULTS

4.4.1 The overall change in archaeal, bacterial, fungal, and functional microbial biomass and abundance since conversion

Microbial biomass decreased after land conversion from forest 5.35E+05 to agriculture 3.08E+05 ng DNA/g dry soil. Microbial abundance varied by time since conversion and by soil depth intervals. The relative abundance of archaeal 16S ($p < 0.05$), and fungal 18S ($p < 0.001$) copies were influenced by time since conversion to agriculture along with soil depths, but Bacteria 16S did not show significant difference. Summary tables are provided for the ANOVA analysis of the functional genes targeted by qPCR, shown as copy numbers per ng-1 DNA (**Error! Reference source not found.**, 4.2) and for detailed statistical results (S11, S12). (phoD and phoC) decreased at 0-5 cm depth interval (Table 4.1) after land conversion but did not change at 5-15cm depth (Table 4.2).

Table 4.1 ANOVA analysis for fungal 18S archaeal 16S copies per ng-1 DNA soil, and functional genes targeted by qPCR on copy number per ng-1 DNA at 0-5 cm depth intervals, in time since conversion. Means values are presented, and significant differences ($p < 0.05$) are denoted by lowercase letters.

Copies ng-1 DNA soil	*p* values	Time since conversion (Categories)								Average (+)
		forest		< 10 y agriculture		>10 to <50 y agriculture		>50 y agriculture		increase/decrease
Archael 16S	< 0.001	3.83E+03	b	6.62E+03	ab	8.49E+03	ab	1.12E+04	a	64%
Bacteria 16S	0.21	1.90E+06	a	2.14E+06	a	2.19E+06	a	2.04E+06	a	2%
Fungal 18S	< 0.001	1.95E+04	b	3.60E+04	ab	3.51E+04	ab	4.07E+04	a	36%
Carbon cycling										
Laccase (multicopper oxidase-LMCO)	0.48	1.98E+04	a	2.04E+04	a	2.27E+04	a	2.24E+04	a	4%
Bglu (β-glucosidase)	0.02	1.83E+03	a	2.26E+03	ab	2.42E+03	ab	2.67E+03	a	15%
Cbhl (cellobiohydrolase)	< 0.001	1.48E+01	b	2.60E+01	a	1.94E+01	ab	2.19E+01	a	16%
Carbon + Nitrogen cycling										
gh11 (glycoside hydrolase)	< 0.001	4.52E+01	b	9.71E+01	a	9.04E+01	a	9.03E+01	a	33%
APR (alkaline metallopeptidase)	0.18	7.10E+03	a	6.11E+03	a	6.38E+03	a	5.88E+03	a	-6%
Nitrogen cycling										
A-amoA- (ammonium monooxygenase)	< 0.001	2.49E+02	c	3.44E+02	bc	1.04E+03	a	7.60E+02	ab	68%
B-amoA- (ammonium monooxygenase)	< 0.001	5.15E+01	b	6.29E+02	a	7.78E+02	a	8.43E+02	a	512%
nxrA (nitrite oxidoreductase)	< 0.001	9.09E+00	b	1.83E+02	a	8.28E+01	a	1.08E+02	a	363%
narG (membrane bound nitrate reductase)	0.84	3.46E+05	a	3.23E+05	a	3.30E+05	a	3.32E+05	a	-1%

nrfA (dissimilatory nitrite reductase)	0.05	8.15E+03	a	5.06E+03	a	7.78E+03	a	7.86E+03	a	-1%
nirK (copper-containing nitrite reductase)	0.63	3.79E+04	a	3.46E+04	a	3.87E+04	a	3.87E+04	a	-1%
aNirK (nitrite reductase)	0.12	5.46E+01	a	3.90E+01	a	4.29E+01	a	5.27E+01	a	-1%
NosZ (nitrous oxide reductase)	0.27	5.70E+03	a	5.18E+03	a	6.16E+03	a	6.39E+03	a	4%
Phosphorus mineralization										
phoC (acid phosphatase)	< 0.001	7.09E+03	a	5.01E+03	b	4.53E+03	b	4.82E+03	b	-11%
phoD (alkaline phosphatase)	< 0.001	2.29E+05	a	1.75E+05	b	1.93E+05	ab	1.76E+05	b	-8%
Phnx (Phosphono-acetaldehyde hydrolase)	0.34	1.44E+03	a	1.44E+03	a	1.53E+03	a	1.69E+03	a	6%
pqqC (pyrroloquinoline quinone)	0.36	1.14E+04	a	1.22E+04	a	1.06E+04	a	1.05E+04	a	-3%

(+) average increased/decreased by time since conversion to agriculture (Categories)

Table 4.2 ANOVA analysis for fungal 18S archaeal 16S copies per ng-1 DNA soil, and functional genes targeted by qPCR on copy number per ng-1 DNA soil at 5-15 cm depth interval, in time since conversion. Means values are presented, and significant differences (p <0.05) are denoted by lowercase letters.

Copies ng-1 DNA soil	***p* values**	**Time since conversion (Categories)**								**Average(+)**
		Forest		**<10 y agriculture**		**>10 to <50 y agriculture**		**>50 y agriculture**		**increased/decreased**
Archael 16S	0.01	4.03E+03	b	6.28E+03	ab	6.77E+03	ab	7.81E+03	a	31%
Bacteria 16S	0.19	1.95E+06	a	2.27E+06	a	2.09E+06	a	2.01E+06	a	1%
Fungal 18S	< 0.001	1.30E+04	b	3.17E+04	a	2.10E+04	ab	2.48E+04	a	30%
Carbon cycling										
Laccase (multicopper oxidase-LMCO)	0.46	2.40E+04	a	2.24E+04	a	2.53E+04	a	2.04E+04	a	-5%
Bglu (β-glucosidase)	0.3	2.87E+03	a	2.22E+03	a	2.81E+03	a	3.53E+03	a	8%
Cbhl (cellobiohydrolase)	0.05	1.18E+01	a	2.74E+01	a	1.44E+01	a	1.43E+01	a	7%
Carbon + Nitrogen cycling										
gh11 (glycoside hydrolase)	< 0.001	3.74E+01	b	8.20E+01	a	6.45E+01	a	6.31E+01	a	23%
APR(alkaline metallopeptidase)	0.71	7.48E+03	a	6.61E+03	a	6.60E+03	a	6.82E+03	a	-3%
Nitrogen cycling										
A-amoA- (ammonium monooxygenase)	< 0.001	4.36E+02	c	4.66E+02	bc	1.18E+03	a	1.05E+03	ab	47%
B-amoA-(ammonium monooxygenase)	< 0.001	9.40E+01	b	3.22E+02	a	3.49E+02	a	4.54E+02	a	128%
nxrA (nitrite oxidoreductase)	< 0.001	1.03E+01	b	9.88E+01	a	4.92E+01	a	9.79E+01	a	285%
narG(membrane bound nitrate reductase)	0.74	3.66E+05	a	3.38E+05	a	3.49E+05	a	3.56E+05	a	-1%
nrfA (dissimilatory nitrite reductase)	0.29	7.93E+03	a	5.56E+03	a	6.87E+03	a	7.62E+03	a	-1%
nirK(copper-containing nitrite reductase)	0.97	3.90E+04	a	3.90E+04	a	4.03E+04	a	3.91E+04	a	0%
aNirK (nitrite reductase)	0.18	4.77E+01	a	4.98E+01	a	8.21E+01	a	1.35E+02	a	61%
NosZ (nitrous oxide reductase)	0.89	5.97E+03	a	5.76E+03	a	5.67E+03	a	6.14E+03	a	1%
Phosphorus mineralization										
phoC (acid phosphatase)	0.11	6.85E+03	a	5.77E+03	a	5.40E+03	a	5.43E+03	a	-7%
phoD (alkaline phosphatase)	0.24	2.47E+05	a	1.95E+05	a	1.95E+05	a	2.07E+05	a	-5%
Phnx (Phosphono-acetaldehyde hydrolase)	0.38	1.48E+03	a	1.48E+03	a	1.18E+03	a	1.69E+03	a	5%
pqqC (pyrroloquinoline quinone)	0.3	1.13E+04	a	1.17E+04	a	9.99E+03	a	1.09E+04	a	-1%

4.4.2 Microbial richness and community composition

A total of 22,734,81 16S rDNA paired-end amplicon sequences were generated from 60 samples, with 23,995 operational taxonomic units (OTUs; 98% nucleotide identity) identified after quality filtering and clustering. A total of 17,238,05 ITS paired-end amplicon sequences were generated, with 16,082 OTU's (98% nucleotide identity) identified after quality filtering and clustering. Detailed information about the bacterial 16S and Fungal ITS OTU data can be found in Supplementary material Table S21. Unique microbial communities developed after land conversion. Bacterial diversity increased over time as the system shifted to one with more readily bioavailable C and N pools, but fungal diversity increased then decreased after land conversion. Bacterial 16S Shannon diversity, assessed by OTU read, significantly increased after land conversion (p <0.05) at both depth intervals (Figure 4.1; Tables S22, S23). Fungal ITS did not change after land conversion (Figure 4.1; Tables S24, S25). Moreover, bacterial 16S Simpson index significantly increased at 0-5 cm depth interval (p <0.001; Figure 4.1; Table S22). Fungal ITS did not show significant change (Table S24, S25).

An NMDS approach with PERMANOVA posthoc was used to evaluate how overall microbial community composition shifted after forest conversion (Figure 4.2; Table S26, S27). For bacterial communities in the surface soils, forest systems and agricultural systems < 10 years and > 50 years since conversion showed significantly different clustering patterns ($p < 0.02$). In the sub-surface soils (5-15cm) different community clusters only occurred for the forest systems and the <10-year system ($p <0.03$). For fungal communities, significantly different clustering patterns were observed between the forest and >50 year since conversion soils ($p < 0.001$).

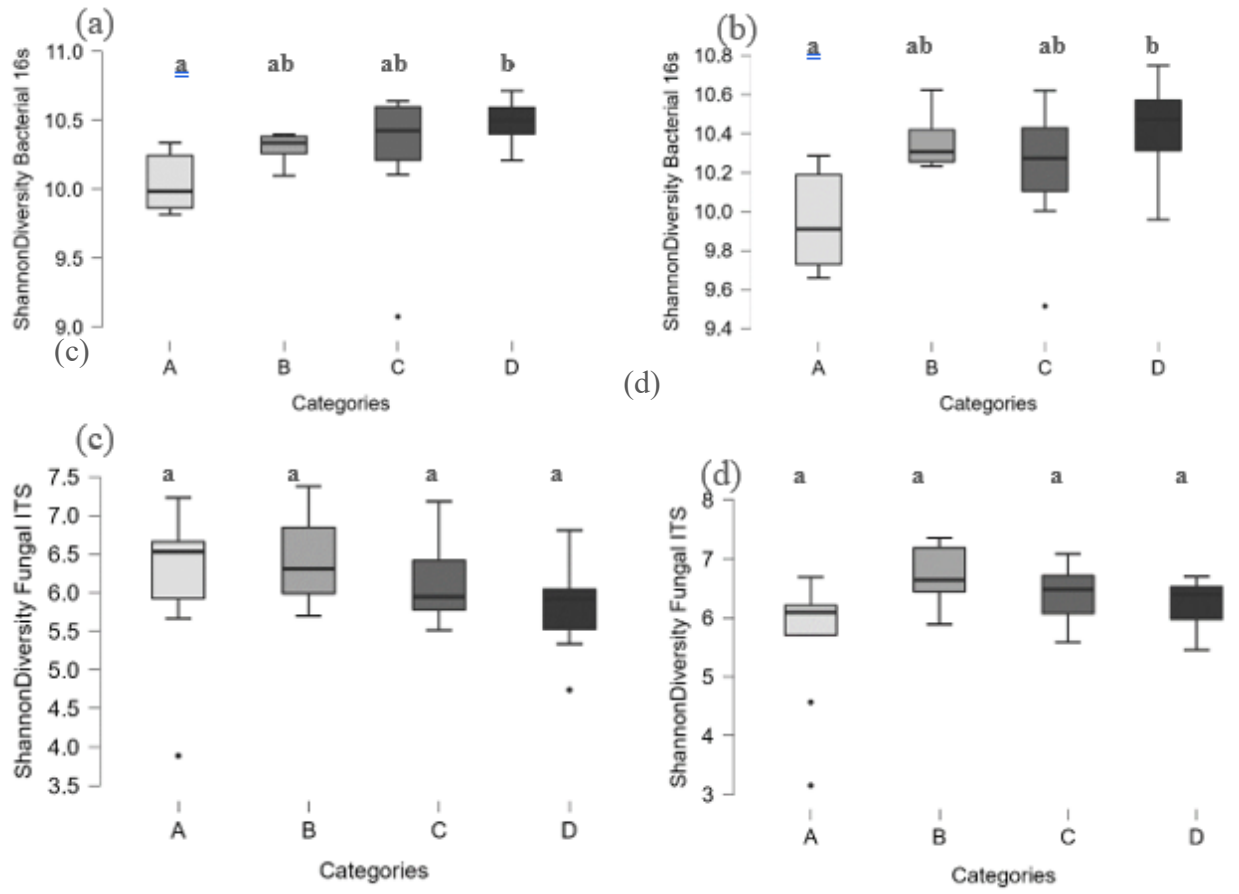

Figure 4.1 Shannon diversity in bacterial 16S at 0-5 cm depth interval (a), and 5-15 cm depth interval (b) and Fungal ITS at 0-5 cm depth interval (c), and 5-15 cm depth interval (d) in mineral soils collected in A = forest sites, B = sites < 10 y, C = >10 and <50 y, and D = > 50 y in agriculture. Bars are means +/- standards errors. Significant differences ($p < 0.05$) are denoted by lowercase letters.

4.4.3 Bacterial taxonomic distribution for time since conversion.

After land conversion, five bacterial phyla showed significant differences: *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, and *Gemmatimonadetes* (data not shown). At the class level, at both depth intervals, soils were dominated by *Acidimicrobiia*, *Actinobacteria, Gammaproteobacteria, Gemm.1, Gemmatimonadetes, MB.A2.108* and *Planctomycetia* (Tables 4.3). The relative abundance of *Gemm.1* and *Gemmatimonadetes* increased at both soil depth intervals. In contrast, the relative abundance of *MB.A2.108* decreased at both soil depth intervals (Tables 4.3).

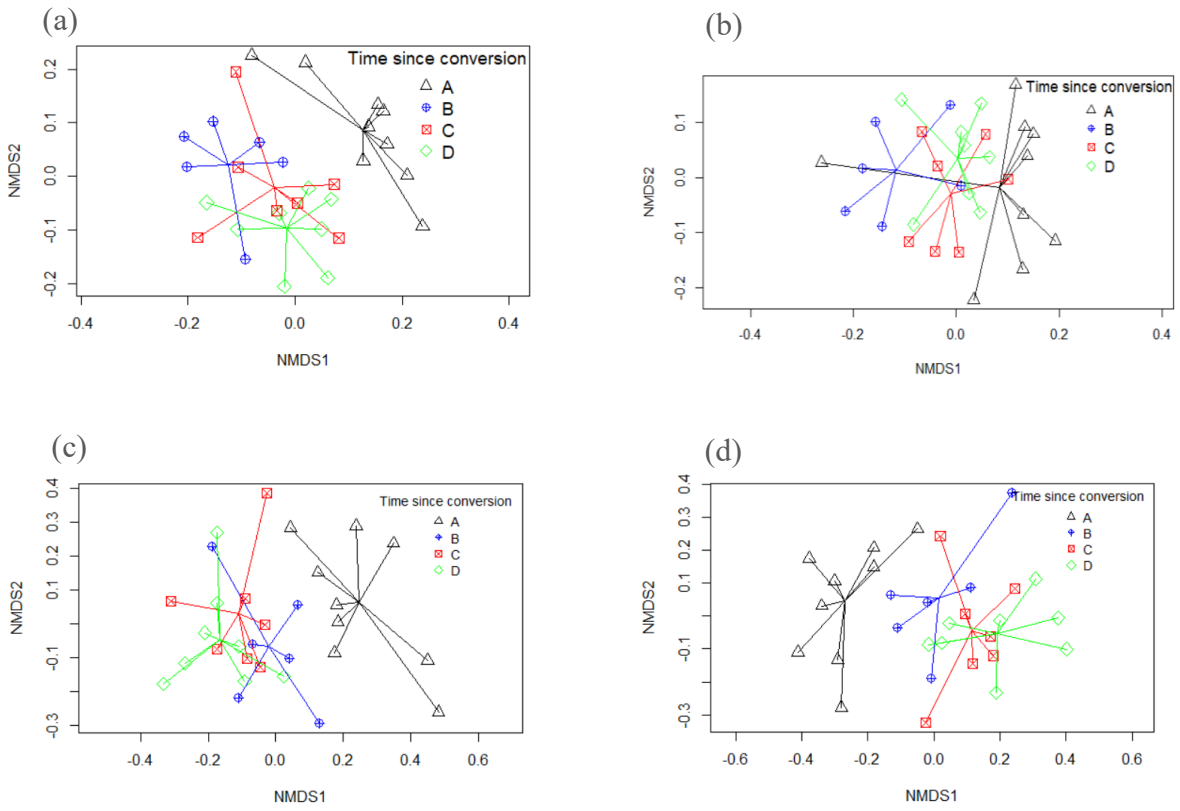

Figure 4.2 Non-metric multidimensional scaling (NMDS) analysis for bacterial 16S at 0-5 cm depth interval (a), 5-15 cm depth interval (b) and fungal ITS at 0-5 cm depth interval (c) and 5-15 cm depth interval (d) assessed by OTU reads by both depths. Time since conversion A= forest sites, and sites B = <10 y, C = >10 and <50 y, and D = >50 y in agriculture. Connected points to group centroid using ordispider produced by vegan package.

Average increased and decreased relative abundances by time since conversion to agriculture are shown in Tables 4.3, with additional information in S28, S29. In general, bacterial communities in the surface soils experienced more significant phylogenetic shifts than those in the sub-surface.

Table 4.3.4.2. ANOVA analysis for bacterial classes by time since conversion at 0-5 cm and 5-15 cm depth intervals. Means values are presented, and significant differences (p <0.05) are denoted by lowercase letters.

Depth 0-5 cm interval	p values	Time since conversion (Categories)				Average
Bacterial Classes		forest	< 10 y agriculture	>10<50 y agriculture	>50 y agriculture	increased/decreased
Actinobacteria - Acidimicrobiia	< 0.001	1.60E-02 a	1.17E-02 b	1.15E-02 b	1.04E-02 b	-12%
Acidobacteria - Acidobacteria.6	< 0.001	1.48E-01 a	8.67E-02 ab	1.14E-01 ab	1.24E-01 b	-5%
Acidobacteria - Acidobacteriia	0.04	6.57E-03 a	1.94E-02 b	1.03E-02 b	7.03E-03 b	2%
Actinobacteria - Actinobacteria	< 0.001	6.04E-02 b	9.44E-02 a	8.79E-02 a	9.21E-02 a	17%
Proteobacteria - Alphaproteobacteria	0.01	1.41E-01 a	1.42E-01 ab	1.08E-01 b	1.07E-01 b	-8%

Firmicutes - Clostridia	0.04	5.37E-03	b	1.25E-02	ab	1.05E-02	ab	1.44E-02	a	56%
Proteobacteria - Deltaproteobacteria	< 0.001	4.34E-02	a	2.88E-02	b	3.40E-02	b	3.34E-02	b	-8%
Chloroflexi - Ellin6529	0.04	1.79E-02	b	2.13E-02	ab	2.21E-02	ab	2.32E-02	a	10%
Gemmatimonadetes - Gemm.1	< 0.001	4.04E-03	b	6.41E-03	ab	7.76E-03	ab	1.00E-02	a	49%
Gemmatimonadetes - Gemmatimonadetes	< 0.001	6.89E-03	b	2.17E-02	a	1.51E-02	ab	1.61E-02	a	45%
Actinobacteria - MB.A2.108	< 0.001	1.36E-02	a	6.29E-03	b	7.31E-03	b	6.58E-03	b	-17%
Planctomycetes - Planctomycetia	0.02	3.37E-02	a	3.03E-02	ab	2.83E-02	ab	2.67E-02	b	-7%
Actinobacteria - Thermoleophilia	0.01	1.38E-01	a	1.30E-01	ab	1.27E-01	ab	9.95E-02	b	-9%
Depth 5-15 cm intervals										
Actinobacteria - Acidimicrobiia	< 0.001	1.44E-02	a	1.16E-02	b	1.07E-02	b	1.11E-02	b	-8%
Actinobacteria - Actinobacteria	0.03	4.73E-02	b	6.91E-02	a	5.49E-02	ab	5.66E-02	ab	7%
Proteobacteria - Gammaproteobacteria	0.03	4.27E-02	b	6.29E-02	ab	4.44E-02	ab	6.40E-02	a	17%
Gemmatimonadetes - Gemm.1	0.01	8.99E-03	b	9.07E-03	b	1.14E-02	ab	1.71E-02	a	30%
Gemmatimonadetes - Gemmatimonadetes	< 0.001	8.91E-03	b	1.60E-02	ab	1.90E-02	ab	1.66E-02	ab	29%
Actinobacteria - MB.A2.108	<0.001	2.05E-02	a	7.75E-03	b	1.19E-02	b	1.15E-02	b	-15%
Planctomycetes - Planctomycetia	0.03	2.62E-02	ab	3.17E-02	a	2.61E-02	ab	2.26E-02	b	-5%

(+) average increased/decreased by time since conversion to agriculture (Categories)

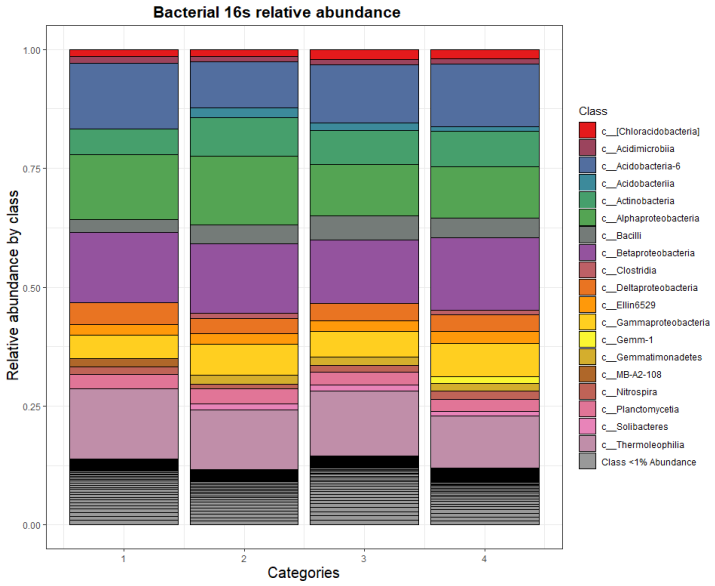

Figure 4.3 Bacteria16S class relative abundance: Sequencing of samples obtained during summer 2019. Time since conversion categories from left to right are forest (1), <10 y in agriculture (2), >10 and <50 y agriculture (3), and > 50 y in agriculture (4) for both depth intervals together.

4.4.4 Fungal taxonomic distribution for time since conversion

Land conversion to agriculture significantly affected fungal phyla *Ascomycota* and *Basidiomycota* (data not shown). At the class level, the soil was dominated by *Agaricomycetes, Dothideomycetes, Eurotiomycetes, Leotiomycetes,* and *Sordariomycetes* (Figure 4.4). At the class level, the relative abundance of *Agaricomycetes* decreased while *Dothideomycetes* and *Sordariomycetes* increased at both depth intervals (Tables 4.4; S30, S31)

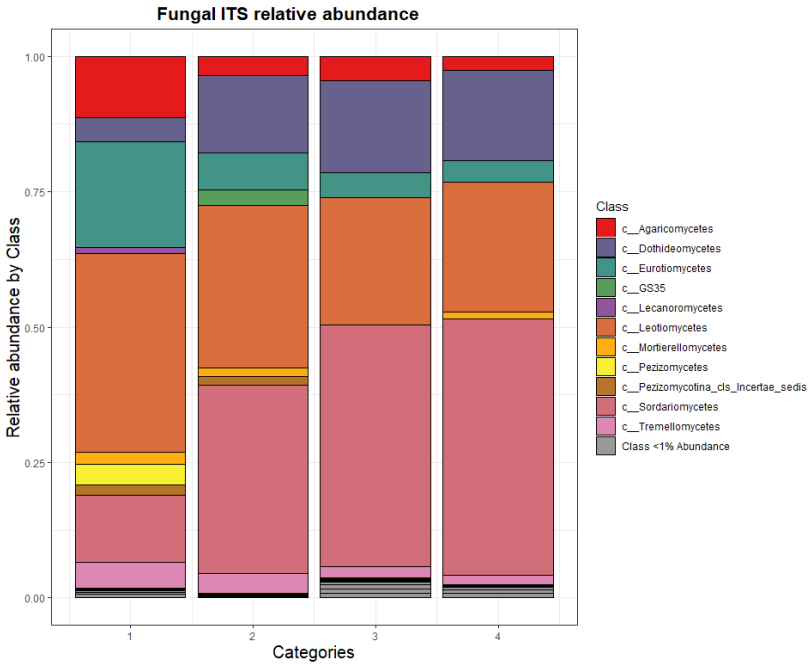

Figure 4.4 Fungal ITS class relative abundance: Sequencing of samples obtained during summer 2019. Time since conversion categories from left to right are forest (1), <10 y in agriculture (2), >10 and <50 y agriculture (3), and > 50 y in agriculture (4) for both depth intervals together.

Table 4.3. ANOVA analysis for fungal OTUs and time since conversion; depth intervals are 0-5 and 5-15 cm. Means values are presented, and significant differences (p <0.05) are denoted by lowercase letters.

0-5 cm depth interval		Time since conversion (Categories)				Average (+)
Fungal classes	p values	forest	< 10 y agriculture	>10<50 y agriculture	>50 y agriculture	increased/decreased
Basidiomycota - Agaricomycetes	< 0.001	1.22E-01 a	2.24E-02 b	7.97E-03 b	2.77E-02 b	-26%
Ascomycota - Dothideomycetes	0.05	6.83E-02 b	1.66E-01 ab	2.52E-01 a	1.93E-01 ab	61%
Ascomycota - Eurotiomycetes	0.01	1.89E-01 a	7.52E-02 ab	3.29E-02 b	3.14E-02 b	-28%
Ascomycota - Sordariomycetes	< 0.001	1.54E-01 b	3.50E-01 ab	4.57E-01 a	4.94E-01 a	74%
5-15 cm depth interval						

Basidiomycota - Agaricomycetes	0.04	1.09E-01	a	4.80E-02	ab	8.40E-02	ab	2.58E-02	b	-25%
Ascomycota - Dothideomycetes	< 0.001	2.17E-02	b	1.20E-01	a	8.77E-02	ab	1.39E-01	a	180%
Ascomycota - Leotiomycetes	0.03	4.40E-01	a	3.34E-01	ab	2.76E-01	ab	2.64E-01	b	-13%
Ascomycota - Sordariomycetes	< 0.001	9.56E-02	b	3.46E-01	a	4.29E-01	a	4.55E-01	a	125%

(+) average increased/decreased by time since conversion to agriculture (Categories)

4.4.5 Relationship between soil organic matter, chemical soil properties and soil biology

Land conversion to agriculture caused several changes in the macro and micro-nutrients associated with soil organic matter and chemistry properties (Table S32). Forests increased soil organic matter (SOM) levels, K, total C, total N, Mg, Fe, and Mn, all of which decreased on land conversion. In forest soil, SOM was 12 %, then decreased to 6% after <10 y agriculture (B), then between >10 <50 y agriculture, SOM was 5.11% followed by an increased of 7.5% after > 50 y agriculture. While pH was more acidic during the first decade after conversion to agriculture, Zn increased after conversion >50 y ago.

We used Spearman's rank correlation to assess the interactions between marker genes, soil organic matter, and chemical properties (Table 4.45). The overall functional potential of the soil communities to break down low molecular weight carbon compounds (B-glu) was negatively correlated with SOM and C+N pools, and positively correlated with pH. In contrast, the cellulolytic (cchl) potential decreased within increasing pH and both cellulolytic and hemicellulolytic potential increased with increased P and K availability. Functions associated with nitrification (amoA, nxrA) were negatively correlated with SOM and total C while those associated with N immobilization (nrfA) and P mineralization (phoC) were positively correlated with SOM and total C. Finally, fungal 18S and archaeal 16S showed the lowest correlation, with three and two correlations each for macronutrients. Additional interactions between the marker genes and micronutrients are described in the supplementary material (Table S33).

Table 4.4. Relationship between soil organic matter, chemical soil properties and functional genes targeted by qPCR using Spearman correlation analysis. Parenthesis show positive (+) or negative (-) correlations (p <0.05); n/f= no correlation found.

Soil properties	Taxonomy classification	Carbon cycling	Carbon + nitrogen cycling	Nitrogen cycling	Phosphorus mineralization, and solubilization
SOM	Fungal 18S (-)	Bglu (-)	Gh11(-)	B-amoA/ A-amoA/ nxrA (-); nrfA (+)	phoC (+)
pH	n/f	Cbhl (-); Bglu (+)	n/f	A-amoA / nrfA (+)	pqqC (-)
P	Archael 16S / Fungal 18S (+)	Cbhl (+)	gh11 (+)	B-amoA / nxrA (+)	n/f
K	Archael 16S / Fungal 18S (+)	Cbhl (+)	gh11 (+)	nc	n/f
Total C	n/f	Bglu (-)	n/f	B-amoA/ A-amoA/ nxrA (-); nrfA (+)	phoC (+)
Total N	n/f	Bglu (-)	n/f	B-amoA/ A-amoA/ nxrA (-)	phoC (+)

The same Spearman's correlation approach was applied to assess the relationship between the relative abundance of different microbial phyla and classes and soil properties.

Two Acidobacteria classes (Acidimicrobiia, Acidobacteria.6), one Actinobacteria class (MB.A2.108) and Planctomycetia were positively correlated with SOM, with most also positively correlated with Total C and N (Table 4.6). A separate Acidobacteria class, Acidobacteriia, and the Gemmatimonadetes class Gemm.1 were negatively correlated with the same parameters. Multiple classes, including Nitrospira, were negatively associated with total C, N, P, and K (Table S34).

Table 4.5. Relationship between soil physical, chemical and biological soil properties and bacterial class OTU's using Spearman correlation analysis. Parenthesis show positive (+) or negative (-) correlations at (p <0.05) . n/f= no correlation found.

Soil properties	Bacterial class OTUs
SOM	Acidimicrobiia/ Acidobacteria.6/ MB.A2.108/ Planctomycetia (+); Acidobacteriia/ Ellin6529/ Gemm.1 (-)
pH	Chloracidobacteria / Acidobacteria.6/ Gemm.1/ MB.A2.108/ Nitrospira (+); Acidobacteriia/ Actinobacteria/ Alphaproteobacteria/ Planctomycetia (-)
P	Gammaproteobacteria/ Actinobacteria (+); Acidimicrobiia/ Deltaproteobacteria/ MB.A2.108/ Nitrospira (-)
K	Actinobacteria (+); Ellin6529/ Nitrospira (-)
Total C	Acidimicrobiia/ Acidobacteria.6/ Planctomycetia (+); Acidobacteriia/ Ellin6529/ Gemm.1/ Nitrospira (-)
Total N	Acidimicrobiia/ Planctomycetia (+); Acidobacteriia/ Ellin6529/ Gemm.1 / Nitrospira (-)

Spearman's correlation showed a positive and negative association between *Ascomycota* and *Basidiomycota* at the phylum level (Table S35). *Sordariomycetes* were negatively associated with SOM and total N, but positively associated with P and K. In contrast, Agaricomycetes were positively associated with SOM and Total C, but negatively associated with P (Table 4.7). We found significant correlations with soil organic matter and micronutrients. Complete chemical properties are described in supplementary material (Table S35).

Table 4.6. Relationship between soil organic matter, chemical soil properties and fungal class OTUs using Spearman correlation. Parenthesis show positive (+) or negative (-) correlations (p <0.05). n/f = no correlation found.

Soil properties	Fungal class OTU's
SOM	Agaricomycetes(+); Sordariomycetes (-)
pH	n/f
P	Dothideomycetes/ Sordariomycetes (+); Leotiomycetes, Agaricomycetes (-)
K	Sordariomycetes (+); Leotiomycetes (-)
Total C	Agaricomycetes (+)
Total N	Sordariomycetes(-)

4.5 DISCUSSION

Land conversion to agriculture has well-recognized impacts on soil microbial abundance and composition (Bevivino et al., 2014; Hirsch et al., 2017). Most agricultural practices, including soil tillage, organic matter addition, crop rotation, cropping systems, and irrigation influence the soil microbial community's, abundance, composition, and activity (Kong et al., 2010).

4.5.1 Overall change in archaeal, bacteria and fungal abundances and functional microbial community associated with C cycling genes

In this study, microbial biomass decreased after land conversion, affecting archaeal, and fungal communities, and the relative functional potential shifted in different ways. Agricultural

soils in the Thunder Bay area had an increased relative abundance of total archaea and fungi compared to forested systems, as estimated by qPCR of taxonomic marker genes (Table 4.1). Conversion to agriculture did not alter overall bacterial abundance. Increased amounts of more bioavailable carbon in the form of crop residues (roots and shoots) resulted in a net increase in overall fungal abundance, with a shift towards communities capable of decomposing those less recalcitrant carbon compounds (e.g., B-glu, chbL, and gh11 gene abundance increased). This shift in functional communities was confirmed with sequencing data, which showed a clear increase in the abundance of Sordariomycetes in agricultural compared to forested systems (Table 4.4). Fungal communities were associated with the decomposition of recalcitrant carbon compounds by saprotrophic fungi suggesting that dead forest roots became the primary sources of carbon (Guo & Gifford, 2002). Other studies have also observed an increase in archaeal 16S gene abundance after land conversion (Lu et al., 2012; Schloter, 2011). Archaea are microorganisms capable of surviving extreme or drought conditions, and their abundance is increased after ammonium nitrate fertilizer, mostly due to increase in archaeal ammonia oxidizers (*A-amoA*) present in acidic soils (Hirsch et al., 2017). However, another study showed that archaea are more dominant in low-N soils, an essential driver of nitrification under nitrogen-poor conditions in grassland soils (Sterngren et al., 2015).

Land conversion had differing effects on the capacity of the soil communities to cycle different carbon compounds. The relative abundance of genes associated with complex organic matter decomposition (laccase: multicopper oxidase-LMCO) did not show significant changes since land conversion. The relative abundance of genes associated with the decomposition of lower molecular weight carbon compounds however, increased as background levels of SOM decreased and nutrient pools such as P and K increased. These functional shifts stabilized within

10 years of conversion. Although we did not directly measure how the composition of carbon compounds changed with age of land conversion, compounds like cellulose, hemicellulose and sugars are common in agricultural fields (Cherubini & Ulgiati, 2010; Ginni et al., 2021) in which these compounds are decompose by saprophytic Basidiomycota and Ascomycota organisms (Barbi et al., 2014). There is potential for these carbon cycling genes to serve as soil health indicators as β-glucosidase gene abundance and enzyme activity, which is an emerging biological indicator, have been shown to be positively related (Pérez-Guzmán et al., 2021).

4.5.2 Functional microbial community associated with N and P cycling genes

The relative abundance of genes associated with nitrification (amoA and nxrA) generally increased following land conversion (Table 4.1and 4.2). However, those associated with denitrification were not impacted. Agricultural production systems typically add N inputs because N is limiting and adding N can promote N utilizing organisms. Indeed, *A-amoA* has shown a positive response to N application in acid soils (Gubry-Rangin et al., 2015), and soil management practices such as tillage, liming, and organic matter amendments promote the abundance of *B-amoA* and *A-amoB* (Banning et al., 2015). Furthermore, tillage and N fertilizer resulted in the increased of nxrA gene abundance (Attard et al., 2010; Hatfield & Sauer, 2020; Liang et al., 2021). Also, nitrifier abundance is associated positively with soils amended/applied with cattle manure (Tatti et al., 2014), a common practice among dairy farmers in the study area (Benalcazar et al., 2022). The relative abundance of genes associated with phosphorus mineralization (phoC and phoD: acid and alkaline phosphatase) decreased followed conversion (Tables 4.1 and 4.2). Acid phosphatase and alkaline phosphatase, which are responsible for recycling organic phosphorus in soil, decreased due to increased mineral P inputs after land conversion (Lang et al., 2021; Ragot et al., 2015). These functions are potential soil health

indicators due to their involvement in the P cycling, although more studies are required (Pérez-Guzmán et al., 2021).

4.5.3 Bacteria and fungal microbial community richness and composition

In Thunder Bay agricultural areas, after land conversion, bacterial diversity increased, while fungal diversity did not experience significant change (Figure 4.1). Soil that receives N flows either by synthetic or organic N inputs increased bacterial and fungal diversity (de Graaff et al., 2019; Chen et al., 2020). Different rates of nitrogen application affected bacteria diversity in Acidobacteria, Gammaproteobacteria (O'Brien et al., 2016). In addition, organic inputs provide more microbial diversity than inorganic input, and changes in microbial diversity depends on tillage, crop rotation, reduce tillage, and pesticide application (de Graaff et al., 2019). Differences in abundances by depth depend on soil mechanical disturbance, specifically tillage, increases bacterial diversity, but reduces fungal diversity (Tardy et al., 2015), that is due to the intra- and inter-soil aggregate pore network (O'Brien et al., 2016) .

4.5.1 Bacteria and fungal abundance by sequencing

Overall, bacterial and fungal abundance evaluated by sequencing changed after land conversion (Table 4.3). The main shifts in bacterial were seen in the phyla Firmicutes, Gemmatimodetes, Actinobacteria, Proteobacteria, and Chloroflexi. The relative abundance of the Firmicutes class Clostridia; Gemmatimodetes classes Gemm.1, Gemmatimonadetes; Proteobacteria class Gammaproteobacteria and Actinobacteria class. Actinobacteria significantly increased at both soil depths, but Actinobacteria classes MBA2.108, Acidimicrobiia significantly decreased at both soil depths. The main shifts in fungi were seen in the phyla *Ascomycota* and *Basidiomycota*. The relative abundance of the Ascomycota classes *Dothideomycetes* and *Sordariomycetes* significantly increased at both soil depths, but *Eurotiomycetes* and *Leotiomycetes* decreased in

the surface and subsurface, respectively. The Basidiomycota classes *Agaricomycetes,* which includes many ectomycorrhizal fungi, significantly decreased as the system shifted from mixed forest dominant to crop dominant.

Previous studies have shown that *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Ascomycota* and *Basidiomycota* are the most predominant phyla in boreal soils (Clemmensen et al., 2013; Lladó et al., 2017). The addition of wood ash and liming in farm fields reduces the abundance of *Acidobacteria*, *Actinobacteria* and *Proteobacteria* (Reid & Watmough, 2014). *Acidobacteria* and *Proteobacteria* soil functions are associated with soil C and N cycling (Merloti et al., 2019). Other soil functions include litter decomposition, N fixation, and symbiosis. *Acidobacteria* and *Proteobacteria* have an active role in litter decomposition in coniferous and deciduous forests (Lladó et al., 2017; López-Mondéjar et al., 2016). *Proteobacteria* is responsible for organic carbon cycling, especially in nutrient-poor environments (Csotonyi et al., 2010). *Alphaproteobacteria* and *Deltaproteobacteria* are responsible for breaking down and recycling organic compounds (Coates & Wyman, 2017), and N fixation in undisturbed natural environments where they live in symbioses or as free-living taxa (van Insberghe et al., 2015). Other studies found that orders of *Alphaproteobacteria* are responsible for denitrification (Coates & Wyman, 2017).

Among the most important fungal phyla found in the forest soils were *Ascomycota* (Sterkenburg et al., 2015), which are responsible for organic matter decomposition (Zhang et al., 2017) and can tolerate synthetic fertilizer, tilling or both (Peltoniemi et al., 2021). However, the *Basidiomycota-Agaricomycete,* and also many ectomycorrhizal classes decreased due to limited lignocellulose sources, a significant component of organic C in wood debris (Tardy et al., 2015).

Finally, after land conversion to agriculture, it takes time for a new stable stage in both microbial community structure and functional potential. For example, shifts in functional potential occurred up to 10 and 50 years post-agricultural conversion, depending on the function. Variability however, generally decreased after that first decade. Land conversion to agriculture affects soils at various spatial and temporal scales by changing or altering the community composition (Paine et al., 1998). Farm management practices modify the quantity and quality of microbial nutrients supplied over time. Tillage practices modify the habitat and soil structure every season, and crop rotation with soil amendments alters microbial diversity for months to weeks. As a result, soil communities change continuously following a soil disturbance until an alternative stable state is achieved (Shade et al., 2012).

4.6 CONCLUSIONS

In this study, we found that forest conversion to agriculture affected microbial community abundance, diversity and composition in boreal soils. Contrary to our hypothesis, we observed that fungal and archaeal community abundance increased with forest to agricultural conversion. Forest conversion to agriculture resulted in microbial indicators of changes to soil organic carbon, nitrogen and phosphorus mineralization (as functional genes showed in C, N and P cycling). The relative abundance of genes associated with lower molecular weights C compound increased, but genes associated with complex organic matter decomposition did not show significant changes, while genes associated with nitrification increased followed land conversion, but those genes associated with denitrification were not impacted. However, relative abundance of genes associated with phosphorus declined followed conversion due to their increased mineral P input required for crop production. In addition, genes associated with denitrification were not impacted. The composition of fungal and bacterial communities changed

after forest conversion, wherein more dominant species for both bacteria (*Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, and *Gemmatimonadetes)* and fungi (*Ascomycota* and *Basidiomycota)* were present. These findings have important implications for understanding C, N, and P cycling that may increase or decrease due management practices. In addition, operational taxonomic showed that soil functions have been altered providing more emphasis in certain soil functions associated with C and N mineralization for the Thunder Bay agricultural diary farm system.

CHAPTER 5 CONCLUSIONS

Soil health assessment and evaluation is an integrative approach in which physical, chemical, biological indicators have been recognized to monitor soil functions. The study presented here provides insights into the impacts of land conversion from boreal forest to agriculture using a standardized soil health framework known as the Comprehensive Assessment of Soil Health (CASH), along with quantitative polymerase chain reaction (qPCR) and gene sequencing to detect bacterial and fungal communities present in forests and agricultural fields under a range of time since conversion. The inference space is the Thunder Bay agricultural areas, predominantly dairy farms that grow forage crops. The CASH framework provides insights into how land conversion caused soil degradation in agricultural fields most quickly during the first ten years. Continued soil amendment (primarily the application of manure) contributes to the recovery of soil organic carbon as soils stabilize after 50 years in agriculture. Soil health metrics that best-described changes following the conversion of boreal forests to agriculture were physical and biological indicators. Land conversion was followed by declines in wet aggregate stability, soil respiration, permanganate oxidizable carbon, and concentrations of organic matter.

Indicators associated with soil functions related to nutrient cycling, carbon sequestration, habitat provision, water quality, climate control and crop production were the best predictors of changes to soil health in conversion from forest to farm. Soil functions under unpredictive extreme weather events require close soil health monitoring in the short and long term. Soil monitoring requires predicting how the current changing climate will influence crop production so that farmers can adjust their sustainable practices for food production. A new agroecosystem that replaces forests enhances carbon and nitrogen mineralization and decomposition of organic

matter. After just ten years of land conversion, changes occurred to microorganism abundance and diversity, while composition stabilized.

With our changing climate, northern agriculture will continue to expand to address food production demands in Canada and worldwide. Consequently, this study is important in showing the implication of land conversion and how physical, chemical, biological changes after forest conversion to agriculture in Thunder Bay Ontario. A comprehensive and holistic soil health assessment and evaluation show how soil functions are affected under dairy farm management practices and the necessity of maintaining environmentally friendly practices adapted for Thunder Bay and northern agriculture. Continued research and future studies in northern Ontario require the establishment of soil plots for monitoring soil health. In addition, complete soil sampling for the area is necessary to have a broad overview of soil health conditions for the region so that future research could reference a complete dataset for soil modelling, taking into account northern climate, soil health conditions, farm practices, and future climate scenarios. Finally, since northern agricultural areas will continue to expand, best management practices for northern regions are necessary, and the promotion and adoption of sustainable soil practices for farmers in the region.

APPENDIX A. Statistical analysis, Chapter 2

Table S1. The F-statistics and probability levels from the marginal means mixed linear model to compare the effects of time of land conversion and soil depth on soil health attributes and CASH scores.

	Surface Hardness				Subsurface Hardness			
Effect	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.
Time	2	15	2.43	0.122	2	15	1.903	0.183

	Wet Aggregate Stability				Organic Matter				ACE Protein				Respiration				Total N				Total C		
Effect	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F
Time	2	14	1.035	0.381	2	14	4.711	0.027	2	14	5.448	0.018	2	14	6.367	0.011	2	14	4.017	0.042	2	14	5.260
Depth	1	16	15.873	0.001	1	16	45.025	0.000	1	16	32.622	0.000	1	16	36.700	0.000	1	16	98.135	0.000	1	16	112.132
Time*Depth	2	16	6.934	0.007	2	16	16.536	0.000	2	16	4.731	0.024	2	16	4.330	0.031	2	16	22.367	0.000	2	16	29.216

	POXC				CASH Score				P			
Effect	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.
Time	2	14	4.437	0.032	2	14	3.475	0.060	2	14	3.749	0.049
Depth	1	16	19.146	0.000	1	16	16.733	0.001	1	17	20.496	0.000
Time*Depth	2	16	0.534	0.596	2	16	1.433	0.268	2	17	0.321	0.730

	pH				Fe				Mn				Zn				K				Sand		
Effect	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F
Time	2	14	3.001	0.083	2	14	1.487	0.260	2	14	0.399	0.678	2	14	0.509	0.612	2	14	0.289	0.753	2	14	0.635
Depth	1	16	9.436	0.007	1	16	5.133	0.038	1	16	34.093	0.000	1	16	14.110	0.002	1	16	27.702	0.000	1	16	16.432
Time*Depth	2	16	0.230	0.797	2	16	2.566	0.108	2	16	2.581	0.107	2	16	0.833	0.453	2	16	0.001	0.999	2	16	2.233

	Mg				Silt				Clay			

Effect	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.	Num df	Denom df	F	Sig.
Time	2	14	2.235	0.144	2	14	0.470	0.634	2	14	0.342	0.716
Depth	1	16	2.766	0.116	1	16	4.099	0.060	1	16	0.024	0.880
Time*Depth	2	16	2.327	0.130	2	16	1.099	0.357	2	16	0.107	0.899

Table S2. The *F*-statistics and probability levels from the marginal means mixed linear model to compare the effect of time of conversion on soil health indicators and scores in the 0-5 and 5-15 cm depth intervals.

	0-5 cm				5-15 cm			
Attribute	Num df	Denom df	F	Sig	Num df	Denom df	F	Sig
Wet Aggregate Stability	2	14	2.640	0.107	2	14	0.209	0.814
Organic Matter	2	14	9.066	0.003	2	14	1.547	0.247
ACE Protein	2	14	9.647	0.002	2	14	1.905	0.186
Respiration	2	14	8.180	0.004	2	14	2.184	0.149
TN	2	14	7.660	0.006	2	14	1.150	0.345
TC	2	14	8.791	0.003	2	14	1.914	0.184

Table S3. The F-statistics and probability levels from a one-way ANOVA model to compare the effect of soil depth on soil health indicators in the time since conversion categories.

	< 10 y agriculture		> 50 y agriculture		Forest	
Attribute	F	Sig.	F	Sig.	F	Sig.
Wet Aggregate Stability	0.058	0.814	0.046	0.835	4.337	0.064
Organic Matter	1.764	0.214	0.070	0.797	8.204	0.017
ACE Protein	3.918	0.076	0.863	0.375	6.269	0.031
Respiration	1.045	0.331	4.256	0.066	16.048	0.002
TN	2.100	0.178	0.959	0.351	12.085	0.006
TC	2.231	0.166	0.713	0.418	9.682	0.011

Table S4.Pearson correlation coefficients for CASH indicators. NS is not statistically significant at alpha=0.05. Correlations are significant at the 0.01 level unless indicated otherwise.

	Sand	Silt	Clay	PR15	PR45	WAS	pH	P	K	Mg	Fe	Mn	Zn	TN	TC	OM	ACE Protein	Resp	POXC
Sand	1.000																		
Silt	-0.875	1.000																	
Clay	-0.689	ns	ns																
PR15	ns	ns	ns	1.000															
PR45	ns	ns	ns	0.906*	1.000														
WAS	0.464	-0.444	ns	ns	ns	1.000													
pH	ns	ns	ns	ns	ns	ns	1.000												
P	ns	ns	ns	ns	ns	ns	ns	1.000											
K	-0.373*	ns	0.371*	ns	ns	ns	ns	0.687	1.000										
Mg	-0.559	ns	0.801	ns	-0.371*	ns	0.395*	ns	ns	1.000									
Fe	ns	ns	ns	ns	-0.337*	ns	-0.353*	-.0367*	ns	ns	1.000								
Mn	ns	ns	ns	ns	ns	0.478	ns	ns	ns	ns	ns	1.000							
Zn	ns	ns	ns	ns	ns	0.332*	ns	0.633**	0.593	ns	ns	ns							
TN	ns	ns	ns	ns	ns	0.684	ns	ns	ns	0.331*	ns	0.641	0.341*	1.000					
TC	ns	ns	ns	ns	ns	0.676	ns	ns	ns	0.365*	ns	0.643	ns	0.982	1.000				
OM	ns	ns	ns	ns	ns	0.697	ns	ns	ns	0.458	ns	0.578	ns	0.937	0.955	1.000			
ACE Protein	ns	ns	ns	ns	ns	0.682	ns	ns	ns	ns	ns	0.743	ns	0.879	0.903	0.862	1.000		
Resp	ns	ns	ns	ns	ns	0.544	ns	ns	0.439	0.420*	ns	0.422*	0.465	0.840	0.807	0.773	0.751	1.000	
POXC	ns	ns	ns	ns	ns	0.430	ns	ns	ns	0.408*	ns	0.534	ns	0.795	0.793	0.787	0.797	0.820	1.000

* Correlation is significant at the 0.05 level (2-tailed).

PR15, Penetration Resistance 0-15 cm; PR45, Penetration Resistance 15-45 cm; WAS, Wet Aggregate Stability; TN, Total Nitrogen; TC, Total Carbon; OM, Organic Matter; ACE-Protein, Soil Protein; Resp, Soil Respiration; POXC, Active Carbon.

Table S5. Farm management history for 2019 soil sample collections, according to CASH sampling assessment

Farm field	Depth	Time	Area	Crop information	
				2016/2017/2018	2019
1	5	B	Murrillo	alfalfa/barley/corn	wheat
2	15	B	Murrillo	alfalfa/barley/corn	wheat
3	5	C	Murrillo	alfalfa/wheat/corn	corn
4	15	C	Murrillo	alfalfa/wheat/corn	corn
5	5	A	Murrillo	forest	forest
6	15	A	Murrillo	forest	forest
7	5	A	Murrillo	forest	forest
8	15	A	Murrillo	forest	forest
9	5	B	Murrillo	alfalfa/alfalfa/corn	corn
10	15	B	Murrillo	alfalfa/alfalfa/corn	corn
11	5	C	Murrillo	wheat//barley/corn	barley
12	15	C	Murrillo	wheat//barley/corn	barley
13	5	A	Murrillo	forest	forest
14	15	A	Murrillo	forest	forest
15	5	B	Murrillo	alfalfa/wheat/corn	corn
16	15	B	Murrillo	alfalfa/wheat/corn	corn
17	5	C	Murrillo	wheat/alfalfa/alfalfa	alfalfa
18	15	C	Murrillo	wheat/alfalfa/alfalfa	alfalfa
19	5	A	Slate River	forest	forest
20	15	A	Slate River	forest	forest
21	5	B	Slate River	wheat/corn/alfalfa	alfalfa
22	15	B	Slate River	wheat/corn/alfalfa	alfalfa
23	5	C	Slate River	wheat/alfalfa/alfalfa	alfalfa
24	15	C	Slate River	wheat/alfalfa/alfalfa	alfalfa
25	5	A	Slate River	forest	forest
26	15	A	Slate River	forest	forest
27	5	C	Slate River	alfalfa/alfalfa/corn	barley
28	15	C	Slate River	alfalfa/alfalfa/corn	barley
29	5	C	Slate River	alfalfa/wheat/corn	corn
30	15	C	Slate River	alfalfa/wheat/corn	corn
31	5	A	Slate River	forest	forest
32	15	A	Slate River	forest	forest
33	5	B	Slate River	wheat/corn/alfalfa	alfalfa
34	15	B	Slate River	wheat/corn/alfalfa	alfalfa
35	5	B	Slate River	alfalfa/alfalfa/corn	corn
36	15	B	Slate River	alfalfa/alfalfa/corn	corn

APPENDIX B. Statistical analysis, Chapter 3

Table S6. Influential biological soil health indicators by time since conversion

Indicator	OM (%)				Soil respiration (mg CO2g-1 4 days-1)				Soil protein (mg g-1)				POXC (mg g-1)			
Time since conversion	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Mean	11.99	5.65	5.11	7.5	1.33	0.83	0.91	0.91	25.4	13.34	9.9	16.47	1343.33	905.89	911.85	1142.66
Std. Deviation	6.03	2.11	1.87	4.31	0.43	0.3	0.44	0.35	9.58	3.92	2.93	8.02	752.27	238.35	115.26	478.26

Forest = A, <10 y agriculture = B, >50 y agriculture =C, >10 y <50 y agriculture =D, all agriculture=BCD.

Table S7. Influential physical indicators by time since conversion

Indicator	Wet aggregate stability (%)				Surface hardness (Kpa)			
Time since conversion	A	B	C	D	A	B	C	D
Mean	64.59	50.11	42.44	46.5	177.78	225	214.29	231.25
Std. Deviation	12.91	10.12	12.04	20.7	25.57	58.39	60.22	57.37

Forest = A, <10 y agriculture = B, >50 y agriculture =C, >10 y <50 y agriculture =D, all agriculture=BCD.

Table S8. Influential chemical indicators by time since conversion

Indicator	Mg (ppm)			
Time since conversion	A	B	C	D
Mean	797.17	448.17	666.23	480.89
Std. Deviation	418.31	284.35	212.31	218.06

Forest = A, <10 y agriculture = B, >50 y agriculture =C, >10 y <50 y agriculture =D, all agriculture=BCD.

Table S9. K-fold cross-validation model for time since conversion in the overall soil health score.

Indicator	Categories					
Time since conversion	A	B	C	D	BCD	All dataset
RMSE	47.9	498.0	43.5	34.9	4.5	6.1
MAE	42.9	495.7	42.6	31.5	3.9	4.6

APPENDIX C. Statistical analysis, Chapter 4

Table S10. qPCR assays: target organisms, amplification conditions and primer sources, and standard calibration identity

Function[a]	**Target gene/qPCR assay name**	**Taxonomic Range**	**Primers**	**Cycling conditions**[b]	**Reference**
Taxonomic classification_Bacteria	Bacterial 16S	General Bacteria	16S_341F/534R	35 cycles of 95 °C/5 s, 60 °C/20 s	(Watanabe, Kodama, & Harayama, 2001)
Taxonomic classification_Fungi	Fungal 18S	General Fungi	Fungal18S-FR1F/FF390-R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Vainio & Hantula, 2000)
Taxonomic classification_Archaea	Archaeal 16S	General Archaea	ARC344F_16S/Arch806R_16S	35 cycles of 95 °C/5 s, 60 °C/20 s	(Raskin, Stromley, Rittmann, & Stahl, 1994); (Takahashi, Tomita, Nishioka, Hisada, & Nishijima, 2014)
Carbon cycling (Polyphenolic C breakdown)	Laccase (multicopper oxidase -LMCO)	Bacteria and Fungi	Cu1A-F/Cu2-R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Kellner, Luis, & Buscot, 2007)
Carbon cycling (Breakdown of oligosaccharides)	Bglu (β-glucosidase)	Bacteria; wide taxonomic range	Bact-bglu2F/bglu4R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Cañizares, Benitez, & Ogunseitan, 2011)
Carbon cycling (Cellulose decomposition)	cbhI (cellobiohydrolase)	Fungi: Basidiomycota, Ascomycota	fungi-cbhI-F/R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Edwards, Upchurch, & Zak, 2008)
C+N cycling (Hemicelluloase decomposition)	GH11 (glycoside hydrolase)	Fungi: Basidiomycota, Ascomycota	fungGH11-F/R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Barbi et al., 2014)
C+N cycling (Nitrogen mineralization)	apr (alkaline metallopeptidase)	Primarily bacterial; dominance of Proteobacteria (i.e. Pseudomonas)	Apr-F/R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Bach, Hartmann, Schloter, & Munch, 2001)
Nitrogen cycling (Nitrification (NH_4^+ to NO_2^-)	*B-amoA*: ammonium monooxygenase	Proteobacteria (BetaProteo eg Nitrosomonas/Nitrosospira)	B-amoA-1F/R	35 cycles of 95 °C/5 s, 60 °C/20 s	(Rotthauwe, Witzel, & Liesack, 1997)

Nitrogen cycling (Nitrification (NH4+ to NO2-)	*A-amoA*: ammonium monooxygenase	Archaea	Gen-AOA-F/R	35 cycles of 95 °C/5 s, 60 °C/20 s	(Meinhardt et al., 2015)
Nitrogen cycling (Nitrification: nitrite to nitrate)	nxrA (nitrite oxidoreductase)	Bacteria: Nitrobacter	norA-1F/1R	45 cycles of 95 °C/5 s, 60 °C/30 s	(Poly, Wertz, Brothier, & Degrange, 2008)
Nitrogen cycling (Ammonification/Denitrification)	narG: membrane bound nitrate reductase	Bacteria: Proteobacteria	narG-F/R	35 cycles of 95 °C/5 s, 60 °C/30 s	(Gregory, Karakas-Sen, Richardson, & Spiro, 2000)
Nitrogen cycling (Dissimilatory nitrate reduction (nitrite to NH4)	nrfA (dissimilatory nitrite reductase)	Bacteria; wide taxonomic range	nrfA-F2aw/7R1	40 cycles of 95 °C/5 s, 60 °C/30 s	(Welsh, Chee-Sanford, Connor, Löffler, & Sanford, 2014)
Nitrogen cycling (denitrification (nitrite to NO)	nirk: copper-containing nitrite reductase	Bacteria	nirK-583-F/909-R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Liu et al., 2003)
Nitrogen cycling (denitrification (nitrite to NO)	A-nirk: nitrite reductase	Archaea	anirKa_58F/578R	45 cycles of 95 °C/5 s, 60 °C/30 s	(Lund, Smith, & Francis, 2012)
Nitrogen cycling (denitrification (N2O to N2)	nosZ: nitrous oxide reductase	Bacteria: Clade I	nosZ2-F/R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Henry, Bru, Stres, Hallet, & Philippot, 2006)
Phosphorous mineralization (Phosphoester and anhydride bond dephosphorylation)	phoD (alkaline phosphatase)	Bacteria; wide taxonomic range	phoD-733F/R10831R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Ragot, Kertesz, & Bünemann, 2015)
Phosphorous mineralization	phoC (acid phosphatase)	Bacteria; wide taxonomic range	phoC-A-F1/R1	40 cycles of 95 °C/5 s, 60 °C/30 s	(Fraser, Lynch, Gaiero, Khosla, & Dunfield, 2017)
Phosphorous mineralization (Phosphoester and anhydride bond dephosphorylation)	phnX (Phosphono-acetaldehyde hydrolase)	Bacteria; Primarily Proteobacteria Firmicutes	phnX-FW/RW	40 cycles of 95 °C/5 s, 60 °C/30 s	(Bergkemper et al., 2016)

Phosphorous solubilization (C-P bond cleavage)	pqqC (pyrroloquinoline quinone)	Bacteria; Primarily Proteobacteria (Alpha-, Beta-, Gamma), Verrucomicrobia, Actinobacteria	pqqC-R/R	40 cycles of 95 °C/5 s, 60 °C/30 s	(Zheng et al., 2017)

All assays started with an initial denaturation step of 95 °C for 3 min and finished with a melt curve from 65 °C to 95 °C; [c] Excluding un-cultured references, Equimolar plasmid pools were used in assays where more than one organism is listed

Sso: Sso Advanced™ Universal SYBR® Green Supermix (Bio-Rad, USA); Sensifast: SensiFAST™ Real-Time master mix (Bioline, UK); Itaq: Itaq Universal SYBR® Green Supermix (Bio-Rad, USA); all assays are run to extinction

Table S11. F statistics and probability levels from the two ways ANOVA table to compare the effects of time since conversion and soil depth for Archael16S, Bacteria16S, and Fungal 18S copies g-1 soil qPCR microbial communities at 0-5 cm depth interval

ANOVA Log Archaeal copies ng -1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.01	3	0.34	6.83	1.52e-3
Residuals	1.28	26	0.05		

Note. Type III Sum of Squares

Post Hoc Archaeal ng -1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.20	0.12	-1.68	0.36
	C	-0.33	0.11	-2.91	0.03 *
	D	-0.47	0.11	-4.37	< .001 ***
B	C	-0.13	0.12	-1.05	0.72
	D	-0.28	0.12	-2.30	0.13
C	D	-0.15	0.11	-1.27	0.59

* p < .05, ** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

ANOVA - Bacterial 16S copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	4.05e+11	3	1.35e+11	1.61	0.21
Residuals	2.17e+12	26	8.36e+10		

Note. Type III Sum of Squares

Post Hoc Fungal 18S copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-16510.98	4526.69	-3.65	6.01e-3 **
	C	-15661.87	4328.35	-3.62	6.46e-3 **
	D	-21267.69	4173.41	-5.10	< .001 ***
B	C	849.11	4778.37	0.18	1.00
	D	-4756.71	4638.48	-1.03	0.74
C	D	-5605.82	4445.13	-1.26	0.59

* p < .05, ** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

Table S12. F statistics and probability levels from the two ways ANOVA table to compare the effects of time since conversion and soil depth for Archael16S, Bacteria16S, and Fungal 18S copies ng-1 DNA soil qPCR microbial communities at 5--15 cm depth interval.

ANOVA - Log Archaeal ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.41	3	0.14	4.45	0.01
Residuals	0.80	26	0.03		

Note. Type III Sum of Squares

Post Hoc Archaeal ng-1DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-0.18	0.09	-1.92	0.24	
	C	-0.23	0.09	-2.55	0.08	
	D	-0.30	0.09	-3.48	9.02e-3	**
B	C	-0.05	0.10	-0.49	0.96	
	D	-0.12	0.09	-1.26	0.60	
C	D	-0.07	0.09	-0.79	0.86	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA - Bacterial 16S copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.94e+11	3	1.31e+11	1.69	0.19
Residuals	2.03e+12	26	7.80e+10		

Note. Type III Sum of Squares

ANOVA - Fungal 18S copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.37e+9	3	4.57e+8	8.33	< .001
Residuals	1.43e+9	26	5.49e+7		

Note. Type III Sum of Squares

Post Hoc Fungal 18S copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-18763.19	3904.74	-4.81	< .001	***
	C	-8012.25	3733.64	-2.15	0.17	
	D	-11856.58	3599.99	-3.29	0.01	*
B	C	10750.94	4121.83	2.61	0.07	
	D	6906.61	4001.16	1.73	0.33	
C	D	-3844.33	3834.37	-1.00	0.75	

* p < .05, *** p < .001

Note. P-value adjusted for comparing a family of 4

Table S13. Carbon cycling functional qPCR assays target organisms at 0-5 cm depth interval

ANOVA - Laccase copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	5.01e+7	3	1.67e+7	0.84	0.48
Residuals	5.16e+8	26	1.99e+7		

Note. Type III Sum of Squares

ANOVA - Bglu copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.15e+6	3	1.05e+6	3.85	0.02
Residuals	7.10e+6	26	273001.97		

Note. Type III Sum of Squares

Post Hoc Bglu copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-423.67	275.38	-1.54	0.43
	C	-589.83	263.31	-2.24	0.14
	D	-835.95	253.89	-3.29	0.01 *
B	C	-166.16	290.69	-0.57	0.94
	D	-412.28	282.18	-1.46	0.47
C	D	-246.11	270.42	-0.91	0.80

* $p < .05$

Note. P-value adjusted for comparing a family of 4

ANOVA - Log Cbhl ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.21	3	0.07	5.42	4.97e-3
Residuals	0.34	26	0.01		

Note. Type III Sum of Squares

Post Hoc Cbhl ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.23	0.06	-3.79	4.18e-3 **
	C	-0.11	0.06	-2.00	0.22
	D	-0.16	0.06	-2.88	0.04 *
B	C	0.11	0.06	1.79	0.30
	D	0.07	0.06	1.11	0.68

Table S14. Carbon cycling functional qPCR assays target organisms at 5-15 cm depth interval

ANOVA - Laccase copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.01e+8	3	3.36e+7	0.89	0.46
Residuals	9.77e+8	26	3.76e+7		

Note. Type III Sum of Squares

ANOVA - Bglu copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	5.97e+6	3	1.99e+6	1.30	0.30
Residuals	3.99e+7	26	1.54e+6		

Note. Type III Sum of Squares

Kruskal-Wallis Test copies Cbhl ng-1 DNA soil

Factor	Statistic	df	p
Categories	7.68	3	0.05

Table S15. Carbon + Nitrogen cycling functional qPCR assays target organisms at 0-5 cm depth interval

ANOVA - APR copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	7.14e+6	3	2.38e+6	1.78	0.18
Residuals	3.47e+7	26	1.33e+6		

Note. Type III Sum of Squares

ANOVA - Log gh11 ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.59	3	0.20	16.29	< .001
Residuals	0.31	26	0.01		

Note. Type III Sum of Squares

Post Hoc gh11 ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-0.33	0.06	-5.69	< .001	***
	C	-0.30	0.06	-5.34	< .001	***
	D	-0.29	0.05	-5.50	< .001	***
B	C	0.03	0.06	0.55	0.95	
	D	0.04	0.06	0.60	0.93	
C	D	1.82e-3	0.06	0.03	1.00	

*** p < .001

Note. P-value adjusted for comparing a family of 4

Table S16. Carbon + Nitrogen cycling functional qPCR assays target organisms at 5-15 cm depth interval

ANOVA - APR copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	4.23e+6	3	1.41e+6	0.46	0.71
Residuals	8.00e+7	26	3.08e+6		

Note. Type III Sum of Squares

ANOVA - Log gh11copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.43	3	0.14	9.34	< .001
Residuals	0.40	26	0.02		

Note. Type III Sum of Squares

Post Hoc gh11copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-0.31	0.07	-4.77	< .001	***
	C	-0.23	0.06	-3.77	4.48e-3	**
	D	-0.22	0.06	-3.67	5.65e-3	**
B	C	0.08	0.07	1.11	0.69	
	D	0.09	0.07	1.35	0.54	
C	D	0.01	0.06	0.22	1.00	

* p < .05, ** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

Table S17. Nitrogen cycling functional qPCR assays target organisms at 0-5 cm depth interval

ANOVA - A-amoA-copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.06e+6	3	1.02e+6	7.06	1.26e-3
Residuals	3.76e+6	26	144505.23		

Note. Type III Sum of Squares

Post Hoc A-amoA-copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-95.27	200.35	-0.48	0.96
	C	-791.77	191.57	-4.13	1.77e-3 **
	D	-511.11	184.71	-2.77	0.05 *
B	C	-696.50	211.49	-3.29	0.01 *
	D	-415.83	205.30	-2.03	0.20
C	D	280.67	196.74	1.43	0.49

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA - Log B-amoA copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	7.81	3	2.60	23.84	< .001
Residuals	2.84	26	0.11		

Note. Type III Sum of Squares

Post Hoc B-amoA copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-1.12	0.17	-6.46	< .001 ***
	C	-1.08	0.17	-6.52	< .001 ***
	D	-1.13	0.16	-7.02	< .001 ***
B	C	0.04	0.18	0.22	1.00
	D	-2.68e-3	0.18	-0.02	1.00
C	D	-0.04	0.17	-0.25	0.99

*** p < .001

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test nxrA copies ng-1 DNA soil

Factor	Statistic	df	p
Categories	20.19	3	< .001

Added categories diferentiation

Post Hoc nxrA copies ng-1 DNA soil comparisons - Categories

	Categories	$p_{bonferroni}$
A	B	0.02**
	C	0.004***
	D	0.004***
B	C	0.4
	D	0.48
C	D	1

* p < .05, ** p < .01, ***p<0.005

ANOVA - narG copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	2.21e+9	3	7.38e+8	0.28	0.84
Residuals	6.85e+10	26	2.64e+9		

Note. Type III Sum of Squares

ANOVA - nrfA copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	4.06e+7	3	1.35e+7	2.91	0.05
Residuals	1.21e+8	26	4.65e+6		

Note. Type III Sum of Squares

ANOVA - nirK copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.20e+8	3	3.99e+7	0.59	0.63
Residuals	1.76e+9	26	6.79e+7		

Note. Type III Sum of Squares

ANOVA - aNirK copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1244.52	3	414.84	2.12	0.12
Residuals	5081.50	26	195.44		

Note. Type III Sum of Squares

ANOVA - NosZ copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	5.86e+6	3	1.95e+6	1.40	0.27
Residuals	3.64e+7	26	1.40e+6		

Note. Type III Sum of Squares

Table S18.Nitrogen cycling functional qPCR assays target organisms at 5-15 cm depth interval

ANOVA - A-amoA-copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.36e+6	3	1.12e+6	6.83	1.52e-3
Residuals	4.27e+6	26	164111.95		

Note. Type III Sum of Squares

Post Hoc - A-amoA-copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-29.57	213.51	-0.14	1.00
	C	-746.62	204.15	-3.66	5.87e-3 **
	D	-610.86	196.85	-3.10	0.02 *
B	C	-717.05	225.38	-3.18	0.02 *
	D	-581.30	218.78	-2.66	0.06
C	D	135.76	209.66	0.65	0.92

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA - Log B-amoA ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.42	3	1.14	11.36	< .001
Residuals	2.61	26	0.10		

Note. Type III Sum of Squares

Post Hoc B-amoA ng-1 DNA soil Comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.70	0.17	-4.19	1.52e-3 **
	C	-0.72	0.16	-4.49	< .001 ***
	D	-0.78	0.15	-5.04	< .001 ***
B	C	-0.02	0.18	-0.10	1.00
	D	-0.08	0.17	-0.44	0.97
C	D	-0.06	0.16	-0.36	0.98

** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

ANOVA - Log nxrA copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	8.43	3	2.81	14.51	< .001
Residuals	5.03	26	0.19		

Note. Type III Sum of Squares

Post Hoc nxrA copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-1.23	0.23	-5.28	< .001	***
	C	-0.95	0.22	-4.27	1.25e-3	**
	D	-1.22	0.21	-5.72	< .001	***
B	C	0.28	0.24	1.14	0.67	
	D	1.95e-3	0.24	8.20e-3	1.00	
C	D	-0.28	0.23	-1.22	0.62	

** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

ANOVA - narG copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.02e+9	3	1.01e+9	0.41	0.74
Residuals	6.33e+10	26	2.43e+9		

Note. Type III Sum of Squares

ANOVA – nrfA copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	2.29e+7	3	7.64e+6	1.33	0.29
Residuals	1.50e+8	26	5.76e+6		

Note. Type III Sum of Squares

ANOVA - nirK copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	8.41e+6	3	2.80e+6	0.08	0.97
Residuals	8.68e+8	26	3.34e+7		

Note. Type III Sum of Squares

Kruskal-Wallis Test aNirK copies ng-1 DNA

Factor	Statistic	df	p
Categories	4.88	3	0.18

ANOVA - NosZ copies ng-1 DNA

Cases	Sum of Squares	df	Mean Square	F	p
Categories	958310.00	3	319436.67	0.21	0.89
Residuals	3.99e+7	26	1.53e+6		

Note. Type III Sum of Squares

Table S19. Phosphorus mineralization functional qPCR assays target organisms at 0-5 cm depth interval

ANOVA - Log phoC copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.20	3	0.07	10.78	< .001
Residuals	0.16	26	6.03e-3		

Note. Type III Sum of Squares

Post Hoc phoC copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	0.16	0.04	3.83	3.79e-3	**
	C	0.20	0.04	5.02	< .001	***
	D	0.17	0.04	4.38	< .001	***
B	C	0.04	0.04	0.91	0.80	
	D	8.57e-3	0.04	0.20	1.00	
C	D	-0.03	0.04	-0.77	0.87	

** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

ANOVA - phoD copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.61e+10	3	5.36e+9	5.28	5.61e-3
Residuals	2.64e+10	26	1.02e+9		

Note. Type III Sum of Squares

Post Hoc phoD copies ng-1 DNA soil comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	54359.88	16794.64	3.24	0.02	*
	C	36175.63	16058.75	2.25	0.14	
	D	53867.27	15483.90	3.48	9.10e-3	**
B	C	-18184.25	17728.38	-1.03	0.74	
	D	-492.60	17209.39	-0.03	1.00	
C	D	17691.65	16492.01	1.07	0.71	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA - pqqC copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.25e+7	3	4.16e+6	1.12	0.36
Residuals	9.67e+7	26	3.72e+6		

Note. Type III Sum of Squares

ANOVA - phnX copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	315921.20	3	105307.07	1.17	0.34
Residuals	2.35e+6	26	90230.11		

Note. Type III Sum of Squares

Table S20. Phosphorus mineralization functional qPCR assays target organisms at 5-15 cm depth interval

ANOVA - Log phoC copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.05	3	0.02	2.21	0.11
Residuals	0.21	26	7.99e-3		

Note. Type III Sum of Squares

ANOVA - phoD copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.49e+10	3	4.97e+9	1.48	0.24
Residuals	8.74e+10	26	3.36e+9		

Note. Type III Sum of Squares

ANOVA - pqqC copies ng-1 DNA soil

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.13e+7	3	3.78e+6	1.29	0.30
Residuals	7.64e+7	26	2.94e+6		

Note. Type III Sum of Squares

Kruskal-Wallis Test Phnx copies ng-1 DNA soil

Factor	Statistic	df	p
Categories	3.07	3	0.38

Table S21. Sequence processing summary for all dataset collected in boreal soils

Bacterial 16S - Dataset	
Sample Number	60
Read 1	23,676,44
Read 2	23,676,44
Primer removal/merging	22,734,81
Filtering (maxee=1)	17,210,30
Trimming (crop=375)	17,210,30
Total Sequence Loss (before clustering)	6,466,14
Cumulative % Sequence Loss (before clustering)	27.31%
Remaining Sequences (after clustering)	12,843,76
Cumulative % Sequence Loss (after clustering)	45.75%
OTU Count (non-rarefied) | 98%	32,01
Min	19,788.00
Max	257,026.00
Median	189,397.50
Mean	183,482.33

Fungal ITS - Dataset	
Sample Number	60
Read 1	17,760,54
Read 2	17,760,54
Primer removal/merging	17,238,05
Filtering (maxee=1)	15,382,39
Trimming (crop=420)	15,382,39
Total Sequence Loss (before clustering)	2,378,15
Cumulative % Sequence Loss (before clustering)	13.39%
Remaining Sequences (after clustering)	14,478,95
Cumulative % Sequence Loss (after clustering)	18.48%
OTU Count (non-rarefied) | 98%	16,48
Min	119,028.00
Max	305,405.00
Median	201,367.00
Mean	206,842.16

Table S22. Bacteria 16S Shannon diversity and Simpson index assessed by OTU reads at 0-5 cm depth interval

ANOVA – Bacterial 16s Shannon diversity

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.93	3	0.31	3.16	0.04
Residuals	2.54	26	0.10		

Note. Type III Sum of Squares

Post Hoc Bacterial 16s comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.24	0.16	-1.48	0.46
	C	-0.20	0.16	-1.24	0.61
	D	-0.47	0.15	-3.06	0.02 *
B	C	0.05	0.17	0.28	0.99
	D	-0.22	0.17	-1.31	0.56
C	D	-0.27	0.16	-1.67	0.36

* $p < .05$

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test Bacterial 16s Simpson index

Factor	Statistic	df	p
Categories	13.74	3	3.28e-3

Post Hoc Bacterial 16s Simpson index

Categories		$p_{bonferroni}$
A	B	1
	C	0.25
	D	0.002**
B	C	0.83
	D	0.05
C	D	1

* $p < .05$, ** $p < .01$, ***$p<0.005$

Table S23. Bacteria 16S Shannon diversity and Simpson index assessed by OTU reads at 5-15 cm depth interval

ANOVA – Bacterial 16s Shannon Diversity

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.09	3	0.36	4.86	8.16e-3
Residuals	1.94	26	0.07		

Note. Type III Sum of Squares

Post Hoc Bacterial 16s comparisons - Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.40	0.14	-2.80	0.04 *
	C	-0.25	0.14	-1.84	0.28
	D	-0.47	0.13	-3.55	7.68e-3 **
B	C	0.15	0.15	0.99	0.76
	D	-0.07	0.15	-0.46	0.97
C	D	-0.22	0.14	-1.54	0.43

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA - Log Bacterial 16s Simpson index

Cases	Sum of Squares	df	Mean Square	F	p
Categories	9.21e-7	3	3.07e-7	2.50	0.08
Residuals	3.19e-6	26	1.23e-7		

Note. Type III Sum of Squares

Table S24. Fungal ITS Shannon diversity and Simpson index assessed by OTU reads at 0-5 cm depth interval

ANOVA - Fungal ITS Shannon diversity

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.43	3	0.48	0.88	0.46
Residuals	14.05	26	0.54		

Note. Type III Sum of Squares

ANOVA – Fungal ITS Simpson index

Cases	Sum of Squares	df	Mean Square	F	p
Categories	4.50e-3	3	1.50e-3	0.48	0.70
Residuals	0.08	26	3.14e-3		

Note. Type III Sum of Squares

Table S 25. Fungal ITS Shannon diversity and Simpson index assessed by OTU reads at 5-15 cm depth interval

ANOVA - Fungal ITS Shannon diversity

Cases	Sum of Squares	df	Mean Square	F	p
Categories	4.38	3	1.46	2.58	0.07
Residuals	14.69	26	0.57		

Note. Type III Sum of Squares

ANOVA - Fungal ITS Simpson index

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.01	3	3.81e-3	1.30	0.30
Residuals	0.08	26	2.94e-3		

Note. Type III Sum of Squares

Table S26. Similarities by time since conversion and depths in bacterial 16S and fungal ITS assessed by OTU using envfit function in the vegan package at 0-5 cm and 5-15 cm depth intervals

Ordination in Bacterial 16S by time since conversion at 0-5 cm depth interval

Vectors	NMDS1	NMDS2	r^2	Pr(>r)	
A	0.76	0.62	0.71	0.001	***
B	-0.98	0.22	0.26	0.02	*
C	-0.84	-0.55	0.04	0.53	
D	-0.12	-0.99	0.28	0.009	**

'***' 0.001, '**'0.01, '*' 0.05

Number of permutations:999

Ordination in Bacterial 16S by time since conversion at 5 -15 cm depth interval

Vectors	NMDS1	NMDS2	r^2	Pr(>r)	
A	0.96	-0.27	0.25	0.03	***
B	-0.99	0.16	0.27	0.01	*
C	-0.28	-0.96	0.03	0.69	
D	0.05	-0.99	0.04	0.52	

Significant codes: '***' 0.001, '**'0.01, '*' 0.05

Number of permutations:999

Ordination in Fungal ITS by time since conversion at 0-5 cm depth interval

Vectors	NMDS1	NMDS2	r	Pr(>r)	
A	0.94	0.33	0.68	0.001	***
B	-0.23	-0.97	0.04	0.60	
C	-0.93	0.36	0.10	0.24	
D	-0.93	0.37	0.27	0.02	*

Significant codes: '***' 0.001, '**'0.01, '*' 0.05

Number of permutations:999

Ordination in Fungal ITS by time since conversion at 5-15 cm depth interval

Vectors	NMDS1	NMDS2	r	Pr(>r)	
A	-0.95	0.30	0.66	0.001	***
B	0.13	0.99	0.03	0.66	
C	0.80	-0.60	0.10	0.21	
D	0.88	-0.47	0.31	0.008	*

Significant codes: '***' 0.001, '**'0.01, '*' 0.05

Number of permutations:999

Table S 27. Pairwise Adonis multilevel comparison by time since conversion and depth in bacterial 16S and fungal ITS assessed by OTU using adonis differences are considered significant if $p < 0.05$.

Bacterial 16S OTU for categories at 0-5 cm depth interval

Source	Df	R^2	Pr(>r)
Categories	3	0.25	0.001***
Residual	26	0.75	

Significant codes: '***' 0.001, '**'0.01, '*' 0.05

Number of permutations = 999

Post hoc Bacteria 16S OTU comparisons by time since conversion at 0-5 cm depth interval

Pairs		F.model	R^2	p.adjusted
A	B	0.22	0.14	0.14
	C	0.16	0.19	0.012 .
	D	0.18	0.20	0.006*
B	C	0.09	0.15	0.11
	D	1.93	0.14	0.14
C	D	0.93	0.06	1.0

Bacterial 16S OTU for categories at 5-15 cm depth interval

Source	Df	R2	Pr(>r)
Categories	3	0.18	0.004***
Residual	26	0.82	

Significant codes: '***' 0.001, '**'0.01, '*' 0.05

Number of permutations = 999

Post hoc Bacteria 16S OTU comparisons by time since conversion at 5-15 cm depth interval

Pairs		F.model	R^2	p.adjusted
A	B	2.46	0.16	0.08
	C	2.04	0.13	0.11
	D	1.94	0.11	0.19
B	C	1.76	0.14	0.33
	D	1.82	0.13	0.32
C	D	0.94	0.07	1.0

Fungal ITS for categories at 0-5 cm depth interval

Source	Df	R2	Pr(>r)
Categories	3	0.21	0.001***
Residual	26	0.79	

Number of permutations = 999

Post hoc Fungal ITS comparisons by time since conversion 0-5 cm depth interval

Pairs		F.model	R^2	p.adjusted
A	B	2.54	0.16	0.01 .
	C	2.85	0.17	0.006*
	D	4.34	0.22	0.006*
B	C	1.41	0.14	0.83
	D	1.27	0.10	1.0
C	D	0.76	0.06	1.0

Fungal ITS for categories at 5-15 cm depth interval

Source	Df	R2	Pr(>r)
Categories	3	0.25	0.001***
Residual	26	0.75	

Significant codes: '***' 0.001, '**'0.01, '*' 0.05

Number of permutations = 999

Post hoc Fungal ITS comparisons by time since conversion 5-15 cm depth interval

Pairs		F.model	R^2	p.adjusted
A	B	2.58	0.17	0.02 .
	C	4.25	0.23	0.01 .
	D	5.20	0.26	0.006*
B	C	1.84	0.14	0.14
	D	1.80	0.13	0.19
C	D	0.85	0.06	1.0

Table S 28. ANOVA analysis Bacterial OTU communities at 0-5 cm depth interval

ANOVA – Acidobacteria-Chloracidobacteria.

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.92e-4	3	6.39e-5	2.53	0.08
Residuals	6.58e-4	26	2.53e-5		

Note. Type III Sum of Squares

ANOVA - p__Actinobacteria.c__Acidimicrobiia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.57e-4	3	5.24e-5	12.30	< .001
Residuals	1.11e-4	26	4.26e-6		

Note. Type III Sum of Squares

Post Hoc Acidimicrobiia comparisons- Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	4.32e-3	1.09e-3	3.97	2.68e-3	**
	C	4.52e-3	1.04e-3	4.34	1.04e-3	**
	D	5.62e-3	1.00e-3	5.60	< .001	***
B	C	1.95e-4	1.15e-3	0.17	1.00	
	D	1.30e-3	1.12e-3	1.17	0.65	
C	D	1.11e-3	1.07e-3	1.03	0.73	

** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

ANOVA – Acidobacteria - Acidobacteria.6

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.01	3	4.64e-3	5.94	3.16e-3
Residuals	0.02	26	7.81e-4		

Note. Type III Sum of Squares

Post Hoc Acidobacteria.6 comparisons-Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	0.06	0.01	4.15	1.68e-3	**
	C	0.03	0.01	2.38	0.11	
	D	0.02	0.01	1.78	0.30	
B	C	-0.03	0.02	-1.77	0.31	
	D	-0.04	0.02	-2.45	0.09	
C	D	-9.37e-3	0.01	-0.65	0.92	

** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA - Acidobacteria - Acidobacteriia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	7.05e-4	3	2.35e-4	3.30	0.04
Residuals	1.85e-3	26	7.11e-5		

Note. Type III Sum of Squares

Post Hoc Acidobacteriia comparisons-Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-0.01	4.44e-3	-2.88	0.04	*
	C	-3.78e-3	4.25e-3	-0.89	0.81	
	D	-4.61e-4	4.10e-3	-0.11	1.00	
B	C	9.03e-3	4.69e-3	1.92	0.24	
	D	0.01	4.55e-3	2.71	0.05	
C	D	3.32e-3	4.36e-3	0.76	0.87	

* p < .05

Note. P-value adjusted for comparing a family of 4

ANOVA - Actinobacteria - Actinobacteria

Cases	Sum of Squares	df	Mean Square	F	p
Categories	6.17e-3	3	2.06e-3	7.15	1.17e-3
Residuals	7.48e-3	26	2.88e-4		

Note. Type III Sum of Squares

Post Hoc Actinobacteria comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-0.03	8.94e-3	-3.80	4.12e-3	**
	C	-0.03	8.55e-3	-3.21	0.02	*
	D	-0.03	8.24e-3	-3.85	3.65e-3	**
B	C	6.49e-3	9.44e-3	0.69	0.90	
	D	2.25e-3	9.16e-3	0.25	0.99	
C	D	-4.24e-3	8.78e-3	-0.48	0.96	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test Alpha-proteobacteria

Factor	Statistic	df	p
Categories	10.70	3	0.01

Post Hoc Alpha-proteobacteria -Categories

Categories		$p_{bonferroni}$
A	B	1.00
	C	0.85
	D	0.36
B	C	0.007***
	D	0.016**
C	D	1.00

* p < .05, ** p < .01, ***p<0.005

Kruskal-Wallis Test Firmicutes - Bacilli

Factor	Statistic	df	p
Categories	4.04	3	0.26

ANOVA - Proteobacteria - Betaproteobacteria

Cases	Sum of Squares	df	Mean Square	F	p
Categories	8.08e-4	3	2.69e-4	0.40	0.76
Residuals	0.02	26	6.81e-4		

Note. Type III Sum of Squares

Kruskal-Wallis Test Firmicutes - Clostridia

Factor	Statistic	df	p
Categories	8.54	3	0.04

Post Hoc Clostridia -Categories

Categories		$p_{bonferroni}$
A	B	1.00
	C	0.85
	D	0.36
B	C	0.007*
	D	0.02*
C	D	1.00

* p < .05, ** p < .01, ***p<0.005

ANOVA – Proteobacteria - Deltaproteobacteria

Cases	Sum of Squares	df	Mean Square	F	p
Categories	8.88e-4	3	2.96e-4	6.61	1.82e-3
Residuals	1.17e-3	26	4.48e-5		

Note. Type III Sum of Squares

Post Hoc Deltaproteobacteria Comparisons –Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	0.01	3.53e-3	4.16	1.66e-3	**
	C	9.40e-3	3.37e-3	2.79	0.05	*
	D	0.01	3.25e-3	3.09	0.02	*
B	C	-5.27e-3	3.72e-3	-1.41	0.50	
	D	-4.62e-3	3.62e-3	-1.28	0.58	
C	D	6.47e-4	3.46e-3	0.19	1.00	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA – Chloroflexi - Ellin6529

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.34e-4	3	4.45e-5	3.28	0.04
Residuals	3.53e-4	26	1.36e-5		

Note. Type III Sum of Squares

Post Hoc Ellin6529 comparisons by categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-3.43e-3	1.94e-3	-1.77	0.31	
	C	-4.17e-3	1.86e-3	-2.25	0.14	
	D	-5.29e-3	1.79e-3	-2.96	0.03	*
B	C	-7.36e-4	2.05e-3	-0.36	0.98	
	D	-1.86e-3	1.99e-3	-0.93	0.79	
C	D	-1.12e-3	1.91e-3	-0.59	0.93	

* p < .05

Note. P-value adjusted for comparing a family of 4

ANOVA – Proteobacteria - Gammaproteobacteria

Cases	Sum of Squares	df	Mean Square	F	p
Categories	2.07e-3	3	6.90e-4	2.36	0.09
Residuals	7.61e-3	26	2.93e-4		

Note. Type III Sum of Squares

ANOVA – Gemmatimonadetes - Gemm.1

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.59e-4	3	5.29e-5	5.29	5.56e-3
Residuals	2.60e-4	26	1.00e-5		

Note. Type III Sum of Squares

Post Hoc Gemm.1- comparisons-Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-2.37e-3	1.67e-3	-1.42	0.50	
	C	-3.72e-3	1.59e-3	-2.34	0.12	
	D	-6.00e-3	1.54e-3	-3.90	3.19e-3	**
B	C	-1.35e-3	1.76e-3	-0.77	0.87	
	D	-3.62e-3	1.71e-3	-2.12	0.17	
C	D	-2.27e-3	1.64e-3	-1.39	0.52	

** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA -Gemmatimonadetes - Gemmatimonadetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	8.53e-4	3	2.84e-4	6.26	2.42e-3
Residuals	1.18e-3	26	4.54e-5		

Note. Type III Sum of Squares

Post Hoc Gemmatimonadetes comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-0.01	3.55e-3	-4.17	1.62e-3	**
	C	-8.24e-3	3.40e-3	-2.43	0.10	
	D	-9.21e-3	3.27e-3	-2.81	0.04	*
B	C	6.56e-3	3.75e-3	1.75	0.32	
	D	5.59e-3	3.64e-3	1.54	0.43	
C	D	-9.68e-4	3.49e-3	-0.28	0.99	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA - Actinobacteria - MB.A2.108

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.02e-4	3	1.01e-4	5.97	3.08e-3
Residuals	4.38e-4	26	1.68e-5		

Note. Type III Sum of Squares

Post Hoc MB.A2.108 comparisons-Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	7.33e-3	2.16e-3	3.39	0.01	*
	C	6.30e-3	2.07e-3	3.05	0.03	*
	D	7.04e-3	1.99e-3	3.53	7.98e-3	**
B	C	-1.02e-3	2.28e-3	-0.45	0.97	
	D	-2.82e-4	2.22e-3	-0.13	1.00	
C	D	7.39e-4	2.12e-3	0.35	0.99	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA – Nitrospirae -Nitrospira

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.15e-4	3	3.82e-5	0.92	0.44
Residuals	1.07e-3	26	4.13e-5		

Note. Type III Sum of Squares

ANOVA – Planctomycetes -Planctomycetia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	2.31e-4	3	7.69e-5	3.79	0.02
Residuals	5.27e-4	26	2.03e-5		

Note. Type III Sum of Squares

Post Hoc Planctomycetia Comparisons by categories

		Mean Difference	SE	t	p_{tukey}	
A	B	3.34e-3	2.37e-3	1.41	0.51	
	C	5.38e-3	2.27e-3	2.37	0.11	
	D	7.00e-3	2.19e-3	3.20	0.02	*
B	C	2.04e-3	2.51e-3	0.81	0.85	
	D	3.66e-3	2.43e-3	1.50	0.45	
C	D	1.62e-3	2.33e-3	0.70	0.90	

* p < .05

Note. P-value adjusted for comparing a family of 4

ANOVA – Actinobacteria - Thermoleophilia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	6.85e-3	3	2.28e-3	4.49	0.01
Residuals	0.01	26	5.08e-4		

Note. Type III Sum of Squares

Post Hoc Thermoleophilia Comparisons-Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	7.62e-3	0.01	0.64	0.92	
	C	0.01	0.01	0.99	0.76	
	D	0.04	0.01	3.51	8.38e-3	**
B	C	3.64e-3	0.01	0.29	0.99	
	D	0.03	0.01	2.53	0.08	
C	D	0.03	0.01	2.33	0.12	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

Table S29. ANOVA analysis Bacterial OTU communities at 5-15 cm depth interval

ANOVA Acidobacteria - Chloracidobacteria.

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.53e-4	3	1.18e-4	2.49	0.08
Residuals	1.23e-3	26	4.74e-5		

Note. Type III Sum of Squares

ANOVA Actinobacteria - Acidimicrobiia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	7.06e-5	3	2.35e-5	6.34	2.26e-3
Residuals	9.65e-5	26	3.71e-6		

Note. Type III Sum of Squares

Post Hoc Acidimicrobiia comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	2.82e-3	1.02e-3	2.78	0.05	*
	C	3.66e-3	9.71e-4	3.77	4.43e-3	**
	D	3.33e-3	9.36e-4	3.55	7.59e-3	**
B	C	8.37e-4	1.07e-3	0.78	0.86	
	D	5.03e-4	1.04e-3	0.48	0.96	
C	D	-3.34e-4	9.97e-4	-0.34	0.99	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA -Acidobacteria - Acidobacteria.6

Cases	Sum of Squares	df	Mean Square	F	p
Categories	4.55e-3	3	1.52e-3	1.83	0.17
Residuals	0.02	26	8.29e-4		

Note. Type III Sum of Squares

ANOVA – Acidobacteria - Acidobacteriia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	6.30e-4	3	2.10e-4	1.38	0.27
Residuals	3.97e-3	26	1.53e-4		

Note. Type III Sum of Squares

ANOVA -Actinobacteria - Actinobacteria

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.72e-3	3	5.73e-4	3.51	0.03
Residuals	4.25e-3	26	1.64e-4		

ANOVA -Actinobacteria - Actinobacteria

Cases	Sum of Squares	df	Mean Square	F	p

Note. Type III Sum of Squares

Post Hoc Actinobacteria comparisons-Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.02	6.74e-3	-3.23	0.02 *
	C	-7.59e-3	6.44e-3	-1.18	0.65
	D	-9.34e-3	6.21e-3	-1.50	0.45
B	C	0.01	7.12e-3	1.99	0.22
	D	0.01	6.91e-3	1.80	0.30
C	D	-1.75e-3	6.62e-3	-0.26	0.99

* $p < .05$

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test Proteobacteria - Alphaproteobacteria

Factor	Statistic	df	p
Categories	7.35	3	0.06

ANOVA -Firmicutes - Bacilli

Cases	Sum of Squares	df	Mean Square	F	p
Categories	1.48e-3	3	4.92e-4	0.65	0.59
Residuals	0.02	26	7.61e-4		

Note. Type III Sum of Squares

ANOVA -Proteobacteria - Betaproteobacteria

Cases	Sum of Squares	df	Mean Square	F	p
Categories	2.52e-3	3	8.41e-4	0.85	0.48
Residuals	0.03	26	9.91e-4		

Note. Type III Sum of Squares

ANOVA – Firmicutes- Clostridia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	5.02e-5	3	1.67e-5	0.68	0.57
Residuals	6.41e-4	26	2.47e-5		

Note. Type III Sum of Squares

Kruskal-Wallis Test Proteobacteria - Deltaproteobacteria

Factor	Statistic	df	p
Categories	4.44	3	0.22

Kruskal-Wallis Test Chloroflexi - Ellin6529

Factor	Statistic	df	p
Categories	4.68	3	0.20

ANOVA -Proteobacteria - Gammaproteobacteria

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.02e-3	3	1.01e-3	3.56	0.03
Residuals	7.36e-3	26	2.83e-4		

Note. Type III Sum of Squares

Post Hoc Gammaproteobacteria comparisons- Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.02	8.87e-3	-2.28	0.13
	C	-1.78e-3	8.48e-3	-0.21	1.00
	D	-0.02	8.17e-3	-2.61	0.07
B	C	0.02	9.36e-3	1.97	0.23
	D	-1.12e-3	9.09e-3	-0.12	1.00
C	D	-0.02	8.71e-3	-2.24	0.14

Note. P-value adjusted for comparing a family of 4

ANOVA -Gemmatimonadetes - Gemm.1

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.39e-4	3	1.13e-4	4.41	0.01
Residuals	6.66e-4	26	2.56e-5		

Note. Type III Sum of Squares

Post Hoc Gemm.1 comparisons - Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-8.02e-5	2.67e-3	-0.03	1.00	
	C	-2.42e-3	2.55e-3	-0.95	0.78	
	D	-8.08e-3	2.46e-3	-3.29	0.01	*
B	C	-2.34e-3	2.82e-3	-0.83	0.84	
	D	-8.00e-3	2.73e-3	-2.93	0.03	*
C	D	-5.66e-3	2.62e-3	-2.16	0.16	

* $p < .05$

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test Gemmatimonadetes - Gemmatimonadetes

Factor	Statistic	df	p
Categories	13.77	3	3.23e-3

Post hoc test Gemmatimonadetes – Categories

Categories		$p_{bonferroni}$
A	B	0.22
	C	1
	D	0.07*
B	C	0.83
	D	1
C	D	0.17

* p < .05, ** p < .01, ***p<0.005

ANOVA -Actinobacteria - MB.A2.108

Cases	Sum of Squares	df	Mean Square	F	p
Categories	6.94e-4	3	2.31e-4	7.76	< .001
Residuals	7.75e-4	26	2.98e-5		

Note. Type III Sum of Squares

Post Hoc MB.A2.108 Comparisons-Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	0.01	2.88e-3	4.45	< .001	***
	C	8.61e-3	2.75e-3	3.13	0.02	*
	D	9.04e-3	2.65e-3	3.41	0.01	*
B	C	-4.18e-3	3.04e-3	-1.38	0.52	
	D	-3.75e-3	2.95e-3	-1.27	0.59	
C	D	4.32e-4	2.83e-3	0.15	1.00	

* p < .05, ** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

ANOVA – Nitrospirae - Nitrospira

Cases	Sum of Squares	df	Mean Square	F	p
Categories	4.17e-4	3	1.39e-4	1.01	0.40
Residuals	3.56e-3	26	1.37e-4		

Note. Type III Sum of Squares

ANOVA – Planctomycetes - Planctomycetia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	2.80e-4	3	9.35e-5	3.59	0.03
Residuals	6.77e-4	26	2.60e-5		

Note. Type III Sum of Squares

Post Hoc Planctomycetia comparisons- Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-5.50e-3	2.69e-3	-2.05	0.20	
	C	4.83e-5	2.57e-3	0.02	1.00	
	D	3.52e-3	2.48e-3	1.42	0.50	
B	C	5.55e-3	2.84e-3	1.96	0.23	
	D	9.03e-3	2.75e-3	3.28	0.01	*
C	D	3.48e-3	2.64e-3	1.32	0.56	

* $p < .05$

Note. P-value adjusted for comparing a family of 4

ANOVA – Actinobacteria - Thermoleophilia

Cases	Sum of Squares	df	Mean Square	F	p
Categories	9.77e-3	3	3.26e-3	2.86	0.06
Residuals	0.03	26	1.14e-3		

Note. Type III Sum of Squares

Table S30. ANOVA analysis of Fungal ITS OTU communities at 0-5 cm depth interval

ANOVA – Basidiomycota - Agaricomycetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.07	3	0.02	5.29	5.55e-3
Residuals	0.11	26	4.25e-3		

Note. Type III Sum of Squares

Post Hoc Agaricomycetes comparisons-Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	0.10	0.03	2.90	0.04	*
	C	0.11	0.03	3.47	9.35e-3	**
	D	0.09	0.03	2.98	0.03	*
B	C	0.01	0.04	0.40	0.98	
	D	-5.26e-3	0.04	-0.15	1.00	
C	D	-0.02	0.03	-0.58	0.94	

* p < .05, ** p < .01

Note. P-value adjusted for comparing a family of 4

ANOVA – Ascomycota - Dothideomycetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.14	3	0.05	2.94	0.05
Residuals	0.42	26	0.02		

Note. Type III Sum of Squares

Post Hoc Dothideomycetes comparisons- Categories

		Mean Difference	SE	t	p_{tukey}	
A	B	-0.10	0.07	-1.45	0.48	
	C	-0.18	0.06	-2.86	0.04	*
	D	-0.12	0.06	-2.01	0.21	
B	C	-0.09	0.07	-1.21	0.62	
	D	-0.03	0.07	-0.40	0.98	
C	D	0.06	0.07	0.89	0.81	

* p < .05

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test Ascomycota -Eurotiomycetes

Factor	Statistic	df	p
Categories	10.82	3	0.01

Post Hoc Test Eurotiomycetes – Categories

	Categories	$p_{bonferroni}$
A	B	1.00
	C	0.19
	D	0.28
B	C	0.13
	D	0.03*
C	D	1.00

* p < .05, ** p < .01, ***p<0.005

ANOVA – Ascomycota - Leotiomycetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.05	3	0.02	1.06	0.38
Residuals	0.41	26	0.02		

Note. Type III Sum of Squares

Kruskal-Wallis Test Ascomycota – Sordariomycetes

Factor	Statistic	df	p
Categories	13.59	3	3.53e-3

Post hoc Sordiomycetes comparisons by categories

	Categories	$p_{bonferroni}$
A	B	0.046
	C	0.047
	D	0.006
B	C	1.00
	D	1.00*
C	D	1.00

* p < .05, ** p < .01, ***p<0.005

ANOVA – Basidiomycota - Tremellomycetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	7.07e-3	3	2.36e-3	2.09	0.13
Residuals	0.03	26	1.13e-3		

ANOVA – Basidiomycota - Tremellomycetes

Cases	Sum of Squares	df	Mean Square	F	p

Note. Type III Sum of Squares

Table S 31. ANOVA analysis of Fungal ITS OTU communities at 5-15 cm depth interval

Kruskal-Wallis Test Basidiomycota - Agaricomycetes

Factor	Statistic	df	p
Categories	8.37	3	0.04

Post hoc Agaricomycetes comparisons by categories

Categories		$p_{bonferroni}$
A	B	1.0
	C	0.68
	D	0.02*
B	C	1.0
	D	0.85
C	D	1.00

* p < .05, ** p < .01, ***p<0.005

ANOVA – Ascomycota - Dothideomycetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.07	3	0.02	9.15	< .001
Residuals	0.06	26	2.44e-3		

Note. Type III Sum of Squares

Post Hoc Comparisons - Dothideomycetes Categories

		Mean Difference	SE	t	p_{tukey}
A	B	-0.10	0.03	-3.79	4.22e-3 **
	C	-0.07	0.02	-2.65	0.06
	D	-0.12	0.02	-4.90	< .001 ***
B	C	0.03	0.03	1.19	0.64
	D	-0.02	0.03	-0.71	0.89
C	D	-0.05	0.03	-2.02	0.21

** p < .01, *** p < .001

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test Ascomycota - Eurotiomycetes

Factor	Statistic	df	p
Categories	4.23	3	0.24

ANOVA – Ascomycota Leotiomycetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	0.16	3	0.05	3.33	0.03
Residuals	0.43	26	0.02		

Note. Type III Sum of Squares

Post Hoc Leotiomycetes comparisons- Categories

		Mean Difference	SE	t	p_{tukey}
A	B	0.11	0.07	1.57	0.41
	C	0.16	0.06	2.55	0.08
	D	0.18	0.06	2.83	0.04 *
B	C	0.06	0.07	0.82	0.85
	D	0.07	0.07	1.01	0.75
C	D	0.01	0.07	0.17	1.00

* p < .05

Note. P-value adjusted for comparing a family of 4

Kruskal-Wallis Test Ascomycota - Sordariomycetes

Factor	Statistic	df	p
Categories	18.00	3	< .001

Post hoc Sordiomycetes comparisons by categories

Categories		$p_{bonferroni}$
A	B	0.05
	C	0.004
	D	<0.001
B	C	1.0
	D	1.0
C	D	1.0

* p < .05, ** p < .01, ***p<0.005

ANOVA – Basidiomycota - Tremellomycetes

Cases	Sum of Squares	df	Mean Square	F	p
Categories	3.07e-3	3	1.02e-3	1.26	0.31
Residuals	0.02	26	8.10e-4		

Note. Type III Sum of Squares

Table S 32. Physical, chemical and biological soil properties since time since conversion by categories A = forest, B = < 10 y agriculture, C= >10<50 y agriculture,D= >50 y agriculture. Mean values and (standard deviation)

Physical and chemical soil properties	Categories								
	A		B		C		D		Average
Clay (%)	29.16	(12.55)	22.22	(11.43)	32.19	(9.78)	24.09	(8.11)	-6%
SOM (%)	11.72	(6.04)	5.65	(2.11)	5.11	(1.87)	7.5	(4.31)	-12%
pH	6.15	(0.38)	5.81	(0.48)	6.19	(0.33)	6.24	(0.31)	0%
P (ppm)	4.21	(2.24)	6.05	(4.38)	8.08	(6.08)	10.39	(10.64)	49%
K(ppm)	154.3	(82.85)	135.96	(59.73)	174.87	(128.78)	190.21	(94.31)	8%
TotalC (%)	7.57	(3.78)	3.56	(1.27)	3.21	(0.94)	4.94	(3.11)	-12%
Total N (%)	0.51	(0.23)	0.27	(0.11)	0.28	(0.11)	0.38	(0.2)	-8%
Mg(ppm)	797.17	(418.31)	448.17	(284.35)	666.24	(212.31)	480.89	(218.06)	-13%
Fe(ppm)	19.95	(14.91)	17.58	(5.4)	16.16	(17.67)	10.95	(4.21)	-15%
Mn(ppm)	12.38	(6.04)	10.4	(3.56)	7.51	(6.1)	9.22	(5.66)	-9%
Zn(ppm)	1.38	(1.18)	1.57	(1.11)	1.06	(0.62)	2.09	(1.53)	17%

Table S 33. Relationship between soil organic matter, chemical soil properties and functional genes targeted by qPCR using Spearman correlation analysis. Correlations (p <0.05) at both depth intervals.

Soil properties	qPCR (copies ng-1 DNA)	Spearman's rho	p	
SOM	Fungal 18S	-0.26	0.04	*
SOM	Bglu	-0.29	0.02	*
SOM	gh11	-0.35	5.90E-03	**
SOM	B-amoA-	-0.45	< .001	***
SOM	A-amoA-	-0.31	0.02	*
SOM	nxrA	-0.46	< .001	***
SOM	nrfA	0.29	0.02	*
SOM	phoC	0.35	5.58E-03	**
pH	CBH	-0.28	0.03	*
pH	Bglu	0.56	< .001	***
pH	A-amoA-	0.37	3.52E-03	**
pH	nrfA	0.57	< .001	***
pH	NosZ	0.3	0.02	*
pH	pqqC	-0.27	0.04	*
P	Archael 16S	0.47	< .001	***
P	Fungal 18S	0.65	< .001	***
P	CBH	0.51	< .001	***
P	gh11	0.56	< .001	***
P	B-amoA-	0.5	< .001	***
P	nxrA	0.42	< .001	***
P	NosZ	0.31	0.02	*
K	Archael 16S	0.28	0.03	*
K	Fungal 18S	0.4	1.34E-03	**
K	CBH	0.27	0.04	*
K	gh11	0.27	0.03	*
K	NosZ	0.32	0.01	*
Total C	Bglu	-0.32	0.01	*
Total C	gh11	-0.28	0.03	*
Total C	B-amoA-	-0.4	1.45E-03	**
Total C	A-amoA-	-0.38	2.71E-03	**
Total C	nxrA	-0.42	< .001	***
Total C	nrfA	0.31	0.02	*
Total C	phoC	0.37	3.26E-03	**

Total N	Bglu	-0.35	5.89E-03	**
Total N	B-amoA-	-0.34	8.35E-03	**
Total N	A-amoA-	-0.35	6.76E-03	**
Total N	nxrA	-0.34	8.15E-03	**
Total N	phoC	0.26	0.04	*
Mg	Laccase	0.3	0.02	*
Mg	nxrA	-0.35	5.94E-03	**
Mg	nrfA	0.49	< .001	***
Mg	NosZ	0.3	0.02	*
Mg	phoD	0.36	4.44E-03	**
Fe	Archael 16S	-0.38	2.48E-03	**
Fe	Bglu	-0.44	< .001	***
Fe	B-amoA-	-0.3	0.02	*
Fe	A-amoA-	-0.4	1.52E-03	**
Fe	nrfA	-0.6	< .001	***
Fe	NosZ	-0.39	2.46E-03	**
Mn	Bglu	-0.5	< .001	***
Mn	A-amoA-	-0.61	< .001	***
Zn	Bacterial 16S	-0.1	0.44	
Zn	Archael 16S	0.26	0.04	*
Zn	Fungal 18S	0.27	0.04	*

* p < .05, ** p < .01, *** p < .001

Table S 34. Relationship between soil organic matter, chemical soil properties and bacterial class OTU's using Spearman correlation analysis. Significant different $p < 0.05$ values are shown for each bacterial class at both depth intervals

Physical, biological and chemical metrics	Phyla – class bacterial 16s OTU	Spearman's rho	p	
SOM	Actinobacteria-Acidimicrobiia	0.58	< .001	***
SOM	Acidobacteria-Acidobacteria.6	0.32	0.01	*
SOM	Actinobacteria-MB.A2.108	0.26	0.05	*
SOM	Planctomycetes-Planctomycetia	0.28	0.03	*
SOM	Acidobacteria-Acidobacteriia	-0.4	< .001	**
SOM	Chloroflexi-Ellin6529	-0.3	0.02	*
SOM	Gemmatimonadetes-Gemm.1	-0.4	< .001	**
pH	Acidobacteria-.Chloracidobacteria.	0.4	< .001	**
pH	Acidobacteria-Acidobacteria.6	0.57	< .001	***
pH	Gemmatimonadetes-Gemm.1	0.45	< .001	***
pH	Actinobacteria-MB.A2.108	0.37	< .001	**
pH	Acidobacteria-Acidobacteriia	-0.5	< .001	***
pH	Actinobacteria-Actinobacteria	-0.3	0.05	*
pH	Proteobacteria-Alphaproteobacteria	-0.7	< .001	***
pH	Planctomycetes-Planctomycetia	-0.6	< .001	***
P	Proteobacteria-Gammaproteobacteria	0.31	0.02	*
P	Actinobacteria-Actinobacteria	0.64	< .001	***
P	Actinobacteria-Acidimicrobiia	-0.3	0.01	*
P	Proteobacteria-Deltaproteobacteria	-0.4	< .001	**
P	Actinobacteria-MB.A2.108	-0.4	< .001	**
K	Actinobacteria-Actinobacteria	0.49	< .001	***
K	Chloroflexi-Ellin6529	-0.4	< .001	**
Total C	Actinobacteria-Acidimicrobiia	0.59	< .001	***
Total C	Acidobacteria-Acidobacteria.6	0.3	0.02	*
Total C	Planctomycetes-Planctomycetia	0.3	0.02	*
Total C	Acidobacteria-Acidobacteriia	-0.4	< .001	**
Total C	Chloroflexi-Ellin6529	-0.3	0.03	*
Total C	Gemmatimonadetes-Gemm.1	-0.4	< .001	***
Total N	Actinobacteria-Acidimicrobiia	0.53	< .001	***
Total N	Planctomycetes-Planctomycetia	0.28	0.03	*
Total N	Acidobacteria-Acidobacteriia	-0.3	0.01	*
Total N	Chloroflexi-Ellin6529	-0.3	0.04	*
Total N	Gemmatimonadetes-Gemm.1	-0.4	< .001	***

Mg	Acidobacteria-Acidobacteria.6	0.38	< .001	**
Mg	Acidobacteria-Acidobacteriia	-0.3	0.02	*
Mg	Chloroflexi-Ellin6529	-0.3	0.01	*
Mg	Proteobacteria-Gammaproteobacteria	-0.5	< .001	***
Mg	Actinobacteria-MB.A2.108	0.46	< .001	***
Fe	Acidobacteria-.Chloracidobacteria.	-0.3	0.02	*
Fe	Acidobacteria-Acidobacteria.6	-0.6	< .001	***
Fe	Acidobacteria-Acidobacteriia	0.58	< .001	***
Fe	Proteobacteria-Alphaproteobacteria	0.59	< .001	***
Fe	Nitrospirae-Nitrospira	-0.3	0.02	*
Fe	Planctomycetes-Planctomycetia	0.34	< .001	**
Mn	Acidobacteria-.Chloracidobacteria.	-0.5	< .001	***
Mn	Actinobacteria-Acidimicrobiia	0.31	0.01	*
Mn	Acidobacteria-Acidobacteria.6	-0.2	0.15	
Mn	Proteobacteria-Alphaproteobacteria	0.36	< .001	**
Mn	Chloroflexi-Ellin6529	-0.3	0.05	*
Mn	Gemmatimonadetes-Gemm.1	-0.5	< .001	***
Mn	Nitrospirae-Nitrospira	-0.4	< .001	***
Mn	Planctomycetes-Planctomycetia	0.4	< .001	**
Zn	Actinobacteria-Actinobacteria	0.3	0.02	*
Zn	Proteobacteria-Alphaproteobacteria	0.27	0.04	*
Zn	Nitrospirae-Nitrospira	-0.4	< .001	**
Zn	Planctomycetes-Planctomycetia	0.31	0.02	*
Zn	Chloroflexi-Thermomicrobia	0.33	0.01	*

Table S35. Relationship between soil organic matter, chemical soil properties and fungal class OTU's using Spearman correlation analysis. Significant different $p < 0.05$ values are shown for each fungal class at both depth intervals.

Biological and chemical metrics	**Phyla – class fungal OTU**	**Spearman's rho**	***p***	
SOM	Basidiomycota-Agaricomycetes	0.32	0.01	*
SOM	Ascomycota-Sordariomycetes	-0.3	0.02	*
P	Ascomycota-Dothideomycetes	0.33	0.01	*
P	Ascomycota-Sordariomycetes	0.49	< .001	***
P	Ascomycota-Leotiomycetes	-0.5	< .001	***
P	Basidiomycota-Agaricomycetes	-0.4	3.92E-03	**
K	Ascomycota-Sordariomycetes	0.31	0.02	*
K	Ascomycota-Leotiomycetes	-0.4	3.68E-03	**
Total C	Basidiomycota-Agaricomycetes	0.31	0.02	*
Total N	Ascomycota-Sordariomycetes	-0.3	0.04	*
Fe	Basidiomycota-Agaricomycetes	0.26	0.04	*
Fe	Ascomycota-Leotiomycetes	0.36	4.93E-03	**
Fe	Ascomycota-Sordariomycetes	-0.3	9.39E-03	**
Mn	Basidiomycota-Agaricomycetes	0.34	8.18E-03	**
Mn	Ascomycota-Sordariomycetes	-0.4	4.16E-03	**

* p < .05, ** p < .01, *** p < .001

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